The Hypothalamus and Hypertension

H. E. DE WARDENER

Department of Clinical Chemistry, Imperial College School of Medicine,
Charing Cross Campus, London, United Kingdom

I. Introduction 1601
II. Disposition of Certain Hypothalamic Nuclei Involved in Hypertension 1601
   A. The AV$_3$V area 1602
III. Functional Disturbances in the Hypothalamus of Hypertensive Animals 1603
IV. Catecholamines 1603
   A. Distribution of catecholamines in the hypothalamus and effect of norepinephrine on the paraventricular nucleus 1603
   B. Hypothalamic catecholamines and normal blood pressure 1603
   C. Hypothalamic catecholamines and hypertension 1604
   D. Summary 1604
   E. Effect on hypothalamic noradrenergic activity of an increase in sodium intake in the young SHR 1604
   F. Evidence for an increase in norepinephrine release from subcortical regions in hypertensive man 1605
V. Cholinergic Mechanisms 1605
   A. Distribution of cholinergic activity in the hypothalamus 1605
   B. Cholinergic interactions with catecholamines, nitric oxide, and AVP 1605
   C. Hypothalamic cholinergic activity and normal blood pressure 1606
   D. Hypothalamic cholinergic activity and hypertension 1606
   E. Summary 1607
VI. Angiotensin 1607
   A. Distribution of angiotensinergic activity in the hypothalamus 1607
   B. Angiotensin interactions with catecholamines and AVP 1608
   C. Hypothalamic angiotensin and normal blood pressure 1608
   D. Hypothalamic angiotensin and hypertension 1608
   E. Summary 1609
VII. Natriuretic Peptides 1609
   A. Distribution of Natriuretic Peptides in the hypothalamus 1610
   B. Effect of ANP on neuronal hypothalamic activity 1610
   C. Interactions between hypothalamic ANP and catecholaminergic and cholinergic mechanisms, AVP, angiotensin, aldosterone, salt intake, and volume changes 1610
   D. Hypothalamic natriuretic peptides and normal blood pressure 1611
   E. Hypothalamic natriuretic peptides and hypertension 1611
   F. Summary 1612
VIII. Vasopressin 1612
   A. Distribution of AVP in the hypothalamus 1612
   B. Interactions between AVP-containing neurons, catecholaminergic and cholinergic mechanisms, ANP, angiotensin, and DOCA 1612
   C. Hypothalamic AVP and normal blood pressure 1612
   D. Hypothalamic AVP and hypertension 1613
   E. Summary 1613
IX. Nitric Oxide 1614
   A. Distribution of NOS in the hypothalamus 1614
   B. Effects of NO 1615
   C. Relation of hypothalamic NOS activity to some other neurotransmitters and neuromodulators 1615
   D. NO and synaptic transmission in the hypothalamus 1616
   E. Hypothalamic NOS and normal blood pressure 1616
   F. Hypothalamic NOS and hypertension 1617
   G. Summary 1617
X. Serotonin 1618
   A. Distribution of 5-HT in the hypothalamus 1618
   B. Hypothalamic 5-HT and normal blood pressure 1618
XI. γ-Aminobutyric Acid
A. Hypothalamic GABA and normal blood pressure 1620
B. Hypothalamic GABA and hypertension 1620
C. Summary 1621

XII. Neuropeptide Y
A. Distribution of NPY in the hypothalamus 1622
B. Interaction of NPY with catecholamines, AVP, NO, cholinergic, and histaminergic mechanisms 1622
C. Hypothalamic NPY and normal blood pressure 1622
D. Hypothalamic NPY and hypertension 1622
E. Summary 1623

XIII. Ouabain-like Substances
A. Distribution of mammalian ouabain in the hypothalamus 1623
B. Control of hypothalamic ouabain, the central effect of ouabain on the sympathetic system, and the interaction of ouabain with other hypothalamic substances 1624
C. Hypothalamic ouabain and normal blood pressure 1624
D. Hypothalamic ouabain and hypertension 1624
E. Summary 1625

XIV. Opioids
A. Hypothalamic opioids and normal blood pressure 1626
B. Hypothalamic opioids and hypertension 1626
C. Summary 1626

XV. Bradykinin
A. Distribution of kallikrein-kinin system in the hypothalamus 1627
B. Bradykinin interaction with catecholamines, prostaglandins, and NO 1627
C. Hypothalamic bradykinin and normal blood pressure 1627
D. Hypothalamic bradykinin and hypertension 1628
E. Summary 1628

XVI. Thyrotropin-Releasing Hormone
A. Distribution of TRH in the hypothalamus 1629
B. TRH interaction with cholinergic, angiotensin, 5-HT, and opiate mechanisms 1629
C. Hypothalamic TRH and normal blood pressure 1629
D. Hypothalamic TRH and hypertension 1629
E. Summary 1630

XVII. Vasoactive Intestinal Polypeptide
A. Hypothalamic VIP and normal blood pressure 1630
B. Hypothalamic VIP and hypertension 1630
C. Summary 1630

XVIII. Tachykinins
A. Hypothalamic substance P and neurokinin B and hypertension 1631
B. Summary 1631

XIX. Histamine
A. Distribution of histamine in the hypothalamus and interaction with other substances 1631
B. Hypothalamic histamine and normal blood pressure 1631
C. Hypothalamic histamine and hypertension 1631
D. Summary 1632

XX. Corticotropin-Releasing Factor
A. CRF and blood pressure 1632
B. Summary 1632

XXI. Compensatory Effects on Hypothalamic Function Induced by a Change in Blood Pressure
A. Summary 1633
XXII. Discussion
A. Summary 1635

De Wardener, H. E. The Hypothalamus and Hypertension. *Physiol Rev* 81: 1599–1658, 2001.—Most forms of hypertension are associated with a wide variety of functional changes in the hypothalamus. Alterations in the
following substances are discussed: catecholamines, acetylcholine, angiotensin II, natriuretic peptides, vasopressin, nitric oxide, serotonin, GABA, ouabain, neuropeptide Y, opioids, bradykinin, thyrotropin-releasing factor, vasoactive intestinal polypeptide, tachykinins, histamine, and corticotropin-releasing factor. Functional changes in these substances occur throughout the hypothalamus but are particularly prominent rostrally; most lead to an increase in sympathetic nervous activity which is responsible for the rise in arterial pressure. A few appear to be depressor compensatory changes. The majority of the hypothalamic changes begin as the pressure rises and are particularly prominent in the young rat; subsequently they tend to fluctuate and overall to diminish with age. It is proposed that, with the possible exception of the Dahl salt-sensitive rat, the hypothalamic changes associated with hypertension are caused by renal and intrathoracic cardiopulmonary afferent stimulation. Renal afferent stimulation occurs as a result of renal ischemia and trauma as in the reduced renal mass rat. It is suggested that afferents from the chest arise, at least in part, from the observed increase in left auricular pressure which, it is submitted, is due to the associated documented impaired ability to excrete sodium. It is proposed, therefore, that the hypothalamic changes in hypertension are a link in an integrated compensatory natriuretic response to the kidney’s impaired ability to excrete sodium.

I. INTRODUCTION

The functional cerebral changes that have been detected in the brains of hypertensive animals occur predominantly in the hypothalamus and medulla. The justification for limiting a review to certain changes that take place in the hypothalamus (and certain areas immediately anterior) stems from the conclusion that hypertension appears to be due to an increase in medullary pressor activity due to a suppression from above of medullary inhibitory activity (94, 95). Furthermore, there is the observation by Julius (288) based on observations made on patients with essential hypertension that “... it appears that hypertension is not a disease of blood pressure regulation. The pressure is set at a high level but around that setting it is regulated in a normal fashion.”

The central nervous system’s medullary control of the arterial pressure stems from a tonic excitatory center, situated in a nucleus in a rostro-ventral position with spinal excitatory fibers to the spinal intermediolateral nucleus which controls sympathetic ganglia and the adrenal medulla. The medulla’s excitatory center is under the influence of the hypothalamus, the midbrain, a medullary inhibitory center slightly more caudal than the excitatory center, and the nucleus tractus solitarius (94, 503).

Functional alterations in particular hypothalamic nuclei either raise or lower the blood pressure by altering sympathetic nervous activity. The nuclei are closely interconnected and also communicate with many other areas in the central nervous system both rostrally and caudally. There are efferent spinal neurons (93, 503, 695) that project to the midbrain and medulla and to spinal sympathetic preganglionic neurons while afferent stimuli come from the midbrain and medulla and from chemo- and pressure-sensitive neurons in the heart, the aorta, the carotids (21, 141, 316, 503) and from the viscera, particularly the kidneys (583). After a brief account of the anatomical layout of the hypothalamic nuclei, their multiple interconnections and the effect of the antero third ventri-
structures of the lamina terminalis there is a relatively large area, the septum, which stretches from the corpus callosum above to the optic chiasma below.

Immediately surrounding the most anterior projection of the floor of the third ventricle, the preoptic recess, there is the periventricular preoptic hypothalamic nucleus with ventrally the anterior part of the medial optic nucleus in the lateral portion of which is embedded the anterolateral preoptic nucleus. More posteriorly and dor-sally there is the most anterior part of the paraventricular nucleus. The ventrally placed medial preoptic nucleus extends posteriorly as far as the most anterior edge of the anterior hypothalamic nucleus which appears lateral and ventral to the more dorsally placed paraventricular nucleus. There are inputs into the preoptic and medial pre-optic areas from the anterior hypothalamic area, the lateral hypothalamus, and the ventral and dorsal medial nuclei. The medial preoptic nucleus also receives afferents from various brain stem nuclei including the locus coeruleus and the median raphe magna (198) and projects to the periaqueductal gray neurons, which themselves project to the medulla (506).

As the paraventricular nucleus extends posteriorly, it enlarges laterally, whereas ventrally it becomes the periventricular nucleus. In the rat the paraventricular nucleus, which is an integrating center for many physiological functions, occupies less than one-third of a millimeter on either side of the third ventricle. It is subdivided into a parvocellular area near the midline, a more lateral magnocellular area, and at least eight subdivisions (595). Out of a total of ~21,000 neurons (both sides), 19% are in the magnocellular area (312). Some neurons in the parvocellular part of the paraventricular nucleus connect directly with the midbrain and others have descending projections which make direct contact with the rostroventrolateral medulla, the nucleus tractus solitarius (116, 504), and spinal sympathetic preganglionic neurons (371, 544). Some of the spinally projecting neurons receive information on extracranial vascular pressures and chemical changes (368). Some fibers from the periventricular area of the paraventricular nucleus project to the median eminence and the arcuate nucleus (622).

Into the parvocellular division of the paraventricular nucleus there are inputs from the anterior hypothalamic area, the lateral hypothalamic, and from the ventromedial and dorsomedial nuclei; there are also a few projections from the preoptic area. The suprachiasmatic nucleus also projects to the periventricular and dorsal parts of the paraventricular area of the paraventricular nucleus. The magnocellular division of the paraventricular nucleus only appears to have projections from the dorsomedial and preoptic nuclei. The SFO projects to most regions of the paraventricular nucleus.

More posteriorly, lateral to the posterior continuation of the anterior hypothalamic nucleus, there is the lateral hypothalamic area and below it the tuber cinereum. And as the area of the tuber cinereum diminishes caudally, the median eminence forms the floor of the ventricle. At this level the ventromedial and arcuate nuclei are evident while dorsal to the dorsomedial nucleus there is the dorsal hypothalamic area. The arcuate nucleus projects to the supraoptic nucleus and the SFO (515). The dorsal, ventromedial, and paraventricular nuclei interconnect and lie medial to the lateral hypothalamus, which mainly contains fibers coming from these nuclei, and there are connections between the dorsomedial nucleus and the circumventricular organs. The dorsomedial nucleus (32) contains direct and indirect connections with sympathetic and parasympathetic systems and is influenced by peripheral afferents via the nucleus solitarius, the parabrachial nucleus, and the sympathetic intermediolateral columns.

The posterior hypothalamic nucleus lies immediately posterior to the dorsal hypothalamic area at a level, where ventrally, the infundibular stalk emerges. The posterior hypothalamic nucleus extends posteriorly for a relatively short distance level with the most rostral edge of the ventrally placed mammillary nuclei. It receives a large number of afferents from other parts of the hypothalamus particularly from the anterior hypothalamic and the ventromedial nuclei and a few from the supraoptic, suprachiasmatic, and paraventricular nuclei. In addition, there are a few from the lateral and dorsal hypothalamic areas and the mammillary nuclei (701). It also receives many afferents from the brain stem including the substantia nigra, the periaqueductal gray matter, the rostral raphe nuclei, and the locus coeruleus (525). Neurons from the posterior hypothalamus have descending projections to several autonomic regulating regions in the midbrain and medulla and to sympathetic preganglionic neurons in the mediolateral cell columns of the spinal cord (529, 651). The function of the posterior hypothalamus is very dependent on the activity of the locus coeruleus which lies more caudally.

A. The AV3V Area

A ventral lesion placed into the anterior part of the third ventricle (AV3V) has been used to study the hypothalamic control of the blood pressure in the rat (60). The lesion, which partially destroys several areas to a varying extent, is produced by skewering, from the front, the anterior ventral corner of the third ventricle in the midline. The most ventral portion of the anterior end wall of the ventricle (the lamina terminalis) between the anterior commissure above and the optic chiasm below is destroyed with the loss of the OVLT. The damage extends posteriorly and horizontally, on either side and close to the midline of the slitlike ventricle, as a narrow tunnel of
destruction into the walls of the anterior and ventral parts of the ventricle. The periventricular hypothalamic nucleus and the median preoptic nucleus are particularly involved, and the lesion may then extend posteriorly into the anterior hypothalamus and paraventricular area. According to Brody and Johnson (60), “the medial preoptic nucleus, the anterior hypothalamic nucleus and the paraventricular nucleus were largely intact, in most cases, and usually sustained little or no apparent damage beyond their medial borders.”

III. FUNCTIONAL DISTURBANCES IN THE HYPOTHALAMUS OF HYPERTENSIVE ANIMALS

In 1969 Yamori and Okamoto (687) sectioned the brains of the spontaneously hypertensive rat (SHR) in vivo in various ways and came to the conclusion that the rise in arterial pressure is due to an increase in hypothalamic “tonic influence.” It has since been established that the rise in sympathetic nervous activity in hypertension is associated with multiple functional abnormalities in certain sites in the hypothalamus, brain stem, and medulla (87). The individual contribution and site of action within the hypothalamus of most of these abnormalities in the SHR and other forms of hypertension are described below.

IV. CATECHOLAMINES

In catecholaminergic neurons, L-tyrosine is converted to L-dopa (dihydroxy-phenylalanine) by tyrosine hydroxylase, a rate-limiting step. Tyrosine hydroxylase activation requires tetrahydropteridine cofactor, oxygen, and ferrous iron. Dopa decarboxylase (L-aromatic amino acid decarboxylase) converts L-dopa to dopamine (dihydroxyphenylacetic acid). In the vesicles dopamine is converted to norepinephrine by dopamine β-hydroxylase, the essential cofactors of which are ascorbic acid and cuprous ion (Cu^{2+}). Norepinephrine is methylated by phenylethanolamine-N-methyltransferase (PNMT) to form epinephrine.

Norepinephrine has a negative feedback action by inhibiting the conversion of L-tyrosine to L-dopa. Following release from storage vesicles into the synaptic cleft, most of the norepinephrine (~80%) reenters the membrane back into the cytoplasmic pool, and then by active transport against a high concentration gradient, it is returned into storage granules. The two main divisions of catecholamine receptors α and β have been further subdivided. Molecular cloning has characterized at least three postjunctional α_{1}-receptors and four prejunctional α_{2}-receptors. Three β-receptors have been identified (497).

A. Distribution of Catecholamines in the Hypothalamus and Effect of Norepinephrine on the Paraventricular Nucleus

With the use of a fluorescent histochemical technique (275) to localize the presence of catecholamines, which correlates quantitatively with their content (320) in neurons and fibers, the distribution of catecholamines is mainly centered on the paraventricular, periventricular, and dorsomedial nuclei. Most of the catecholaminergic fibers ascend from the medulla and travel to the paraventricular nucleus (444). The medial preoptic, anterior hypotalamic, and posterior hypothalamic nuclei demonstrate scattered fluorescence, while the ventromedial nucleus appears almost clear of activity. In neurons from the paravascular area of the paraventricular nucleus, which can be either neuroendocrine or preautonomic, norepinephrine regulation occurs for the most part either as a β-adrenoreceptor-mediated inhibition or via α_{1}-receptor-mediated activation of intrahypothalamic glutaminergic circuits (127). In the magnocellular portion of the paraventricular nucleus, norepinephrine has an inhibitory effect on vasopressin-secreting neurons and an excitatory effect on oxytocin-secreting neurons (280).

B. Hypothalamic Catecholamines and Normal Blood Pressure

The earliest observations were in vitro studies of catecholamine turnover, vesicular storage capacity, release, uptake, catabolism, density of innervation and of varicosities, enzyme function, and receptor binding capacity on tissue sections and synaptosomes. The results tended to be incongruous (256). The position was clarified by the use of in vivo injection and microperfusion techniques (460). Microperfusion of catecholamines or α_{2}-agonists into the anterior hypothalamic area lowers the blood pressure (47, 587) and when injected into the paraventricular nucleus raises the blood pressure (237). Conversely, injections of 6-hydroxydopamine (6-OHDA) into the anterior hypothalamic area lowers the blood pressure (28). 6-OHDA causes degeneration of catecholaminergic nerve endings, particularly noradrenergic and adrenergic (227); hypothalamic dopamine neural group however are resistant (700). The pressor effect of catecholamines injected into the paraventricular nucleus is due in part to the release of arginine vasopressin (AVP). The injection of a catecholamine, phenylephrine, into the posterior hypothalamus also raises the arterial pressure (420), and the injection of interleukin-1β into the lateral ventricles decreases norepinephrine secretion from the posterior hypothalamus and lowers the blood pressure (85).

The locus coeruleus caudal to the hypothalamus in the
floor of the fourth ventricle strongly influences the function of the posterior hypothalamus. The injection of the neuro-stimulating amino acid L-glutamate into the locus coeruleus increases the norepinephrine content of the posterior hypothalamus and raises the blood pressure. These effects are attenuated by a prior injection of the catecholaminergic neurotoxin 6-hydroxytryptamine (6-OHD) (305) into the posterior hypothalamus.

The extensive functional interconnection between catecholamines and most of the other neurotransmitters and neuromodulators that are involved in the control of the blood pressure are described below.

C. Hypothalamic Catecholamines and Hypertension

There are three in vivo microperfusion-dialysis studies in the whole rat which show that in the SHR there is an increased norepinephrine release from the paraventricular nucleus (488, 673) and from the posterior lateral hypothalamus (440). In one study, the increased release at 7–10 wk exceeded the raised levels at 12–14 wk (488). In another in vivo study at 9 wk, however, the release of norepinephrine (and epinephrine) from the posterior hypothalamus of the SHR was lower than from the Wistar-Kyoto rat (WKY), although the release of dopamine was raised (631). The precision of the in vivo microperfusion experiments and the focal nature of the changes in these experiments is evident by the finding that in the areas immediately surrounding the paraventricular nucleus and the dorsomedial area norepinephrine release is not raised (673).

Another microperfusion study has been carried out in which the perfusate levels of 4-hydroxy-3-methoxymandelic acid (MOPEG), the major metabolite of norepinephrine, has been used as an index of norepinephrine release. It was found that the release of MOPEG from the anterior hypothalamic nucleus of the 9- to 10-wk-old SHR is substantially greater than from the WKY (99). The release of MOPEG from the posterior hypothalamus, however, was no different from that of the WKY.

The first study that demonstrated the importance of cerebral catecholamines in hypertension was the effect of the central administration of 6-OHD (229). In SHR, in which the blood pressure had risen to 160–170 mmHg and would subsequently plateau at 190–210 mmHg at 12–14 wk, two intracerebroventricular injections of 6-OHD at 7 wk caused a sustained fall in arterial pressure to ~140 mmHg until the 12th week when the pressure then gradually rose to the same levels as in control SHR (162, 229). If, however, 6-OHD was administered to the SHR at 12 wk, it only produced a transient and moderate fall in pressure lasting <1 wk, a response similar to that of a normal animal (229).

An increase in norepinephrine release from the paraventricular and posterior hypothalamic nuclei has also been measured, with microperfusion, in the hypertension, which results from a constricting ligature around the aorta just above the kidneys (598) and from 5/6 removal of renal mass (86), but it does not occur in the DOCA plus salt (489). The injection, however, of 6-OHD intracerebroventricularly 7–10 days before the administration of DOCA and salt or the application of a clip on a renal artery prevents the blood pressure from rising (180, 208).

In vitro studies of hypothalamic slices from 8- to 10-wk-old SHR have also shown there is an increased secretion of norepinephrine from the paraventricular nucleus (408).

D. Summary

Catecholamine-containing neurons are mainly concentrated in the paraventricular, periventricular, and dorsomedial nuclei. Some of the neurons from the paraventricular and anterior nuclei have descending projections to spinal sympathetic preganglionic neurons. The blood pressure of a normal rat is raised by microperfusion of norepinephrine into the paraventricular and posterior hypothalamic nuclei, and it is lowered by microperfusion of the anterior hypothalamic nucleus. In the young SHR, there is a pressor increase from the release of norepinephrine from the paraventricular nucleus. There is a suggestion that this rise diminishes with age, which is in keeping with the finding that the hypotensive effect and reduction in sympathetic nervous activity of injecting 6-OHD intracerebroventricularly is greater in the young SHR. The direction of the change in catecholamine release that takes place in the anterior and posterior hypothalamic nuclei of the SHR is less clear cut; depressor increases in catecholamine activity in the anterior hypothalamus and depressor decreases in the posterior hypothalamic nuclei have been described. It is probable that the changes in the posterior hypothalamic nuclei are substantially influenced by changes in the activity of the locus coeruleus. In renal ischemic hypertension and the hypertension that follows 5/6 removal of the renal mass, there is an increased release of norepinephrine from the paraventricular and posterior nuclei. These changes do not occur in DOCA plus salt hypertension.

E. Effect on Hypothalamic Noradrenergic Activity of an Increase in Sodium Intake in the Young SHR

The usual rise in arterial pressure in the SHR occurs on a “normal” intake of sodium. A rise in sodium intake from 1 to 8% in the 9- to 10-wk-old SHR, however, causes a further rise in arterial pressure and a diminution of the preexisting compensatory depressor increase in MOPEG.
(and therefore, probably, norepinephrine) release from the anterior hypothalamus (99, 435, 677). It is probable that this reduction is due, in part, to the simultaneous increase in the local concentration of the neuroinhibitor atrial natriuretic peptide (691).

F. Evidence for an Increase in Norepinephrine Release From Subcortical Regions in Hypertensive Man

The venous drainage of the human brain is asymmetrical. Usually the right internal jugular vein drains the sagittal sinus as its main tributary, and the cerebral cortex is its predominant field of drainage. The left internal jugular is typically a continuation of the straight sinus and drains predominantly the subcortical area. Ferrier et al. (175) report that in patients with essential hypertension norepinephrine release from the subcortical region is significantly greater and that this increase correlates with the increase in total body spillover of norepinephrine (i.e., overall sympathetic nervous activity) and with renal norepinephrine spillover. The subcortical region includes the medulla,pons, and the hypothalamus, and most norepinephrine is released from the locus coeruleus in the floor of the fourth ventricle. These findings indicate that in essential hypertension, as in hypertension in animals, there is a central disturbance of catecholaminergic metabolism in the inferior part of the brain, which is related to the increase in sympathetic nervous activity, but the contribution of the hypothalamus is not distinguishable.

V. CHOLINERGIC MECHANISMS

Acetylcholine is synthesized from acetyl coenzyme A and choline by the action of choline acetyltransferase. It is packaged into synaptic vesicles at high concentrations by an active transport process and is released from the vesicles by exocytosis in response to calcium entry. Once released, acetylcholine is rapidly broken down by acetylcholinesterase that is present in high concentrations wherever acetylcholine acts as a neurotransmitter. Destruction is rapid; ~90% of the released acetylcholine may be hydrolyzed before it gets across the synaptic cleft onto its receptor on the postsynaptic membrane (497). The acetic acid produced by the hydrolysis is rapidly removed by various biochemical pathways while the choline is actively transported back into the nerve terminal where it can be recycled to synthesize acetylcholine (296).

All five subdivisions of muscarinic receptors (M1–5) and the nicotine receptor are found in the brain; they are diffusely present in the hypothalamus.

A. Distribution of Cholinergic Activity in the Hypothalamus

The intracerebroventricular injection of carbachol or neostigmine induce strong fos-like immunoreactivity in the paraventricular, posterior, ventral premamillary, and the supra, median, and medial preoptic area. Only moderate activity is present in the SFO and the OVLT (57, 353, 517). With the use of Koelle’s (314) thiocholine method for the localization of acetylcholinesterase, the highest concentrations are in the dorsomedial and paraventricular nuclei with high concentrations in the median eminence, premamillary, and posterior hypothalamic nuclei (275). A monoclonal antisera against choline-acetyltransferase, however, reveals a rather different pattern. In the preoptic area, immunostaining is found particularly in the medial and median preoptic areas, whereas in the hypothalamus there are numerous immunoreactive cells in the lateral hypothalamic area, the dorsal premamillary, ventromedial hypothalamic nuclei, and the area surrounding the fornix. Substantial numbers of immunoreactive cells are also seen in the dorsomedial, posterior, and ventral premamillary nuclei. There are also many such cells between the periventricular zone and the anterior hypothalamic nucleus and between the paraventricular zone and the anterior hypothalamus (498).

B. Cholinergic Interactions With Catecholamines, Nitric Oxide, and AVP

1. Catecholamines

The integrity of central catecholaminergic neurons appears to be critical for expression of the cholinergic response (56, 459). The rise in arterial pressure and increase in sympathetic nervous activity induced by an increase in central cholinergic activity is linked to activation of central catecholaminergic mechanisms. For instance, muscarinic receptor agonists and acetylcholinesterase inhibitors increase the activity of tyrosine hydroxylase (349) and the synthesis and release of brain catecholamines (306). Microperfusion and dialysis of the paraventricular nucleus of the normal rat with nicotine stimulates norepinephrine release (548), and superfusion of the posterior hypothalamus of the cat with nicotinic drugs or acetylcholine elicits a rise in arterial pressure that is abolished by hypothalamic superfusion with β-adrenoreceptor blocking agents (39). There is also some evidence that clonidine exerts an inhibitory effect on central cholinergic neurons involved in the regulation of the arterial pressure (68). Some neurons contain both norepinephrine and acetylcholinesterase, and the prolonged administration of muscarinic agonists oxotremorine and pilocarpine increase markedly the number of α2-adrenoreceptors throughout the rat brain (251). The in-
crease in blood pressure observed after the central administration of carbachol is inhibited by the prior injection into the lateral ventricle of 6-OHD (208) and by the administration of bethanidine and guanethidine. β-Adrenoceptors may also be involved in that the effects of the central administration of carbachol can be blocked by the central administration of propranolol. Finally, the inhibitory effect of catecholaminergic agonists on acetylcholine release from cholinergic terminals provides evidence of a feedback system between catecholaminergic and cholinergic systems (56, 151).

2. Nitric oxide

In rat neuronal cultures, carbachol causes a time- and concentration-dependent increase in cGMP levels, which is antagonized by atropine. This response is depressed by nitric oxide (NO) synthase inhibitors, which suggests that, as elsewhere, muscarinic receptor stimulation increases NO production in the cerebral cortex (91).

3. AVP

The injection of acetylcholine into the supraoptic nucleus of an anesthetized dog during a water diuresis has no effect on the blood pressure but inhibits the urine flow from denervated kidneys (471), which suggests there has been an increase in plasma AVP. Intracerebroventricular administration of carbachol into conscious rats causes an increase in AVP secretion and blood pressure that is blocked by the muscarinic blocker atropine but not by a nicotinic blocker (271).

C. Hypothalamic Cholinergic Activity and Normal Blood Pressure

In the normal rat, injection of choline intracerebroventricularly or of carbachol or neostigmine into either the posterior area of the paraventricular nucleus, the dorsal portion of the anterior hypothalamic nucleus, the posterior hypothalamic nucleus and the ventromedial nuclei increases the arterial pressure and renal sympathetic nerve activity (12, 56, 59, 67, 385, 475). Injections of carbachol into the dorsomedial nuclei, however, evoke a fall in blood pressure (53, 242) while neostigmine either causes an increase in pressure or has no effect (53). Injections into the lateral hypothalamic nucleus only evoke insignificant responses (59). Because of the relative absence of M1 muscarinic receptors in the posterior hypothalamic nuclei (200) and the observation that the hypertensive effect of carbachol can be partially blocked by the injection into the nucleus of the M1+M2+M3 receptor antagonist 4-diphenylacetoxy-N-methyl-piperidine methiodide (4-DAMP), it has been considered that the hypertensive effect of carbachol on the posterior hypothalamus is due principally to stimulation of M2-M3 receptors (52, 678). The injection of carbachol into the third or lateral ventricle of unanesthetized rats also releases AVP, which contributes to the early rise in arterial pressure (250, 331, 676). Intravenously administered physostigmine raises the blood pressure by its effect on the rostral ventrolateral medulla (483).

D. Hypothalamic Cholinergic Activity and Hypertension

The intravenous injection of atropine in the conscious SHR evokes an age-dependent fall in blood pressure, the younger rats (11 wk) being significantly less sensitive than the older rats (88) (15–20 wk). It has no significant effect in the WKY. The intravenous injection of methyl atropine, however, which does not readily enter the brain, induces a transient rise in blood pressure. This suggests that the depressor effect of intravenous atropine in the SHR is due to a central action that suppresses increased cholinergic activity.

High-affinity choline uptake (330, 684) in freshly prepared crude synaptosomes from the hypothalamus of the SHR, although greater than that of the Wistar-Lewis rat, is not different from the WKY (625). And choline acetyltransferase (ChAT) activity as measured in sections obtained by micropuncture is reduced in the paraventricular, dorsomedial, and posterior hypothalamic nuclei, a change which is more pronounced with age (242). In contrast, the hypotensive effect of hemicholinium (HC-3), ethyl-choline aziridium, and 4-DAMP intracerebroventricularly and into the posterior hypothalamus and paraventricular nucleus indicate that there is an increase in hypothalamic cholinergic activity particularly in the posterior hypothalamus.

HC-3 is a synthetic substance which, at low concentrations, selectively blocks sodium high-affinity neuronal uptake of choline and reduces acetylcholine synthesis (330, 684). At high concentrations it can block first nicotinic and then muscarinic receptors. Single acute injections of HC-3 into the lateral ventricle of young and adult WKY have no effect on the blood pressure (54). In the 12-wk-old SHR, such injections reduce the blood pressure, an effect which becomes more pronounced with age until 18–20 wk (201). Five- to eight-week lateral ventricle injections of HC-3 cause no significant reduction in blood pressure. Single central injections of HC-3 also reduce the blood pressure in the DOCA salt, aortic constriction, and Grollman, renal hypertensive rats. The magnitude of the depressor response in the rat with aortic constriction increases with age (201).

Chronic lateral ventricle infusions of HC-3 for 21 days in the 5-wk-old SHR decreases hypothalamic acetylcholine levels and suppresses the development of hyperten-
tion during the 21 days (647). In contrast, a similar infusion at 18 wk only reduces the blood pressure for the first 8 days, after which the blood pressure returns toward control values during which time hypothalamic acetylcholine levels also return to normal despite the continuing HC-3 injection.

Microinjection of HC-3 into the lateral septal area, the paraventricular, and the posterior hypothalamic nuclei of the adult SHR reduce the blood pressure (58, 537). About 60% of the acute depressor effect of HC-3 injected into the posterior hypothalamic nucleus can be abolished by an injection of choline into the nucleus (58). When ethylcholine aziridium, a cholinergic neurotoxin, is injected into the posterior hypothalamus of 1- and 3-mo-old SHR and WKY, there is a substantial fall in arterial pressure, although not to normal in the SHR (158). When injected into the anterior hypothalamic nucleus of the 4-wk-old SHR, it does not prevent the onset or severity of the hypertension (158).

4-DAMP mustard, an M₁, M₂, and M₃ receptor antagonist which decreases the density of muscarinic receptors in the hypothalamus by ~60%, has no significant effect on receptors in the brain stem (55). Following a bilateral injection of 4-DAMP mustard bilaterally into the posterior hypothalamic nuclei of the SHR, there is a fall in blood pressure (although not to normal) that takes several days to return to the original level (55). These findings suggest that the hypertensive effect of the increase in cholinergic activity in the hypothalamus including the posterior hypothalamus is independent of changes in cholinergic activity in the brain stem and medulla.

The increase in cholinergic activity in the SHR which most of these observations suggest is mediated at least in part through enhanced density of muscarinic receptors. Muscarinic receptor sites in the SHR are increased as early as 1 wk of age and continue to increase until 11 wk (244). mRNA encoding of five muscarinic receptors in the whole hypothalamus has revealed that there is 40–50% increase in excitatory M₁ subtype and a decrease in the inhibitory M₄ subtype during the establishment of the hypertension (662). The changes are more pronounced at 12 wk of age. The levels of the other subtypes M₂, M₃, and M₅ in the SHR were not significantly different from the WKY (662). A change in muscarinic receptor density does not occur in renal hypertension (681), but the density is increased in the Dahl salt-sensitive rat (154).

E. Summary

Acetylcholinesterase activity is widely distributed throughout the normal rat hypothalamus. The principal areas of localization vary according to the localizing technique used. The rise in arterial pressure and increase in sympathetic nervous activity that accompanies an increased increase in cholinergic activity in the paraventricular, posterior, and ventromedial nuclei is closely linked to central catecholaminergic mechanisms. An induced increase in cholinergic activity in the dorsomedial nucleus lowers the blood pressure. In vitro measurements of high-affinity choline uptake and of choline acetyltransferase activity in the SHR hypothalamus either show no rise or a fall in cholinergic activity. In vivo, however, the hypertension in the SHR, the DOCA plus salt and the renal ischemic hypertensive rat is reduced by agents, introduced either into the cerebral ventricles or directly into certain hypothalamic nuclei, which either block neuronal uptake of choline or hypothalamic muscarinic receptors. In hypothalamic preparations of SHR and the Dahl salt-sensitive rat, but not in the renal ischemic hypertensive rat, there is an overall increased density of the excitatory M₁ muscarinic receptor and a decreased density of the inhibitory M₄ receptor. The evidence suggests that in the SHR the rise in arterial pressure is due in part to an increase in cholinergic activity that becomes more prominent with age and that may be due solely to an increase in hypothalamic M₁ receptors and a decrease in M₄ receptors.

VI. ANGIOTENSIN

All the components of the renin-angiotensin system, renin, angiotensinogen, angiotensin-(1–7), angiotensin converting enzyme, angiotensin II, and angiotensin II receptor subtypes have been identified in brain neurons and glial cells (182, 189, 363). The angiotensinogen gene is exclusively expressed in astrocytes (363). Angiotensin II modulates voltage-activated ion channels of neurons which leads to depolarization and an increase in intraneuronal calcium (170, 689).

There are two distinct subtypes of angiotensin II receptors, AT₁ and AT₂. Most of the known actions of angiotensin II are mediated by AT₁ receptors, which belong to the G protein-coupled receptor superfamily.

A. Distribution of Angiotensinergic Activity in the Hypothalamus

Ligand binding, immunochemistry, and electrophysiological techniques agree that in the hypothalamus angiotensin has its major impact on the blood pressure by its effect on the structures at the anterior part of the third ventricle, particularly those that form part of the lamina terminalis, i.e., the SFO, the median optic nucleus, and the OVLT (360, 430, 517). “The OVLT ‘sees’ both CSF and blood angiotensin, whereas the SFO only detects bloodborne angiotensin” (465). The angiotensin pressor area appears to be mainly below the level of the anterior commissure where the OVLT is the most prominent structure, and it includes the margins of the preoptic and
of AT1 receptors. There is some evidence that the pressor administration of angiotensin II is mediated by activation (167, 539, 619, 661). The pressor effect of the central associated with an increase in sympathetic nervous activ-

ity (167, 539, 619, 661). The pressor effect of intracerebroventricular administered angiotensin II (279, 283).

B. Angiotensin Interactions with Catecholamines and AVP

Intracerebroventricular angiotensin II increases turnover of norepinephrine in certain hypothalamic nuclei, particularly the most rostrally placed (589) and the paraventricular nucleus (361, 465, 579). The turnover of dopamine is not affected.

In cultured neurons from the rat brain, angiotensin II not only causes a significant increase of angiotensin II receptors and in the concentration of neuronal and media norepinephrine, it also stimulates norepinephrine re-
take, thus mitigating the rise of norepinephrine in the extraneuronal environment (494). There is also evidence of a negative feedback loop in that catecholamines inhibit angiotensin II release and reduce the number of angiotensin II receptors (494).

An intracerebroventricular injection of angiotensin II (0.7–5.7 fmol) causes an increased release of AVP (465), an effect which is diminished by the injection of a GABA agonist (51).

C. Hypothalamic Angiotensin and Normal Blood Pressure

The intracerebroventricular administration of angiotensin II (539, 589) and the microinjection of doses of angiotensin II as low as 50 fmol (464) into the OVLT/median optic nucleus area cause a rise in blood pressure associated with an increase in sympathetic nervous activ-

ity (167, 539, 619, 661). The pressor effect of the central administration of angiotensin II is mediated by activation of AT1 receptors. There is some evidence that the pressor effect of an acute intracerebroventricular injection of angiotensin II is due in part to the release of AVP (249, 593). In the rat, intracerebroventricular transection of the human angiotensin-converting enzyme gene increases blood pressure and heart rate and is associated with increased hypothalamic production of AVP, the transgene is widely expressed in periventricular cells, the cortex, hypothalamic nuclei, and the brain stem (417).

The pressor effect of intracerebroventricular angiotensin II is exaggerated in 12-wk-old normal rats fed a high-sodium diet from the age of 2–3 wk. The exaggera-
tion of the pressor effect is associated with acute retention of sodium, is abolished by renal denervation, and does not occur in a 10-wk-old normal rat given a high-
sodium diet for 2 wk (437).

D. Hypothalamic Angiotensin and Hypertension

In the SHR, there are more than twice as many cells and fibers in the supraoptic and paraventricular nuclei which stain for angiotensin II-like immunoreactivity (665), and the turnover (188) of angiotensin II and its concentration (468) are greater in the hypothalamus of the adult SHR than in the WKY. Angiotensinogen mRNA in the preoptic area of the SHR is increased, a rise which is apparent at 4 wk of age and becomes more pronounced with age (555). In the two-kidney, two-clip (2K2C) renal hypertensive rat, the angiotensinogen concentration in the hypothalamus is increased and the rise in pressure occurs despite central deple
tion of catecholamines with 6-OHD (23). In the 2K2C renal hypertensive Wistar rat, Basso et al. (23) found that the angiotensinogen concentration in the hypothalamus, 6 wk after placing clips, was also increased (and that the rise in arterial pressure occurred despite central depletion of catecholamines with 6-OHD), whereas Lou et al. (367) in the two-kidney, one-clip (2K1C) hypertensive Sprague-Dawley rat reported a decrease in hypothalamic mRNA at 19 days, but not at 40 days.

The pressor response to the injection of angiotensin II into the preoptic area in the SHR is two to three times greater than in the WKY (388). Both the 4- and 14-wk-old SHR have significantly greater concentrations of angio-
tensin II binding sites in the median preoptic nucleus, the SFO and the paraventricular nucleus than the WKY (224). In the DOCA plus salt hypertensive rat angiotensin II binding in the median preoptic nucleus, the SFO, and the paraventricular nucleus is increased (502).

Hypothalamic neuronal cultures have been established from 1-day-old WKY and SHR strain rats (370, 493, 702). They contain paraventricular and supraoptic nuclei, anter-
ior, lateral and posterior, and dorsal and ventromedial nu-

clei, and the cultures consist of 85–90% neuronal cells with 10–15% astroglial cells. Neuronal cultures from the SHR hypothalamus express two to four times higher levels of functional AT1 receptors with parallel increases in mRNAs for both AT1A and AT1B receptor subtypes (233, 493, 495, 582). And in such cultures angiotensin II stimulates tyrosine hydroxylase (TH) activity, TH mRNA, norepinephrine up-
take, c-fos mRNA, and norepinephrine uptake significantly more when the neurons originate from SHR rather than WKY (370, 702). Stimulation of TH mRNA is mediated by AT1 receptor subtypes, which results in an increase in its transcrip
tion and involves activation of phospholipase C and protein kinase C (702). In vivo confirmation of the stimulat-
ing effect of angiotensin II on TH activity and TH mRNA has been obtained in adult WKY and SHR when the level of stimulation in the SHR has been found to be significantly greater than in the WKY (702). Overall, these observations are consistent with the increase in AT₁ receptor gene expression in the SHR (493). The greater level of angiotensin II receptors found in the SHR hypothalamus does not include AT₂ receptor subtype (590). The level of angiotensinogen mRNA in the preoptic area of the SHR is higher than in the WKY at 7 and 16 wk, although at 4 wk the difference is insignificant (555).

Intervention in the hyperactivity of the hypothalamic angiotensin system lowers the blood pressure in the SHR (30). The intracerebroventricular or anterior hypothalamic injection of an angiotensin-converting enzyme inhibitor or angiotensin receptor antagonist in the SHR (31, 269, 469, 629), Dahl salt-sensitive rat (614), DOCA plus salt (273) and 2K1C renal hypertensive (165) rat lowers the blood pressure but is without effect on the blood pressure of normal rats. In the SHR, the depressor effect is greater in an animal on a high salt intake (686). In the Dahl salt-sensitive rat, an intracerebroventricular infusion of an angiotensin receptor blocker also prevents the usual increase in sympathetic nervous activity (265). Injection of an angiotensin receptor antagonist into the posterior hypothalamic nucleus of the SHR produces no significant effect on the blood pressure (692), which supports the conclusion that the hypothalamic localization of the increase in angiotensin hyperactivity is in the anterior hypothalamus.

More recently, antisense oligodeoxynucleotides, targeted to angiotensinogen mRNA and AT₁ receptor mRNA, injected into the lateral ventricle have significantly reduced the blood pressure of SHR (294, 467, 470, 667). Antisense oligodeoxynucleotides targeted to angiotensinogen do not lower the blood pressure of normotensive rats (466).

The bilateral injection of antisense oligonucleotide targeted solely to angiotensinogen into the paraventricular nuclei of the SHR does not reduce the blood pressure (294), suggesting that the increase in angiotensin in the paraventricular nucleus does not contribute to the sustained rise in blood pressure. There is some fragmentary evidence, however, that the effect of the excess angiotensin in the paraventricular nucleus on the blood pressure is to exaggerate baroreflex responses. In normal dogs in which the sinoaortic nerves and the vagi have been sectioned, the introduction of angiotensin into the paraventricular nucleus increases the renal sympathetic nerve activity response to electrical stimulation of the left cardiac sympathetic nerve (660).

E. Summary

Angiotensin’s principal site of action in the hypothalamus of the rat, as regards the control of the blood pressure, is the anterior part of the third ventricle including particularly the organum vasculosum, the lamina terminalis, as well as the preoptic and the anterior hypothalamic nuclei. Angiotensin appears to increase the number of angiotensin receptors and the neuronal secretion and reuptake of norepinephrine, whereas catecholamines inhibit angiotensin release and the mRNAs of angiotensin receptors and tyrosine hydroxylase. A high-salt diet for 10 wk exaggerates these effects.

A pressor increase in hypothalamic angiotensin activity occurs in the SHR, the Dahl S rat, renal hypertension, and DOCA plus salt hypertension. It takes place mainly in the anterior hypothalamic area and includes an increase in angiotensinogen, AT₁A and AT₁B receptor mRNAs. These changes can be detected in the first week and do not diminish with age. The introduction of antisense oligonucleotides targeted to angiotensinogen or AT₁ receptor mRNA into the lateral ventricle reduces the blood pressure of the SHR and of some other forms of hypertension but not of the normal rat. The evidence suggests that the effect of the antisense oligonucleotide is due to a direct effect on angiotensinogen production in the hypothalami.

VII. NATRIURETIC PEPTIDES

Four so-called natriuretic peptides have been identified, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP), and urodilatin. ANP is natriuretic, is present in plasma and in the hypothalamus, and is most abundant in the auricle. BNP is natriuretic, is present in plasma, and is most abundant in the ventricle of the heart (427). There is no BNP mRNA in the hypothalamus. CNP, which is neither natriuretic nor present in plasma, is found predominantly in the brain, particularly in the hypothalamus (243) and vascular endothelial cells (586). Urodilatin, which is natriuretic, is only present in the urine. It is produced in the kidney (540).

There are three main natriuretic peptide receptors (NPRs). NPR-A and NPR-B are particulate guanyl cyclases with an extracellular binding site, a region which spans the membrane and an intracellular tail that catalyzes the conversion of GTP to cGMP. NPR-C, which lacks an intracellular catalytic tail, does not appear to be involved in the generation of cGMP. It is thought to be a clearance receptor for all forms of natriuretic peptides, which controls plasma natriuretic peptide concentration. If the effect of natriuretic peptides on the brain is related to the production of cGMP, it will depend on astrocytes, since these are the only brain cells that generate cGMP upon exposure to natriuretic peptides. CNP also reduces astrocyte glutamate reabsorption (138), which should stimulate neuronal activity. ANP and CNP are equally potent in...
their ability to generate cGMP in rat brain (206). In cell cultures, cGMP is rapidly exported out of astrocytes and acts extracellularly to inhibit sodium/hydrogen exchange and reduce intracellular pH (623). Hypothalamic ANP, like other hypothalamic neurotransmitter and neuromodulator peptides, is released in a Ca$^{2+}$-dependent manner (554).

Stimulation of the AV$_3$V region induces a rapid rise in plasma ANP (20), whereas lesions of the AV$_3$V region are followed by a marked fall in plasma ANP and a significant suppression of the normal rise that occurs with volume expansion (10). Because these changes are accompanied by little or no change in atrial ANP, it suggests that brain ANP can alter plasma ANP (20).

A. Distribution of Natriuretic Peptides in the Hypothalamus

The distribution of ANP immunoreactive cells is particularly dense in the anterior part of the hypothalamus (399, 530, 581), including much of the area included in the AV$_3$V area, the OVLT, the SFO, the periventricular nucleus, the medial preoptic nucleus, the preoptic suprachiasmatic nucleus, and the anterior hypothalamic nucleus. The density of ANP-containing cells is less in the paraventricular nucleus, the dorso- and ventromedial nucleus extending to the arcuate nucleus and the medial mammillary nucleus. The axons of ANP-containing cells project to the median eminence and neural lobe (10).

ANP mRNA is widely distributed in the preoptic area, the paraventricular and arcuate nucleus, and throughout the lateral inferior and posterior areas of the hypothalamus, including the mamillary nucleus. The distribution of CNP mRNA is less widely distributed, overlapping that of ANP mRNA in the preoptic and arcuate nuclei (243). In contrast to the distribution of ANP immunoreactive cells, there is a surprising paucity of cells containing ANP or CNP mRNA in the anterior hypothalamic area (see below). The paraventricular nucleus, which contains a relatively low density of ANP mRNA cells, contains, however, much ANP immunoreactivity (243). The significance of this discrepancy between the distribution of ANP mRNA and immunoreactivity, particularly in the paraventricular nucleus, is not understood. Herman et al. (243) have suggested that, as ANP antibodies cross react with other species possessing cysteine rings near the carboxy terminus, it is possible that the protein product detected by immunocytochemistry is derived from some other molecule than ANP. In addition to the natriuretic peptides formed in the brain, those present in the blood and cerebrospinal fluid (CSF) can affect neurons in organs that lack a blood-brain barrier, such as the SFO and OVLT.

There is a certain overlap, but the principle receptor for ANP is NPR-A and for CNP is NPR-B (96, 668). The clearance receptor NPR-C has much less stringent structural requirements for ligand binding than either NPR-A or -B. It is present in such rostral areas as the olfactory bulb, the medial frontal cortex, the cingulate gyrus, and the lateral septal nucleus; there are no NPR-C receptors in AV$_3$V structures such as the median, preoptic, supraoptic and paraventricular nuclei and SFO or in the rest of the hypothalamus. NPR-A is distributed in the same area as the NPR-C receptors and in the SFO, the median preoptic, supraoptic, the paraventricular nucleus and certain areas caudal to the hypothalamus (63). No NPR-B sites have been detected in the rat brain (63), nor has any high-affinity guanylyl cyclase-linked receptor that is specific for BNP (668).

B. Effect of ANP on Neuronal Hypothalamic Activity

In the anesthetized rat, microinjections into the paraventricular nucleus of ANP in doses as low as 1.3 fmol have an inhibitory effect on the electrical activity of single neurons within the nucleus (580). In hypothalamic slice preparations in vitro, ANP has a direct inhibitory effect on neurons in the AV$_3$V area and the paravascular part of the paraventricular nucleus but no effect on the electrical activity of magnocellular neurons in the paraventricular and supraoptic nucleus (431, 672). However, ANP applied to the rat SFO in vitro, in concentrations as low as $10^{-10}$ M, induces a brief period of excitation (76). In vivo the injection of 100 ng ANP into the cerebral ventricles increases afferent renal sympathetic nerve activity (512).

C. Interactions Between Hypothalamic ANP and Catecholaminergic and Cholinergic Mechanisms, AVP, Angiotensin, Aldosterone, Salt Intake, and Volume Changes

In vitro, ANPs inhibit the release of norepinephrine from hypothalamic neurons (183), and injections of ANP (2–5 μg) into the lateral ventricles of the normal rat decrease the amount of norepinephrine, dopamine, and their metabolites in the hypothalamus (419). Microperfusion of ANP into the anterior hypothalamus of conscious WKY, however, elicits an unexplained small rise in the concentration of norepinephrine metabolite MOPEG, whereas microperfusion of ANP into the anterior hypothalamus of the SHR induces a substantial fall in the concentration of MOPEG (451). Conversely, microinjections of norepinephrine into AV$_3$V region stimulates the release of ANP by stimulating cholinergic neurons to stimulate ANP neurons (8). Microinjection of carbachol into the AV$_3$V region increases the content of ANP in the hypothalamus and raises plasma ANP (20).

An infusion of either ANP or BNP into the third...
ventricle inhibits vaspressin secretion, and the effect is greater with BNP (685). Conversely, AVP stimulates ANP gene expression and secretion from hypothalamic neurons (348). There is also evidence that ANP inhibits the release of ACTH, prolactin, and growth hormone (397).

The central administration of ANP blocks some of the actions of centrally administered angiotensin II such as its effect on water intake, salt intake, and AVP release, although the rise in blood pressure is not always affected (9, 89, 414, 418, 601). The topical application of ANP on the SFO selectively depresses the excitatory action of angiotensin II but has no effect on the excitatory action of angiotensin II on the supraoptic nucleus (685).

In normal rats aldosterone, but not dexamethasone, increases ANP in the supraoptic, paraventricular, perifornical, and lateral hypothalamic nuclei. A high salt intake has a variable effect on hypothalamic content of ANP, decreasing it in one study (611) and increasing it in two others (282, 601). Volume loading raises and depletion lowers the concentration of ANP in the OVLT, paraventricular and medial preoptic nucleus, and the SFO (443).

D. Hypothalamic Natriuretic Peptides and Normal Blood Pressure

The effect on the blood pressure of natriuretic peptide injected into the lateral ventricles of normal animals is not consistent. One group found that rat ANP [atriopeptin III and ANP-(5—28)] raised the blood pressure of conscious rats, whereas human ANP-(1—25) did not (691). This same group, however, found that microperfusion of the anterior hypothalamus of the conscious rat did not raise the blood pressure (451). Others (340, 512) using similar amounts of rat ANP injected into the lateral ventricles of the anesthetized and the conscious rat and the conscious sheep also obtained no significant change in blood pressure, but microinjection of 2–4 pmol ANF into the suprachiasmatic nucleus raised the blood pressure of the anesthetized rat (560).

E. Hypothalamic Natriuretic Peptides and Hypertension

The evidence in the SHR is also inconsistent. In contrast to the normal rat, microperfusion of ANP into the hypothalamus of the conscious SHR raises the blood pressure (451). Most investigators have found the content of immunoreactive ANP in the hypothalamus of the SHR to be raised at 4 wk, before the onset of hypertension, and at 8, 12, and 18 wk, when it is established (131, 272, 282, 317, 520). And microinjection of 0.055–0.55 μg of a blocking antibody to ANF into the anterior hypothalamus of the SHR lowers the blood pressure and decreases the local concentration of the major norepinephrine metabolite 3-methoxy-4-hydroxyphenylglycol (691). The ANP blocking antibody does not lower the blood pressure when injected into the posterior hypothalamus of the SHR or into the anterior hypothalamus of the WKY. Similarly, some investigators have found that the content of immunoreactive ANP is raised in the anterior part of the hypothalamus in the Dahl salt-sensitive rat (197, 585), the salt-loaded reduced renal mass rat (195), and the DOCA salt rat (196). The distribution of the increased content of ANP in the DOCA salt rat, however, was found to be different in that it is entirely focused on the OVLT and the SFO (196). In the reduced renal mass rat, the rise in ANP concentration in the hypothalamus was demonstrated to be independent of the blood pressure in that it occurred in rats in which the pressure was controlled with the angiotensin-converting enzyme inhibitor quinapril, which does not cross the blood-brain barrier in SHR (195, 520). However, it was reversed by lisinopril, which does cross the barrier, suggesting that the hypothalamic rise in ANP is in part due to local angiotensin II-related mechanisms.

In contrast, however, Bahner et al. (17) found a decrease in ANP in the SFO, the perifornical and periventricular hypothalamic nuclei and the medial preoptic nuclei, and the paraventricular and striae terminalis of the 4- and 12-wk-old SHR. In the median eminence, an increase in ANF occurred at 4 wk. The same authors reported that in both the Dahl salt-sensitive and salt-resistant rats a high salt intake induced a decrease in the content of ANP in the OVLT, the SFO, and the supraoptic nucleus and that in the salt-sensitive rat there was, in addition, a significant increase in ANP in the paraventricular nucleus (197).

There is also some disagreement on the content of ANP mRNA in the SHR. Komatsu et al. (317) found an increased content in the 17-wk-old SHR mainly localized to the anterior hypothalamic area, and the AV3V area, including segments of the periventricular nucleus, the paraventricular nucleus, the suprachiasmatic nucleus, and the rostro-dorsal part of the ventromedial nucleus (317). But Chen et al. (103) found no difference in atrial peptide mRNA in anterior, posterior, or ventral hypothalamus between 10-wk-old SHR and WKY fed 1 or 8% salt diet for the preceding 3 wk (103).

Hypothalamic ANP receptors in the SHR are quantitatively and qualitatively different from ANP receptors in the WKY. There is a reduced number of receptors, particularly in the SFO, the paraventricular nucleus, and the choroid plexus (62, 221, 521, 522). In addition, the affinity of the ANP receptors in SHR is reduced, but they have an increased sensitivity to ANP (62) in that they generate larger quantities of cGMP (221). In contrast, in DOCA plus salt and Dahl salt-sensitive forms of hypertension there is an increase in ANP binding sites in the SFO (585, 669).
F. Summary

ANP immunoreactive cells are particularly prominent in the anterior hypothalamus. In vitro ANP suppresses the release of norepinephrine and vasopressin from hypothalamic neurons. Induced changes in the concentration of ANP in the anterior hypothalamus do not appear to affect the blood pressure of the normal rat, but microinjections of ANP into the suprachiasmatic nucleus raise the blood pressure. Reports on the hypothalamic changes that occur in hypertension are not unanimous. The increase in the concentration of ANP which all but one group has reported to be present in the anterior hypothalamus in some forms of hypertension, including the SHR, probably contributes to the rise in pressure by decreasing the local release of norepinephrine which, at this site, has a depressor role. But this conclusion awaits further work on the content of ANP mRNA in the SHR for the only two reports available are contradictory.

Nevertheless, the proposal that in hypertension there is a pressor increase in ANP in the anterior hypothalamus is supported by the depressor effect of a microinjection of a blocking antibody to ANP into the anterior hypothalamus of the SHR. A procedure that has no effect on the blood pressure of a normal animal. It is probable that, in the SHR, when the norepinephrine neurons in the anterior hypothalamus are freed of the restraining effect of the raised levels of ANP by the blocking antibody the associated fall in blood pressure is initially due to the prevailing hypertension causing a compensatory supranormal release of norepinephrine within the anterior hypothalamus.

VIII. VASOPRESSIN

A. Distribution of AVP in the Hypothalamus

Circulating AVP is produced in the supraoptic nucleus and magnocellular neurons in the paraventricular nucleus. It is transported on its carrier neurophysin II to the posterior pituitary gland from where it is released into the circulation. Release is dependent on both osmotic and nonosmotic pathways (547, 650). Osmolality activates neurons throughout the anterior hypothalamus including the magnocellular neurons, the SFO, the OVLT and the median preoptic nucleus. Projections from the SFO, the OVLT, and the median preoptic nucleus activate both parvocellular area of the paraventricular nucleus and from the suprachiasmatic nucleus to the brain stem and to the spinal cord (114, 115, 399, 595) where the fibers make direct synaptic contact with sympathetic preganglionic neurons.

AVP receptors V1a, V1b, and V2 are G protein coupled. V1a and V1b receptors act through phosphatidylinositol hydrolysis, whereas V2 receptors are coupled to adenylate cyclase (402). There are V1 receptors in the suprachiasmatic (150) nucleus, the SFO, the OVLT, the arcuate nucleus, and astrocytes (291). Oxytocin receptors are found predominantly in the ventromedial nucleus (150), the SFO, and the OVLT (291). There is no evidence to suggest that there are any V2 receptors in the brain.

B. Interactions Between AVP-Containing Neurons, Catecholaminergic and Cholinergic Mechanisms, ANP, Angiotensin, and DOCA

The intracerebroventricular administration of AVP to a normal rat reduces the content of norepinephrine in the anterior hypothalamus, the median eminence, and the arcuate nucleus (612). Conversely, the pressor effect of the central administration of an α1-adrenergic agonist to a normal rat does not occur in a rat with congenital diabetes insipidus, suggesting that the pressor effect of central catecholamines in a normal rat is contingent on the intraneuronal presence of AVP (247).

In vitro, acetylcholine is a potent stimulus for AVP release from hypothalamo-neurohypophysial explants in organ culture from normal rats (574). In vivo, the central administration of acetylcholine or carbachol in the intact rat stimulates muscarinic receptors, possibly in the paraventricular nucleus, to increase AVP secretion (271). These findings support the evidence that AVP neurons are cholinergically innervated (561).

Intracerebroventricular injection of ANP reduces the concentration of plasma AVP (591) and prevents the hypertensive effect of an intracerebroventricular injection of AVP (584) (see below) in both the WKY and SHR.

The pressor effect of centrally administered angiotensin II may be in part due to the pressor effect of AVP (51).

C. Hypothalamic AVP and Normal Blood Pressure

Microinjection of V1 agonists into the lateral ventricle or third ventricle of conscious or anesthetized rats causes a brisk rise in blood pressure with marked increases in efferent splanchnic and renal nerve activity, whereas V2 agonists and oxytocin have no effect on the blood pressure (179, 510, 576, 584, 640, 705, 706). The hypertensive effect of the central administration of a V1 agonist also occurs in the anesthetized Brattleboro con-
genital diabetes insipidus rat, indicating that the rise in arterial pressure is a central effect and not due to the release of endogenous AVP into the circulation (473).

In contrast, microinjection of 5 pmol AVP into the SFO of the anesthetized rat lowers the blood pressure (576). This central depressor effect of AVP in the anesthetized rat appears to act by enhancing baroreflex-induced bradycardia and reducing cardiac output (401). A central depressor effect of AVP was first suspected when it was found that, although the rise in plasma AVP is the same whether AVP is administered via the inferior vena cava or the vertebral arteries, the rise in arterial pressure is less with the arterial infusion (356).

D. Hypothalamic AVP and Hypertension

Overall the evidence suggests that in hypertension there is an increase in AVP release from the hypothalamus. Plasma AVP is raised in the SHR (123, 400), the Dahl salt-sensitive rat (389), essential hypertension (436), and in the DOCA plus salt rat (409). Urinary excretion of AVP is also raised in the SHR (123) and essential hypertension (311). In the SHR, the rise in plasma AVP is accompanied by a fall in the content of AVP (and oxytocin) in the hypothalamus (140, 186, 239, 337, 406, 407, 409, 573). In the 4-wk-old SHR AVP mRNA levels in the paraventricular and supraoptic nuclei are two- to threefold higher than in the WKY, and at 10 wk, the level is still 30–40% higher. At 24 wk the content of radioimmunoassayable AVP (and oxytocin) in the paraventricular nucleus is reduced (406). Oxytocin mRNA levels, however, in the SHR paraventricular and supraoptic nuclei at 4 wk are not different from those in the WKY, and at 10 wk they are ~10% lower than in WKY (646).

AVP release has also been measured in vitro from either a triangular slice of tissue from the basal hypothalamus which includes the supraoptic nucleus with its AVP axons and their extension to the median eminence and their termination in the attached neural lobe, or a punched out region (441) from the paraventricular and supraoptic nuclei (29, 408). At 4 wk, basal release of AVP and oxytocin is less in the SHR than in the WKY (29), but from 5 to 8 wk, although there is now no difference in basal release, which remains low, stimulated release of AVP with either acetylcholine (569) or potassium (140) is significantly greater in the SHR than in the WKY explants. At 18 wk this difference is no longer detectable. Hypersensitivity does not occur with an osmotic stimulus (572). There is one study (570) that has provided in vivo evidence that in young SHR the AVP system is hypersensitive to at least one physiological stimulus. It was found that the rise in plasma AVP in response to a decrease in plasma volume is greater in the SHR (570). There is a similar increase in hypothalamic release of AVP in vivo in the DOCA plus salt rat which appears independent of the blood pressure and the salt intake (104).

It is not clear whether these changes in central AVP contribute to the rise in pressure. The development of stroke-prone SHR (SHRSP) homozygous for hypothalamic diabetes insipidus has yielded an animal in which AVP is not detectable in the plasma or the hypothalamus but which nevertheless has severe hypertension (190). Although the experiments were performed in SHRSP rats, it was concluded that AVP is not essential for the development or maintenance of the hypertension in the SHR. The extension of this conclusion to the SHR, however, has been contested because AVP levels in the SHRSP differ from those in the SHR. In contrast to the SHR, plasma AVP at 6, 9, and 12 wk in the SHRSP is lower than in the WKY, only rising above that of the WKY at 24 wk, and there is the remarkable finding that the introduction of AVP into the lateral ventricle of the SHRSP lowers the blood pressure (396). Furthermore, the intravenous administration of an AVP pressor antagonist, which blocks the action of both V1 and V2 receptors and lowers the blood pressure of the SHR, does not lower the blood pressure of the SHRSP rat (366, 499, 500, 571). In attempting to assess the importance of the increase in AVP release on the hypertension of the SHR, it was unfortunate to use the stroke SHRSP, which does not appear to have an increase in AVP release.

The central administration of 6-OHDA prevents the DOCA salt rat from developing hypertension but has no effect on plasma AVP, which is raised (429). The presence of AVP appears to be essential for the rise in arterial pressure in the DOCA salt rat in that the DOCA plus salt Brattleboro diabetes insipidus rats do not develop hypertension unless they are given an antidiuretic form of AVP (524). Furthermore, the blood pressure of the DOCA plus salt hypertensive rat is lowered by the oral administration of the V1 receptor antagonist OPC-21268 (78). Share (547) has therefore proposed that AVP is essential for the development of DOCA salt hypertension because of its antidiuretic action, which makes possible the expansion of blood volume that appears to be required for the initiation of this model of hypertension.

E. Summary

AVP originates in the paraventricular and supraoptic nuclei. In addition to hormonal AVP, there are also AVP-containing neural pathways from the paraventricular and suprachiasmatic nuclei to the brain stem and the spinal cord. V1 receptor agonists placed intracerebroventricu-
There is a small rise in plasma AVP in the SHR, the Dahl salt-sensitive rat, essential hypertension, and the DOCA plus salt rat but not into the range of vasopressor concentration. In essential hypertension and the SHR, there is also a rise in urine AVP. These changes are accompanied by a reduction in the content of AVP in the hypothalamus and an increase in mRNA levels in the paraventricular and supraoptic nuclei in the SHR, which appears to diminish with age. There is also some evidence that in the SHR the AVP system is hyperresponsive.

These findings suggest that in many forms of hypertension, including the SHR, the rise in arterial pressure may be due in part to an increase in AVP in the hypothalamus. The opposite assertion, based on the SHRSP homozygous for hypothalamic diabetes insipidus, that AVP does not contribute to the rise in pressure in the SHR is questionable.

IX. NITRIC OXIDE

The principal isoform of NO synthase (NOS) in the brain is neural NOS (nNOS), and ~5% is endothelial NOS (eNOS). Both are constitutive enzymes regulated by changes in intracellular calcium. The normal brain contains no inducible NOS, although under certain abnormal circumstances such as trauma and infection it is expressed in large quantities by reactive astrocytes and infiltrating neutrophils (105). NOS catalyzes the formation of NO from the terminal guanidino nitrogen of arginine with the production of citrulline. nNOS and eNOS are dependent on calmodulin, the action of which is regulated by calcium in the presence of nicotinamide adenine dinucleotide phosphate (NADPH), tetrahydrobiopterin and flavinadenine dinucleotide (FAD), and flavin mononucleotide (FMN) (541). The neuronal concentration of arginine is maintained by an arginine pool in glial cells (216). The rise in intracellular calcium that stimulates brain NOS is due to increased entry initiated principally by glutamate binding onto N-methyl-D-aspartate (NMDA) receptors. These receptors, which occur postsynaptically, are found on certain proteins (PSD-95), which themselves are bound to nNOS (105). A small proportion of the brain nNOS is regulated by calcium entry through voltage-dependent calcium channels.

NO is a free radical (it has an unpaired electron) and is therefore highly active. In contact with tissues it is rapidly oxidized and has a half-life of 1 or 2 s. It is a hydrophobic molecule that diffuses down a concentration gradient across all membranes. NO, therefore, spreads rapidly oxidized and has a half-life of 1 or 2 s. It is a free radical (it has an unpaired electron) and is therefore highly active. In contact with tissues it is rapidly oxidized and has a half-life of 1 or 2 s. It is a hydrophobic molecule that diffuses down a concentration gradient across all membranes. NO, therefore, spreads rapidly. It is highly reactive and can react with a wide range of molecules. NO is oxidized by superoxide to peroxynitrite, which is converted to nitrite and nitrate that are excreted by the kidneys (359). NO also reacts with a wide range of metal- and of thiol-containing proteins and with low-molecular-weight thiols such as glutathione and cysteine (359). The main control of NO concentration, however, is by the regulation of NOS activity. NO, itself, exercises two feedback mechanisms that suppress NOS activity. It interacts with the iron-containing heme of NOS (217), thus directly inhibiting its activity, and also with a thiol constituent of the NMDA receptor which results in closure of the calcium channels. This diminishes calcium entry into the cells, which reduces the rate of NOS activity (364). Phosphorylation of NOS by cAMP-dependent protein kinase, protein kinase C, and Ca$^{2+}$/calmodulin-dependent protein kinase II also control NOS activity (48).

In addition, the content of NOS in the hypothalamus is regulated by changes in gene transcription leading to longer lasting changes in the concentration of NOS (423). For instance, a number of endocrine, immunological, osmolar, and volume stimuli such as thyroid hormone (634), corticotropin-releasing factor (344), hypernatremia induced by water deprivation or chronic salt loading (291) and normonatraemic hypovolemia with hemoconcentration and increase in plasma protein (635) increase NOS gene expression in the paraventricular and supraoptic nucleus. There does not appear to be information on the effect on hypothalamic NOS of an increase in volume unaccompanied by hyperosmolarity.

It is possible that hypothalamic NOS activity is also controlled by endogenous NOS inhibitors present in the brain such as asymmetrical dimethyl arginine and monomethyl arginine which in the human hypothalamus are present in concentrations of 0.39 and 1.7 μmol/g brain tissue, respectively (295). In the bovine hypothalamus, the free concentrations of these two inhibitors are ~1,000 times less (322). There is also some evidence of another, as yet unidentified, hypothalamic NOS inhibitor (405), the concentration of which varies with a prolonged increase in isosmolar salt intake. Hypothalamic NOS activity may also be controlled by circulating levels of other endogenous inhibitors such as methyl guanidine, all of which are excreted by the kidney and thus rise to high levels in renal failure (45, 199, 359, 641).

A. Distribution of NOS in the Hypothalamus

In the rat brain, immunoreactivity staining to antisera raised against purified NOS, or NADH-diaphorase histochemical visualization with the histochemical marker nitro blue tetrazolium, which reacts with NOS-containing neurons, colocalizes exclusively in neurons and nerve endings in the median preoptic area, paraventricular nucleus, suprachiasmatic nucleus, and lateral hypothalamic area.
fibers (652, 658). In the rat and primate (49), in situ hybridization for NOS mRNA is present in the same sites. NOS is found in neurons which, apart from tending to have long and ramifying processes, cannot be differentiated by any other biochemical or anatomical feature. NOS is present at higher levels in the brain than in any other tissue (105), and the density of NOS-containing neurons and nerve fibers in the hypothalamus is second only to that in the cerebellum. These NOS-containing cells form a population of nonpyramidal local circuit neurons. There are approximately a billion synapses in every cubic millimeter of neuropil, and 10 million of these may be expected to stain heavily for NOS. Although these make up only 1–2% of the total neuronal population in the telencephalon, they are broadly dispersed and project a ramifying meshwork of processes.

NOS-containing cells are spread diffusely throughout the hypothalamus (509). The most prominently stained areas lie anteriorly in the supraoptic nucleus, the paraventricular nucleus, in both the magnocellular and non-magnocellular cells, the OVLT, the SFO, and the periventricular nucleus. The anterior commissure has a moderately dense plexus of fibers. The lateral hypothalamus contains many positively stained cells and a particularly dense network of stained processes. The ventral and dorsomedial nuclei and the posterior hypothalamus contain a scattered number of stained cells. In situ hybridization reveals that eNOS mRNA in the brain is associated exclusively with blood vessels (134).

Immunoreactivity and diaphorase staining are comparable in all hypothalamic nuclei except for the arcuate nucleus that stains positive for immunoreactivity but not for diaphorase reactivity (424). It appears, however, that immunocytochemistry and diaphorase histochemical visualization underestimate the true level of NOS activity in the hypothalamus. In the suprachiasmatic nucleus these techniques have either revealed no NO, or only a few positive cells, but recently, monitoring the conversion of L-[3H]arginine to L-[3H]citrulline in extracts of the nucleus has demonstrated that its NOS activity is comparable to that in the cerebellum. In addition, confocal microscopy has revealed that the nucleus contains a dense plexus of cell processes that stain for nNOS (100).

B. Effects of NO

NO, at the high concentrations that are formed after stimulation of inducible NOS, is toxic. In addition, the concentration of peroxynitrite also rises with a breakdown to hydroxyl radicals, which are more toxic than the high levels of NO. Under normal circumstances, thiol-containing agents (glutathione, albumin, cysteine) convert the peroxynitrite anion to nitrosothiols and related products (514). When the buffering thiol pool becomes depleted, enzymes involved in producing energy may be inactivated; for instance, inhibition of glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase results in irreversible ADP-ribosylation. NO also has a rapid inhibitory action on mitochondrial metabolism (61), and high concentrations of NO deaminate DNA leading to mutagenesis and ultimately cell death (359). NO's effect on mitochondrial electron transport and oxygen consumption, however, is usually less drastic and is reversible. Normal tissue concentrations of NO reversibly suppress metabolic activity so that NOS inhibitors increase oxygen consumption of the whole body as well as the skeletal muscle, heart, and kidneys of conscious dogs (33, 339, 549–551).

One of the principal targets of NO is soluble guanylyl cyclase (GC); it enhances the enzyme's catalytic activity to form cGMP (359, 538). It is therefore interesting that, in the hypothalamus, with the possible exception of the supraoptic nucleus (690), hybridization histochemistry localization of GC has revealed low levels in the medial preoptic area and “very low” levels in the other hypothalamic nuclei such as the paraventricular nuclei (77) which contain large amounts of NOS, which suggests that there are other effector pathways for NO in these areas. The localization of GC in the brain, however, does closely resemble that of heme oxygenase (HO), which forms carbon monoxide (CO) by degrading heme. Other possibilities include an interaction with other enzymes. It is notable that with the exception of GC, NO inactivates most other enzymes, which accounts for its suppression of metabolic activity. There is also the possibility that the feedback effect of NO on the NMDA receptor (mentioned above), which diminishes calcium entry, may have an effect on other cellular processes that are controlled by intracellular calcium.

C. Relation of Hypothalamic NOS Activity to Some Other Neurotransmitters and Neuromodulators

In the hypothalamus, NOS colocalizes focally with angiotensin, AVP (321, 565), and pituitary adenylate cyclase activity peptide (688), particularly in the supraoptic and paraventricular nuclei. It is not clear if more than one of these colocalizes with NOS in a single neuron.

The intracerebroventricular injection of an NO donor or L-arginine in the conscious rat causes a dose-related increase in plasma AVP and ACTH (343, 438); the ACTH response can be abolished by antibodies against corticotropin-releasing factor and blunted by AVP. These effects appear to be due to upregulation of corticotropin-releasing factor and AVP mRNA in the parvocellular portion of the paraventricular nucleus (288). In conscious dehydrated rats, the intracerebroventricular administration of large amounts of NOS inhibitors enhances the rise in...
plasma oxytocin but has no effect on plasma AVP (588).
The administration of NO donors and inhibitors into the posterior hypothalamus with a push-pull cannula influences the local release of serotonin in a biphasic and cGMP-dependent way (293).

The systemic administration of a NOS inhibitor augments the effect of secretagogues on ACTH and cortisone plasma levels. NO is also involved in the cyclic release of prolactin, luteinizing hormone (403), growth hormone (299), and β-endorphin (33). In addition, the prolonged administration of N^\text{G}-nitro-L-arginine methyl ester (L-NAME) in the drinking water increases the turnover of norepinephrine in the paraventricular nucleus and posterior hypothalamus (696). At present, it is not possible to distinguish whether this response is due to the central effect of the NOS inhibitor reducing the concentration of NO in the hypothalamus, and therefore diminishing its dampening effect on excitatory signals (see below), or whether it is a compensatory response to the rise in arterial pressure that accompanies the systemic administration of a NOS inhibitor.

**D. NO and Synaptic Transmission in the Hypothalamus**

Studies in the paraventricular nucleus have revealed that NO is an important mediator of synaptic transmission (19). NO microdialyzed into the paraventricular nucleus of the normal rat increases the rate of release of GABA, and glutamate (254), with an increase of inhibitory synaptic activity (19). Bains and Ferguson (19) point out that these observations demonstrate that NO participates in the regulation of neuronal excitability in the paraventricular nucleus by activating an inhibitory feedback loop that may dampen excitatory signals.

**E. Hypothalamic NOS and Normal Blood Pressure**

A decrease in hypothalamic NOS activity raises the blood pressure, and it is reduced by an increase in activity. These effects were first revealed when it was observed that NOS inhibitors, administered either as an intravenous bolus or by mouth, cause a rise in arterial pressure accompanied by a paradoxical increase in renal nerve activity (527), and that this effect can be abolished by ganglionic blockade (528, 621) or attenuated by sympathectomy (528). It was then demonstrated that acute and chronic injections of a NOS inhibitor either intracerebroventricularly or directly into the paraventricular nucleus raise the arterial blood pressure (79, 159, 394, 526, 707) and cause an increase in renal nerve sympathetic nervous activity (159, 526), which can be eliminated by a C_1-2 transection or by pentolinium (527). The rise in arterial pressure is more rapid the higher the salt intake (526). Recently, it has been shown that central NOS inhibition in conscious rats raises the blood sugar by stimulating the adrenal secretion of epinephrine and norepinephrine, and induces a state of insulin resistance with defective insulin secretion. This suggests that central NOS-dependent efferent pathways may be responsible for the association of hypertension and some forms of insulin resistance (546, 633). Conversely, the injection of NO (254) or NO donors such as desethylamiodarone (DEA)/NO (79) or sodium nitroprusside (707), into the paraventricular or intracerebroventricular nucleus lowers the arterial pressure and reduces renal nerve sympathetic nervous activity (707). The injection of interleukin-1β into the lateral ventricles, which increases NOS mRNA gene expression in the paraventricular nucleus, the posterior hypothalamic nucleus, and the locus coeruleus, lowers the blood pressure (85). The arterial pressure also increases after the intracerebroventricular administration of R_p-8-bromo-cGMP to block the action of cGMP on cGMP-dependent protein kinase and falls after the injection of calcium chloride to stimulate NOS activity (79). Injections of NOS inhibitors intracisternally have no effect on the blood pressure (654). The localization of these effects to the paraventricular nucleus is apparent from the finding that injections into areas adjacent to the nucleus produce no significant change in arterial pressure or renal nerve activity (707).

It is not clear which NOS isoform is involved in the central control of the blood pressure. An intracerebroventricular injection of the highly selective nNOS inhibitor 7-nitroindazole into a normal rat has no effect on the blood pressure (484). Furthermore, the blood pressure of mutant mice that lack nNOS is normal (268). However, the blood pressure of mice in which the gene encoding eNOS is disrupted is raised (553), but paradoxically the blood pressure can be lowered by the systemic acute and chronic administration of L-NAME or the acute administration of 7-nitroindazole. Kurihara et al. (333) have suggested that the rise of blood pressure in eNOS-mice is due to a pressor effect of NO derived from the intact nNOS but that, given the peripheral vasodilatory action of NO, this putative pressor effect of NO derived from nNOS probably occurs at the level of the central nervous system or baroreceptor pathways. In view of NO's depressor effect when introduced into the hypothalamus, any putative central nervous system pressor effect of NO would appear to have to take place at some other site.

Although lowering the blood pressure of an SHR with an angiotensin-converting enzyme inhibitor lowers the increased rate of hypothalamic NOS activity (486) (a compensatory pressor response), lowering the blood pressure of a normal rat by reducing the blood volume isomotically with an intraperitoneal injection of polyethylene glycol increases hypothalamic NOS activity, a depressor response which aggravates the fall in blood pressure.
caused by the intraperitoneal injection; within 3–6 h, there is an increase in fos-like activity in NADPH diaphorase-positive cells and in NOS mRNA expression throughout the paraventricular and supraoptic nuclei (635). This effect, which is induced by an isosmotic reduction in blood volume, suggests that an isosmotic increase in blood volume might result in a decrease of hypothalamic NOS activity, a pressor response, at least initially.

F. Hypothalamic NOS and Hypertension

1. SHR

Exogenous NO administered intracerebroventricularly induces a greater fall in arterial pressure in the SHRSP than in the normotensive WKY. Attempts, however, to increase or decrease the concentration of endogenous NO by either stimulating NOS with intracerebroventricular calcium or inhibiting it with a NOS inhibitor induce changes in pressure that are less in the 12-wk-old SHRSP than in the WKY (80). Thus the SHRSP is more responsive than the WKY to NO acting centrally but is less responsive to interventions that attempt to change NOS activity. It has been proposed that this suggests that in the SHRSP the activity of NOS and thus the production of NO is reduced, which would raise the blood pressure (80). The finding that the 9- to 11-wk-old SHR hypothalamus has a greater concentration of a NOS inhibitor of unknown structure is in line with the proposal that SHR hypothalamic NOS activity is reduced (405). The physicochemical properties of this hypothalamic substance suggest that it contains a quarternary or quarternizable nitrogen; dimethyl methylene immonium has very similar though not identical properties (404).

That there may be a transient reduction in hypothalamic NOS activity in the SHR is also supported by the finding that total nitrate/nitrite concentration of hypothalamic extracts from the 9-wk-old SHR is significantly lower than in the WKY (5). In contrast, however, others have found that hypothalamic NOS activity in the 11- to 12-wk-old SHR (485) and NOS mRNA in the paraventricular nucleus of the 14-wk-old SHR (474) are greater than in the WKY. The finding that lowering the blood pressure of the 11- to 12-wk-old SHR with an angiotensin-converting enzyme inhibitor diminishes the increased hypothalamic NOS activity suggests that the increase in NOS activity in the 11-wk-old SHR is a compensatory mechanism attempting to reduce the hypertension (486).

2. Mineralocorticoid hypertensive rats

In rats given DOCA and saline in the drinking water for 6 wk, the rise in blood pressure is associated with a diminished constitutive NOS mRNA in the hypothalamus (422). Similarly, rats infused chronically with 19-noraldosterone for 4 wk develop hypertension associated with a significant decrease in NOS mRNA in the hypothalamus (606).

3. 2K1C hypertension

Three weeks after the application of the clip, the consequent rise in blood pressure is associated with a decrease in nNOS mRNA in the hypothalamus (327). However, at 6 wk, there is a significant increase in NOS mRNA. Soluble isofrom guanylyl cyclase mRNA, however, also falls at 3 wk but, in contrast to nNOS mRNA, remains low at 6 wk.

4. Hypertension following operative removal of % of the renal mass

This form of hypertension, sometimes referred to as chronic renal failure hypertension, is abolished by renal denervation (86), which demonstrates that the associated increase in sympathetic nervous activity is predominantly driven by afferent stimulation of the hypothalamus. It is interesting therefore that in this form of hypertension 5 wk after the reduction in renal mass there is, as in the 11- to 12-wk-old SHR, an increase in hypothalamic NOS mRNA expression in the paraventricular and posterior hypothalamic nucleus and an increase in NO\textsubscript{2}/NO\textsubscript{3} content (696). This compensatory depressor response of NOS activity may be due to the associated rise in interleukin-1β mRNA for this substance increases NOS activity (see above). Furthermore, the injection into the lateral ventricles of a chronic renal failure rat of a specific antibody to interleukin-1β causes a further increase in blood pressure and a decrease in NOS mRNA gene expression in the posterior hypothalamus.

G. Summary

The hypothalamus has numerous NOS-containing neurons that tend to have long ramifying processes. Such neurons are particularly dense in the supraoptic and paraventricular nuclei and the structures that form the anterior wall of the third ventricle. NO diffuses rapidly and centrifugally through all membranes affecting a much greater area than that close to the neuron from which it originates. With the exception of guanylyl cyclase, normal concentrations of NO reduce the activity of most other enzymes and thus oxygen consumption. Microdialyzed into the paraventricular nucleus of the normal rat, NO increases the release of GABA and glutamate, which results in an increase of inhibitory synaptic activity and a lowering of the blood pressure. Conversely, the injection of a NOS inhibitor into this nucleus raises the blood pressure.

Hypothalamic NOS activity is increased in the 11-
14-wk-old SHR, which is consistent with a compensatory depressor response to the hypertension. In mineralocorticoid and 2K1C hypertension and the hypertension that follows 5/6 removal of the renal mass there is a tendency, within 3–6 wk of the start of the experimental interference, for an early pressor decrease in hypothalamic NOS activity to change subsequently to a depressor increase. Overall, these findings suggest that, in the various forms of hypertension studied, changes in hypothalamic NOS activity at first contribute to the rise in arterial pressure and subsequently attempt to reduce it.

X. SEROTONIN

Serotonin, 5-hydroxytryptamine (5-HT), is a free amine formed from the amino acid L-tryptophan (300). It is a neurotransmitter the synthesis of which can be blocked by p-chlorophenylamine and p-chloroamphetamine. The free amino acid is stored and rapidly inactivated by monoamine oxidase. Storage of 5-HT can be depleted by reserpine in much the same manner as it depletes catecholamines from vesicles in catecholaminergic neurons. The actions of serotonin are mediated through numerous and varied membrane receptors, several of which still lack any recognized physiological function. Sumatripan is a 5-HT receptor agonist, and Ketanserin is a 5-HT1c and 5-HT2c receptor antagonist. Melatonin (N-acetyl-5-methoxytryptamine) is a metabolite of 5-HT.

A. Distribution of 5-HT in the Hypothalamus

The principal sites of 5-HT-containing neurons in the rat brain are the raphe nuclei in the midbrain. The neurons in these nuclei send many ascending and descending fibers. Most of the serotonin in the hypothalamus lies within ascending fibers from the most rostral and superior group of the raphe nuclei (276, 457). In addition, using histopharmacological and microspectrofluometric analysis to identify 5-HT, the hypothalamus does contain a few scattered neurons, the perikarya of which contain 5-HT, in the arcuate and dorsomedial nuclei and the median eminence (276, 308). The administration of tryptophan increases the concentration of 5-HT in these sites, suggesting that it can be synthesized by these neurons. The presence of 5-HT-containing neurons has also been demonstrated with the use of 5-[^3H]HT in vivo autoradiography when a single cluster of labeled cells was found in the dorsomedial nuclei (26), and with immunocytochemical techniques, in rats pretreated with paraglyine (a monoamine oxidase inhibitor) and L-tryptophan (185). A high concentration of 5-HT2c receptor-like immunoreactivity has been identified in the suprachiasmatic nucleus of the rat (411).

Plexuses of ascending fibers and terminals are situated in the lateral hypothalamus, the suprachiasmatic, ventromedial, and perifornical nuclei. In many of these fibers, 5-HT is contained within varicosites. These are particularly numerous in the suprachiasmatic and arcuate nuclei and the medial basal area. The third ventricle is also surrounded at all levels with 5-HT-containing varicosites. Some are scattered in the supraoptic nucleus. Such varicosites are abolished by the administration of p-chlorophenylalanine. The terminal distribution of 5-HT fibers in the hypothalamus has been demonstrated in the suprachiasmatic nucleus and the medial mammillary nucleus. Afferents from the hypothalamus to the raphe nuclei come mainly from the medial preoptic and lateral hypothalamus.

Electrical stimulation of dorsal raphe nuclei increases the rate of 5-HT accumulation in several hypothalamic nuclei, the median preoptic, the arcuate nuclei, the median eminence, and the paraventricular nucleus but not in the suprachiasmatic nucleus (454). Electrical stimulation of median, more caudal, raphe nucleus also increases in 5-HT accumulation in the paraventricular and arcuate nuclei but not in the suprachiasmatic, medial preoptic, or median eminence (454). Serotonergic terminals either make specialized synaptic contact or release their 5-HT in a manner that allows it to diffuse over distances as great as several hundred microns. The distribution of these two alternatives in the hypothalamus has not been delineated (276). It is notable that 5-HT-containing neurons, or 5-HT neuronal fibers, and varicosities have not been described in the paraventricular and anterior hypothalamic nuclei, yet micropunched pieces of paraventricular nuclei contain 5-HT (454). 5-HT is transferred into an inactive form by NO (184), and the intracerebroventricular injection of leptin causes an increase in hypothalamic 5-HT secondary to leptin’s inhibitory effect on NOS (81). In the nucleus accumbens, 5-HT inhibits the release of acetylcholine (490).

B. Hypothalamic 5-HT and Normal Blood Pressure

In rats the intracerebroventricular injection of 50–100 ng 5-HT raises the blood pressure (334), an effect which appears to be mediated in part through the release of corticotropin-releasing factor (132). Intrahypothalamic administration of DOI (a 5-HT2 agonist), but not the injection of 5-HT1 or 5-HT3 agonists, also raises the blood pressure and heart rate. Electrical stimulation of, or the microinjection of kainic acid or glutamate into, the dorsal raphe nuclei enhances 5-HT release in the hypothalamus, which raises the blood pressure and the heart rate (358). These changes can be prevented by intrahypothalamic injections of 5-HT2 antagonist (671).

Microinjections of 5-HT have been made into various...
nuclei in the hypothalamus from the posterior hypothalamus caudally to the preoptic area rostrally. 5-HT causes a dose-dependent increase in blood pressure only when injected into what is described as the anterior hypothalamus/preoptic region (577, 671). The lowest dose of 5-HT needed to obtain an effect is 3–10 ng, which is comparatively low in view of the usual concentrations of endogenous 5-HT in the rat hypothalamus (42, 523).

C. Hypothalamic 5-HT and Hypertension

The concentration of 5-HT in several punched out nuclei from the hypothalamus and midbrain, including the paraventricular and medullary raphe nuclei, from 4- and 16-wk-old SHR, does not differ from the WKY (169, 603). At 28 wk, however, the 5-HT content of the whole hypothalamus and of the brain stem is raised (603). The administration of 5,6-dihydroxytryptamine intracerebroventricularly to the 6-wk-old SHR to destroy 5-HT neurons, retards the development of hypertension for ~6 wk and when given at 14–15 wk even induces a transient fall in pressure (71).

Evidence in line with the suggestion from these results that hypothalamic 5-HT activity in the SHR may be raised is the finding that the activity of tryptophan hydroxylase, the 5-HT synthesizing enzyme, in the whole hypothalamus, is increased at 5, 8, and 15 wk (412). It is also raised in the hypothalamus of the renal hypertensive rat but not in the DOCA rat (412).

The most definitive studies are those of Koulu et al. (324, 325) who investigated the association between 5-HT metabolism in punched out nuclei from the forebrain, hypothalamus, and raphe nuclei and the development of hypertension in the SHR. The basal levels of 5-HT and its main metabolite 5-hydroxy-indole acetic acid (5-HIAA) were measured in all nuclei studied. Three different methods were used to analyze the turnover of 5-HT: its accumulation after the administration of the monoamine oxidase inhibitor paraglyline; the reduction of 5-HIAA after paraglyline; and the accumulation of 5-hydroxytryptophan (5-HTP), the immediate precursor of 5-HT, after the administration of m-benzylhydrazine an inhibitor of L-aromatic amino acid decarboxylase inhibition. In the more caudal raphe nucleus (the nucleus raphe magnus), however, there are any differences after the administration of L-aromatic amino acid decarboxylase inhibition. In the more caudal raphe nucleus (the nucleus raphe magnus), however, there were no differences after the administration of L-aromatic amino acid decarboxylase inhibition, however, the young SHR had significantly faster accumulation of 5-HTP in several nuclei including the supraoptic periventricular, paraventricular, and dorsomedial nuclei. These changes were only present in the 4-wk-old SHR; they were no longer evident at 14 wk.

In the most rostral of the raphe nuclei in the midbrain, which sends most of the ascending fibers to the hypothalamus, there were no differences between SHR and the WKY in the concentration of 5-HT, 5-HIAA, and 5-HTP, nor were there any differences after the administration of L-aromatic amino acid decarboxylase inhibition. In the hypothalamus, which give rise to most of the ascending fibers to the hypothalamus, seems to be independent of the neurons in the more caudal raphe nuclei that give rise to most of the descending fibers. And the onset and maintenance of the hypertension, which is associated with an increase in hypothalamic 5-HT activity, is not accompanied by any detectable change in 5-HT metabolism in the rostral raphe nuclei. This suggests that the hypothalamic changes are not stimulated from the midbrain.

These observations demonstrate that young SHR have an increase in 5-HT activity in certain areas in the hypothalamus and the forebrain that subsides when the blood pressure is established. It is difficult to distinguish the contribution of the raphe nuclei to these changes, but the accumulated evidence suggests that the marked increase in activity that occurs at 14 wk in the median eminence of the SHR is not due to stimulation from the raphe nuclei.

D. Summary

Most of the 5-HT present in the hypothalamus is contained within neuronal projections from the dorsal
XII. γ-AMINOBUTYRIC ACID

GABA is an inhibitory neurotransmitter formed from glutamate with the loss of a carboxyl group by the action of glutamic acid decarboxylase (GAD). The concentration of GABA in the human brain, although less than glutamate, is 200–1,000 times greater than that of neurotransmitters such as norepinephrine and acetylcholine. It is present in abundance in the hypothalamus, particularly in the anterior hypothalamus, the medial preoptic nucleus, and the paraventricular nucleus (157, 643). After release, GABA is removed by GABAergic neurons that have a GABA uptake system, and to a lesser extent it is also destroyed by a transamination reaction catalyzed by GABA-transaminase (GABA T) to yield glutamate and succinic acid (497). There are two principle types of GABA receptors, A and B. A is a ligand-gated chloride ion channel, and B is a member of the G protein-coupled receptor proteins that are concerned with the transport of potassium and calcium. The two receptors affect different functions, but both have an effect on the cardiovascular system (392). Muscimol and picrotoxin are GABA<sub>A</sub> receptor agonists, baclofen is a GABA<sub>B</sub> receptor agonist, and saclofen is a GABA<sub>B</sub> receptor antagonist. Bicuculline methiodide is a GABA postsynaptic antagonist, 3-mercapto-propionic acid (3-MP) is a GABA synthesis inhibitor, and aminooxycetic acid is a selective inhibitor of GABA degradation (497).

It was pointed out earlier that microdialysis of NO into the paraventricular nucleus increases the release of GABA and glutamate (254) and increases inhibitory synaptic activity (19).

A. Hypothalamic GABA and Normal Blood Pressure

The injection of a GABA<sub>A</sub> agonist intracerebroventricularly causes a fall in blood pressure and sympathetic nervous activity, whereas the injection of the GABA<sub>B</sub> antagonist into the whole hypothalamus, the posterior hypothalamus, or the paraventricular nucleus of the conscious or anesthetized rat raises the blood pressure, heart rate, sympathetic nervous activity, as well as plasma epinephrine and norepinephrine (142, 143, 380, 382, 383, 607, 608, 666). Injection of the GABA<sub>A</sub> agonist muscimol into the posterior hypothalamus of the conscious, but not the anesthetized rat, lowers the blood pressure, heart rate, and sympathetic nervous activity (666).

GABA<sub>B</sub> agonists, injected intracerebroventricularly in the anesthetized rat, cause a rise in blood pressure, heart rate, and sympathetic nervous activity that is abolished by an injection of a GABA<sub>B</sub> antagonist (383). When injected into the anterior hypothalamus of the anesthetized rat, agonists of the GABA<sub>B</sub> receptor cause an increase in blood pressure that is abolished by phentolamine. Agonists of GABA<sub>A</sub> receptors, however, injected into the anterior hypothalamus have no effect on the blood pressure (383). Injected into the ventromedial nucleus GABA reduces blood pressure and sympathetic nervous activity through both GABA<sub>A</sub> and GABA<sub>B</sub> receptors (383). Injected into the cerebral ventricles or preoptic area GABA suppresses the increase in blood pressure induced by intravenous angiotensin II (1).

B. Hypothalamic GABA and Hypertension

Czyzewska et al. (126) and Hambley et al. (231) describe a decrease in GAD activity in the posterior hypothalamus of the 4-, 8-, and 14-wk-old SHR, with a lower content of GABA in the whole hypothalamus by the 14th wk. In keeping with these findings, Horn et al. (253) have recently found that in the posterior hypothalamic nucleus and the adjoining dorsomedial nucleus of the 11- to 16-wk-old SHR there are 42% less immunochemically stainable neurons for GAD and a 33% reduction in GAD mRNA. It is probable that the accompanying diminution in the concentration of GABA in the posterior hypothalamic nucleus contributes to the raised neuronal activity of this nucleus in the adult SHR (558).

Sasaki et al. (534) found that although the basal content of GABA in the SHR hypothalamus is not significantly different from the WKY, the amount of GABA that accumulates after an injection of an inhibitor of GABA degradation (aminooxycetic acid) is less in the SHR. They also demonstrated that the administration of a...
GABA<sub>A</sub> receptor agonist intracerebroventricularly for 2 wk lowered the blood pressure of the WKY to a much smaller extent than that of the SHR, which suggested that the GABAergic stimulant was attenuating the development of spontaneous hypertension by reducing hypothalamic hyperactivity (533). Similar results were obtained in the 6- to 10-mo-old SHRSP (637).

Microinjections of 3-MP, a GABA synthesis inhibitor, into the posterior hypothalamus raises the blood pressure and heart rate of young (6–8 wk) and adult (11–16 wk) WKY and young SHR, but it has no effect on the blood pressure of the adult SHR, which suggests a deficiency of GABAergic activity (557). The fall in blood pressure, heart rate, and sympathetic nervous activity after an injection of baclofen (608), the GABA<sub>B</sub> receptor agonist, into the ventromedial nucleus is much larger in the SHR. Interestingly, when baclofen is injected into the anterior hypothalamic nucleus, there is a rise in blood pressure and heart rate which is not significantly different in the SHR and WKY. There are several reports that the fall in blood pressure, heart rate, and sympathetic nervous activity induced by the injection of GABA directly into cerebroventricles is much greater in the SHR than in the WKY (50, 531, 532).

Hambley et al. (231) found that the number of muscimol binding sites (GABA<sub>A</sub> receptor sites) in the whole hypothalamus were significantly lower in the 10- to 20-wk-old SHR than in the WKY. In addition, the content of GABA in the whole hypothalamus of the SHR was significantly lower than in the WKY. Focal densities of GABA<sub>A</sub> receptor binding sites in the SHR were significantly lower in all hypothalamic nuclei examined at 4 wk, and they were still lower in the paraventricular nucleus at 12 wk (332). In addition, compared with the WKY, GABA binding onto GABA<sub>B</sub> receptors is significantly less in the posterior hypothalamus of the 4- and 11-wk-old SHR, and the suppression of adenylate cyclase activity by GABA<sub>B</sub> receptor agonists at this site, at those ages, is also less (269).

At the onset of one model of renal ischemic hypertension (one normal kidney and a figure of eight renal wrap of the other kidney), bilateral injections of bucculine (a GABA postsynaptic antagonist) into the paraventricular nuclei had no significant effect on the blood pressure, whereas it raised the blood pressure of control animals. In contrast, the injection of muscimol (GABA<sub>A</sub> receptor agonist) reduced the blood pressure of the hypertensive animals but had no effect on the blood pressure of the controls. These responses suggest that GABA function in the paraventricular nuclei of the renal wrap hypertension is reduced (381). Later, however, there is a compensatory depressor increase in GABA function in the paraventricular nucleus (240a).

In the DOCA plus salt hypertensive rat, however, Zappia et al. (704) found an increased density of GAD containing terminals in the areas lateral to the paraventricular nucleus and the medial preoptic nucleus of the DOCA plus salt hypertensive rat 4 wk after the initiation of the DOCA. This increase did not occur in the paraventricular nucleus, the lateral septum, or the suprachiasmatic nucleus (704). In the light of the depressor effect of GABA when injected into the preoptic area (1) (see above) of the normal rat, this finding in the DOCA plus salt rat suggests that the increase in GAD in the medial preoptic nucleus may be a compensatory response.

C. Summary

GABA is an ubiquitous inhibitory neurotransmitter that is present in abundance throughout the hypothalamus, particularly the anterior hypothalamic area, the medial preoptic nucleus, and the paraverntricular and posterior hypothalamic nuclei. GABA is formed from glutamate by the action of glutamic acid decarboxylase. GABA<sub>A</sub> receptor agonists injected intracerebroventricularly or into the posterior hypothalamus lower the blood pressure and reduce sympathetic nervous activity, whereas GABA<sub>B</sub> receptor agonists injected intracerebroventricularly or into the anterior hypothalamus raise the blood pressure and increase sympathetic nervous activity. Both GABA<sub>A</sub> and GABA<sub>B</sub> receptor agonists lower the blood pressure when injected into the ventromedial nucleus.

The main abnormalities of GABA metabolism in the SHR hypothalamus are a general pressor diminution in GABA activity, which is particularly pronounced in the posterior hypothalamus where there is diminished GAD activity, a reduction in the concentration of GABA, and a diminution in GABA<sub>A</sub> receptor sites.

XII. NEUROPEPTIDE Y

Neuropeptide Y (NPY) belongs to the pancreatic peptide family, each of which contains 36 amino acid residues (384, 556). It is one of the most abundant neuropeptides in the mammalian nervous system (106, 136, 137, 152). It has a wide range of activities including effects on food intake, ethanol consumption, central endocrine secretion, anxiety, psychomotor activity, and cardiovascular effects. There are indications that its major physiological function is to protect neural circuits from excessive stimulation. There are at least five classes of G protein-coupled receptors Y<sub>1</sub>, Y<sub>2</sub>, Y<sub>4</sub>, Y<sub>5</sub>, and Y<sub>6</sub> (152). These receptors transmit a signal initiated by NPY binding to an intracellular protein complex that binds GTP. This in turn activates a variety of second messenger systems including a decrease in cAMP and an increase in intracellular calcium. NPY receptors may also be involved with phosphoinositid and phospholipase A activity (556). NPY directly constricts blood ves-
sels in some vascular beds and potentiates the peripheral pressor effects of other vasoactive agents (556).

A. Distribution of NPY in the Hypothalamus

The hypothalamus contains the highest concentration of NPY in the brain. Immunoreactive NPY staining is found in the paraventricular, ventromedial, dorsomedial, posterior, arcuate, supraoptic, anterior, and medial preoptic nuclei (106, 255, 384). The paraventricular and arcuate nuclei contain the highest density of immunoreactive fibers and perikarya, respectively (106), and the arcuate nucleus contains the greatest density of Y2 receptors (223). Most of the NPY immunoreactive fibers in the paraventricular and dorsomedial nuclei originate from NPY immunoreactive neurons in the arcuate nucleus (372). The suprachiasmatic nucleus, the median eminence, and dorsomedial also show high concentrations, whereas moderate concentrations are present in the nucleus of the striae terminalis. Some of the hypothalamic NPY is colocalized in neurons containing catecholamines (372, 630), and there is an abundant network of NPY immunoreactive axons surrounding neurons that contain galanin (255). With age there is striking diminution of NPY in the hypothalamus, particularly in the ventromedial nucleus (374, 410, 447).

B. Interaction of NPY With Catecholamines, AVP, NO, Cholinergic, and Histaminergic Mechanisms

In hypothalamic slices the release of norepinephrine by electrical stimulation is inhibited by NPY (629, 630). Similarly, the amount of norepinephrine sampled by microdialysis from the paraventricular nuclei decreases sharply after exposure to NPY, and NPY decreases the potassium-induced release of norepinephrine from slices of posterior hypothalamic nucleus (663). NO appears to participate in these responses in that the NOS inhibitor L-n-monomethyl-L-arginine blocks the inhibitory effect of NPY on norepinephrine release in tissue slices (40). The ß-blocker propranolol causes a profound decrease on the basal and potassium-evoked release of NPY from slices of rat hypothalamus (107). Norepinephrine therefore stimulates the release of NPY, which inhibits the release of norepinephrine.

The rise in blood pressure induced by the intracerebroventricular injection of NPY is shortened by the administration of propranolol and the ß-blocker prazocin (257), but the preliminary injection of 6-hydroxydopamine intracerebroventricularly does not prevent the pressor response of a subsequent injection of NPY (642). The rise in pressure induced by the intracerebroventricular administration of NPY is not affected by a specific AVP antagonist, but the rise does not occur in the Brattleboro rat (642). The rise in blood pressure induced by an injection of NPY into the posterior hypothalamic nucleus is attenuated by prior installation of either atropine or chlorpheniramine into the nucleus (384), suggesting that the effect of NPY at this site involves local cholinergic and histaminergic activity.

C. Hypothalamic NPY and Normal Blood Pressure

The results are contradictory. On the one hand some investigators have found that the intracerebroventricular injection of NPY in the conscious or anesthetized rat causes a dose-dependant rise in blood pressure (3, 257, 642) with an associated increase in vascular resistance in the hindlimb and the mesenteric vessels with no change in the vascular resistance of the renal vascular bed. And there are several reports that the injection of NPY into either the posterior or the paraventricular nucleus of the anesthetized and conscious rat induces a rise in arterial pressure and an increase in vascular resistance in the hindquarters that is prevented by pretreatment with pentolinium while the response of the renal vasculature varies (384–386, 455, 663, 664). On the other hand, others who have injected NPY either intracerebroventricularly or into the paraventricular nucleus of the rat have reported either no change or a fall in blood pressure with a decrease in sympathetic nervous activity (120, 235, 236, 644). Van Ness et al. (645) found that an NPY receptor antagonist injected into the paraventricular nucleus increased the blood pressure of conscious rats during and after a period of food restriction.

These inconsistencies may be due to the state of consciousness. For instance, it has been found that the increase in NPY mRNA in the arcuate nucleus of the conscious SHR is suppressed if the rat is restrained (328). Furthermore, in all seven studies in which NPY was administered centrally to anesthetized rats, the blood pressure rose, whereas it rose in only two of the seven studies in conscious rats.

D. Hypothalamic NPY and Hypertension

In the SHR quantitative densitometry and in situ hybridization by two groups has shown that, compared with normotensive strain rats, NPY mRNA is increased in the arcuate nucleus (328, 398). If, however, the SHR is restrained, this difference in gene expression in the arcuate nucleus is not evident (328). In the SHR, NPY immunoreactivity is greater in the anterior hypothalamus and dorsomedial and ventromedial areas and lower in the lateral preoptic area (375). There is another finding which suggests that there is an increase in NPY activity. In the SHR (and the aortic banded renal ischemic hypertensive rat) the amount of norepinephrine obtained from the paraven-
tricular nucleus is not influenced by exposure to exogenous NPY, in striking contrast to the reduced amounts of norepinephrine retrieved from the WKY. Similarly, NPY inhibition of norepinephrine released by electrical stimulation of slices of SHR hypothalamus is much less than its inhibitory effect on slices of hypothalamus from the WKY (629). In addition, the hypothalamus of the SHR has an increase in Y2 receptors, which have a depressor effect, in contrast to the number of Y1 receptors, which have a pressor effect (3). This may be responsible for the finding that the rise in arterial pressure induced by the intracerebroventricular injection of NPY in the SHR occurs with smaller doses than in the WKY, and lasts longer (3).

E. Summary

The hypothalamus contains the highest concentration of NPY in the brain. The paraventricular nucleus has the greatest number of NPY immunoreactive fibers, most of which originate from the arcuate nucleus which contains the greatest number of NPY immunoreactive neurons. The suprachiasmatic nucleus, the median eminence, and the dorsomedical nucleus also contain high concentrations of immunoreactive NPY neurons.

NPY and norepinephrine coexist in some neurons. Norepinephrine stimulates NPY release, whereas NPY inhibits the release of norepinephrine. There is a close connection between cholinergic and NPY functions in the posterior hypothalamus. NPY administration into the cerebral ventricles, the paraventricular nucleus, and the posterior nucleus of the anesthetized rat consistently raises the blood pressure, but in the conscious rat the results are variable.

Findings in the SHR suggest that there is an increase in hypothalamic NPY activity. There is an increase in NPY mRNA in the arcuate nucleus and in NPY immunoreactivity in the anterior hypothalamus and dorsomedical and ventromedial nuclei. There is also a relative increase in Y2 “pressor” receptors. The return to normal of NPY mRNA in the arcuate nucleus, when the conscious SHR is restrained, which would appear to be a depressor response to stress, is paradoxical.

XIII. OUABAIN-LIKE SUBSTANCES

Since the demonstration in the 1970s that plasma contains a Na+-K+-ATPase inhibitor controlled by volume changes (70, 111), two families of mammalian ouabain steroids have been identified that are probably synthesized by endogenous mammalian pathways. Both bind onto Na+-K+-ATPase; one, the cardenolides (ouabain and digoxin) (210), resembles cardenolides found in plants, and the other, the bufodienolides (bufalin and marinobufogenin) (149), resembles bufodienolides found in toads. Up to 1998 most of the work on the relation of ouabain-like substances to hypertension has concerned ouabain. It has to be kept in mind that although it is usual to consider that ouabain is a Na+-K+-ATPase inhibitor, at low concentrations (10−9 to 10−10 M) ouabain stimulates the pump (173).

The hypothalamus contains ouabain (301, 632) and also bufalin and marinobufagenin (69). The endogenous isomer of ouabain has the same basic structure as plant ouabain, but as Kawamura et al. (301) point out with reference to endogenous ouabain, “the polyhydroxylated ouabain molecule serves as a polydendrite ligand to many inorganic series.” This has caused the identity of the endogenous isomer to be masked by the purification and identification procedures, which is the reason it has not yet been identified (301). The isomer upon which the structural investigations have been carried out has been a ouabain-borate complex from contact with borosilicate glassware. Ouabain is a polar compound that does not cross the blood-brain barrier rapidly (232). The biosynthetic pathway of endogenous mammalian ouabain is not known, and in contrast to plant ouabain, which selectively inhibits the α2-isoform of Na+-K+-ATPase, the particular endogenous isomer of ouabain that emerges after purification procedures inhibits the α1-subunit (69).

Mammalian ouabain appears to originate from the adrenal (338, 545), the hypothalamus (632), and the midbrain (130).

A. Distribution of Mammalian Ouabain in the Hypothalamus

Ouabain-like activity in the hypothalamus is greater than in the cortex and midbrain and less than in the striatum and hippocampus (501). Histological studies with a ouabain-specific antibody have been performed on the rat and macaque hypothalamus (680) and have revealed no significant differences. In both species ouabain immunoreactive neurons were observed concentrated particularly in the paraventricular and supraoptic nuclei. The neurons have the appearance of magnocellular neurons. Their nerve fibers, some of which contain varicosities, are abundantly distributed in the AV3V area including the OVLT, SFO, and the median eminence. Immunoreactive neurons are also scattered in the periventricular, perifornical, lateral hypothalamic, anterior hypothalamic, and preoptic areas. The distribution of ouabain immunoreactive neurons is very similar to that of digoxin immunoreactive neurons (679).
B. Control of Hypothalamic Ouabain, the Central Effect of Ouabain on the Sympathetic System, and the Interaction of Ouabain With Other Hypothalamic Substances

Because of its scarcity, few experiments have been carried out with endogenous mammalian ouabain. The administration of plant ouabain intracerebroventricularly or into the caudal hypothalamic nuclei causes an increase in sympathetic nervous activity in conscious and anesthetized rats (266, 274, 600). The most sensitive site for eliciting this response is the posterior hypothalamic nucleus (274, 535). Plant ouabain, in vitro, inhibits monoamine oxidase (518), the catabolic enzyme for noradrenaline, dopamine, and 5-HT. The instillation of 6-hydroxydopamine into the lateral ventricles of the rat diminishes the hypothalamic content of a ouabain-like factor, as measured by an enzyme-linked immunosorbent assay, the elution pattern of which on high-performance chromatography is similar to that of plant ouabain (319). These isolated results suggest that ouabain may increase the hypothalamic activity of catecholamines and 5-HT and that catecholamines increase hypothalamic ouabain activity.

The increase in sympathetic nervous activity and rise in arterial pressure (see sect. xivb) associated with the intracerebroventricular administration of ouabain in normal animals is dependent on angiotensin II. The effect on the blood pressure and sympathetic nervous activity is either abolished or significantly suppressed by the administration of an angiotensin inhibitor (saralasin) or receptor blocker (losartan) (83, 260, 511, 600) into the cerebral ventricles but not when given intravenously.

C. Hypothalamic Ouabain and Normal Blood Pressure

The introduction of ouabain into the lateral ventricles of the hypothalamus or posterior hypothalamus of rats causes an increase in blood pressure which, like the associated rise in sympathetic nervous activity, can be prevented by the central administration of fab fragments of digoxin antibody (Digibind) that binds ouabain and related steroids with high affinity (83, 252, 258, 259, 266, 274, 284). Intracerebroventricular injection of an AVP antagonist does not prevent the rise in arterial pressure (258). These relatively acute effects of ouabain given centrally do not occur if given intravenously (597). The administration of a crude extract of rat hypothalamus containing ouabain-like activity into the lateral ventricles of the rat also causes a rise in arterial pressure that can be suppressed by central Digibind (258).

Microinjections of 20 ng ouabain in 200 nl CSF when injected into the median preoptic nucleus, the paraventricular nucleus, and the anterior and posterior hypothalamus of anesthetized rats (284) induce rises in pressure from 5 to 25 mmHg. The paraventricular nucleus has the greatest sensitivity. Ouabain at 0.01–1.0 μg has also been injected into the posterior hypothalamus, the anterior preoptic hypothalamus, and the ventromedial nucleus (274). Microinjections into the posterior hypothalamus produce a dose-dependent increase in arterial pressure, but ouabain injections as great as 1.0 μg into the anterior preoptic and ventromedial nucleus have no significant effect. The AV3V region may also be sensitive to ouabain for the intracerebroventricular injection of ouabain into a conscious AV3V lesioned rat depresses the usual rise in pressure by ~30% (648).

A continuous infusion into the lateral ventricle of the conscious rat of a CSF with a sodium concentration 6 mmol greater than normal CSF (152 vs. 146 mmol) increases the blood pressure, renal sympathetic nerve activity, and the concentration of the ouabain-like material in the hypothalamus. The rise in arterial pressure and renal sympathetic nervous activity can be prevented by the intracerebroventricular injection of either Digibind or losartan, an angiotensin II receptor blocker (267).

D. Hypothalamic Ouabain and Hypertension

The most striking finding is that of Ferrandi et al. (172) in the Milan hypertensive strain rat. Using the same extraction technique as Haupert and co-workers (632), who had previously shown that the ouabain-like material they extracted from the hypothalamus is an isomer of plant ouabain, Ferrandi et al. (172) found that the yield of this material from the Milan hypertensive strain rat hypothalamus is ~5- to 10-fold greater than from the hypothalamus of the Milan normotensive strain rat. This was the first demonstration of a quantitative relationship between a form of hereditary hypertension and a brain-derived sodium pump inhibitor. Furthermore, they observed that this mammalian hypothalamic material inhibits renal Na\(^+\)-K\(^+\)-ATPase with a potency that is ~1,000 greater than plant ouabain. This finding suggests that mammalian ouabain might, after all, be natriuretic.

The potency of the ouabain-like activity of a crude hypothalamic extract from the SHR is greater than from a similar extract obtained from the WKY (345, 347). The difference is apparent at 4 wk of age and is more pronounced at 8 wk. A high sodium intake (a food content of 8% salt) increases both the ouabain-like potency of hypothalamic extracts obtained from the SHR (345, 347). The administration of Digibind (264) into the lateral ventricles of SHR on a normal sodium intake does not lower the blood pressure or diminish renal sympathetic nerve activity.
activity, but it does both in SHR on a high-sodium diet (264). That this hypotensive response may indeed be due to an effect on the hypothalamus was demonstrated by observing that the introduction of Digibind directly into the median preoptic nucleus of the SHR on a high-sodium diet significantly lowers the blood pressure (72). This finding also suggests that, in the SHR, the increase in hypothalamic ouabain, induced by a high-salt diet, may take place preeminently in the median preoptic nucleus.

In SHR on a high-sodium diet centrally infused with Digibind, the pressor and sympathoexcitatory response to the central administration of angiotensin II is greater than when given to SHR on a high sodium intake not given Digibind. Huang and Leenen (263) have proposed that this is consistent with a decrease in neuronal angiotensin II occupancy in the SHR receiving Digibind and that, therefore, in the SHR, among the pathways mediating sympathoexcitatory and pressor effects, angiotensin II stimulation may be "downstream," in a metabolic sense, of the ouabain effect.

Similar observations have been made in the Dahl salt-sensitive rat (the Dahl S rat) (261, 262). Four hours after a 5-min injection of Digibind into the lateral ventricle of Dahl S hypertensive rats on a high-sodium diet the hypertension and raised renal sympathetic nerve activity are unchanged. However, 18 h after the injection, there is a significant fall in both the blood pressure and renal nerve activity. Alternatively, a chronic infusion of Digibind into the lateral ventricle of a Dahl S rat prevents the rise in blood pressure usually associated with a high-sodium diet, but once the Digibind infusion is discontinued the blood pressure rises. Huang and Leenen (261) suggest that "a high sodium intake may cause hypertension in Dahl S rats by increasing endogenous brain ouabain-like activity, thereby increasing sympathetic outflow and basal blood pressure..." This concept is supported by these investigator's observation that, in the Dahl S rat, a high sodium intake increases the hypothalamic content of ouabain-like activity before the rise in pressure but not, or only to a minor extent, in the Dahl R rat (346) and by the finding that the pressor and sympathoexcitatory effects of an injection of ouabain into the lateral ventricle of a Dahl salt-sensitive rat or a SHR, on a high-sodium diet are much less than in the Dahl salt-resistant rat which is consistent with increased receptor occupancy by endogenous ouabain-like substance in the SHR (261).

### E. Summary

The hypothalamus contains ouabain, bufalin, and marinobufagenin. The isomeric structure of the endogenous ouabain present in the hypothalamus is not known. This does not prevent quantitative assessments but determines the relevance of qualitative studies. Most functional studies have been carried out with plant ouabain. Hypothalamic ouabain is present particularly in the neurons of the paraventricular and supraoptic nuclei, while ouabain fibers are found in the OVLT, SFO, and median eminence. The administration of plant ouabain or of a crude hypothalamic extract containing ouabain-like activity intracerebroventricularly or into the paraventricular nucleus or the posterior hypothalamus increases the blood pressure and sympathetic nervous activity. These effects can be suppressed by fab fragments of digoxin antibody (Digibind) or an angiotensin inhibitor or a receptor blocker. A sustained 6-nmol rise in CSF sodium concentration causes an increase in blood pressure and sympathetic nervous activity that is accompanied by a rise in the concentration of a ouabain-like material in the hypothalamus; all these changes can be prevented by the central administration of Digibind or losartan.

The yield of a highly purified ouabain-like material from the hypothalamus of the Milan hypertensive rat is ~5- to 10-fold greater than from the Milan normotensive rat. Similarly, the potency of a much cruder preparation from the SHR hypothalamus is greater than from the WKY hypertalamicus. Such increases suggest a pressor role for hypothalamic ouabain in the Milan hypertensive rat and the SHR. The central administration of Digibind, however, has no effect on the blood pressure of the SHR, although it does prevent the blood pressure rise associated with an increase in salt intake in both the SHR and the Dahl salt-sensitive hypertensive rat.

### XIV. OPIOIDS

"Opioids” stem from three distinct genes the products of which are preproopiomelanocortin (POMC), preproenkephalin (or preproenkaphalin A), and preprodynorphin (preproenkaphalin B). The derived mediators about which most is known are β-endorphins, Met-enkephalin, and dynorphin. Their action is mediated by three distinct types of receptors: δ, μ, and κ. β-Endorphins and Met-enkephalin act mainly on μ-receptors, Leu-enkephalin on δ, and dynorphin on κ (497). All the receptors are linked to G proteins and affect ion gating, intracellular Ca²⁺, and protein phosphorylation. The opioids have an inhibitory effect on neurons, both presynaptically when they close voltage-gated Ca²⁺ channels and postsynaptically when they hyperpolarize by opening K⁺ channels. Naloxone is an antagonist for all types of receptors, but there are specific agonists. In the normal rat brain the highest concentration of immunoreactive dynorphin A(1—8) is found in the substantia nigra and in the lateral preoptic area (703). High concentrations are found in the ventral premammillary nucleus, the lateral hypothalamicus, anterior hypothalamicus, dorsomedial nucleus, and
the arcuate nucleus. Lesser amounts are found in the mid and hindbrain (113, 703).

A. Hypothalamic Opioids and Normal Blood Pressure

In conscious 9-wk-old WKY β-endorphin injected into the paraventricular nucleus raises the blood pressure and plasma epinephrine (281). The same response is obtained by the injection of dynorphin into the anterior hypothalamic and preoptic nucleus (148), and there is one study of a small rise in arterial pressure after the injection of demorphin into the caudal anterior hypothalamic nucleus of the halothane-anesthetized rat (148). DAGO (a μ-receptor agonist) and DADLE (a δ-receptor agonist) injected into the paraventricular nucleus of the anesthetized normal rat decreases the arterial pressure (592). Naxolone alone, injected into the paraventricular nucleus, lowers the arterial pressure of the anesthetized normal rat (592). In the conscious rabbit the intracerebroventricular injection of enkephalins causes a dose-dependent increase in blood pressure and sympathetic nervous activity (393). The increase in blood pressure and sympathetic nervous activity induced by the intracerebroventricular administration of leptin is mediated by POMC and its breakdown products α-melanocyte stimulating hormone and β-endorphin (153).

B. Hypothalamic Opioids and Hypertension

Genetic studies in the SHR demonstrate an increase in hypothalamic opioid activity. Proencephalin mRNA is increased significantly in the anterior and the lateral hypothalamus of the 14-wk-old SHR (44), and preproencephalin mRNA levels that are not different from the WKY at 3 wk are increased in the SHR at 12 wk (248).

The binding and density of β, δ, and κ-opioid receptors in the SHR hypothalamus are greater than in the WKY (37, 222, 698, 699). A change that is probably responsible for the observation that the intracerebroventricular injection of (D-Ala²)methionine enkephalin into the SHR causes a greater rise in arterial pressure, in the first phase of the usual biphasic blood pressure response, than when injected into the WKY (508). In addition when DAGO (a highly selective ligand for the μ-opiate receptor) is injected into the medial preoptic nucleus of SHR, the rise in arterial pressure is greater than when it is injected into the WKY, although at higher doses the effect in the SHR is depressor (178). Whatever the effect on the blood pressure, DAGO raises plasma norepinephrine and epi- nephrine and reduces plasma AVP in both the SHR and WKY (178).

Injection of β-endorphin into the paraventricular nucleus of the conscious SHR causes a greater increase in arterial pressure than in the WKY. Ganglionic blockade significantly reduces this increase but only that induced by the lower doses of β-endorphin (281). In contrast to the normal rat, naxolone injected into the paraventricular nucleus of the anesthetized SHR does not lower the arterial pressure (592).

Some investigators have found that dynorphin A-like and B-like immunoreactivities in the hypothalamus of the 8-wk-old SHR are greater than in the WKY (38, 613), while the extent of immunoreactivity to enkephalin is not different (38). Another group found no difference in dynorphin A-(1—8) immunoreactivity in the lateral preoptic and lateral hypothalamic areas of the hypothalamus between SHR and WKY aged 6–14 wk (113). In other studies, SHRs were found to have lower levels of opioids in the hypothalamus. In one the immunoreactivity concentrations of dynorphin-(1–8), dynorphin-(1–13), and Leu-enkephalin were decreased in the suprachiasmatic nucleus, and there were low levels of dynorphin A-(1–8) in the paraventricular nucleus (177). In line with this finding, there are three other studies which found that the immunoreactivity of dynorphin A-(1—8) β-endorphin and Leu-enkephalin of the whole hypothalamus in SHR and SHRSP rats four to 24 wk of age was lower than in the WKY (186, 354, 355). These changes were not secondary to the hypertension in that they persisted in animals treated chronically with clonidine to reduce their blood pressure to normal (186).

C. Summary

Opioids stem from three distinct genes, the best known derived mediators of which are β-endorphin, Met-enkephalin, and dynorphin. There are three receptors. β-Endorphin and Met-enkephalin act mainly on μ-receptors, Leu-enkephalins on δ-receptors, and dynorphin on κ-receptors. Naloxone is an antagonist of all types of receptors. DAGO is a highly selective ligand for μ-receptors and DADLE a δ-receptor agonist.

In the adult SHR there is an increase in pro- and prepro-enkephalin mRNA in the anterior and lateral hypothalamus and an increase in binding and density of all three receptors. Injection of β-endorphin into the paraventricular nucleus of the conscious SHR causes a greater rise in blood pressure than in the WKY. And, in contrast to the anesthetized WKY, the injection of naloxone into the paraventricular nucleus of the anesthetized SHR lowers the blood pressure. In general, the concentration of immunoreactive opioids in the SHR and SHRSP hypothalami is lower than in the WKY.

Overall the findings suggest that in the hypothalamus of the SHR there is a pressor increase in opioid activity.
XV. BRADYKININ

Kininogenases such as glandular and plasma kallikreins that generate kinins by hydrolyzing kininogenases are enzymes that belong to a family of serine proteases. Kinins are oligopeptides that contain bradykinin in their structure. They are destroyed by kininases, the best known of which is angiotensin converting enzyme or kininase II, which convert angiotensin I to II and “inactive” kinin, substance P, and other peptides. Kinins act mainly as local hormones. Two main subtypes of kinin receptors have been characterized B₁ and B₂. They are cell surface, G protein-coupled receptors of the seven-transmembrane domain variety (230). Most of the effects of bradykinins are mediated by B₂ receptors (481, 502), a potent synthetic antagonist of which is the analog DARG₀-HYP₅-THI⁵-OIC⁸ bradykinin, known as HOE-140 (670); there are others.

A. Distribution of Kallikrein-Kinin System in the Hypothalamus

All the components of the kallikrein-kinin system are present in the brain (452, 542). Brain kallikrein appears to be identical to urinary kallikrein (97, 98), and the presence of kallikrein mRNA demonstrates that bradykinin is formed in the brain. The highest concentration of bradykinin in the brain is in the hypothalamic area where a concentration of 4.8 pmol/g has been found in contrast to a whole brain concentration of 0.6 pmol/g. A specific monoclonal antibody against kallikrein localizes in the ependyma of the third ventricle, the supraoptic, paraventricular, ventromedial, and arcuate nuclei (566). Kininogen immunoreactivity is concentrated in the neurons of the periventricular nucleus (particularly those with projections to the median eminence); the parvocellular neurons of the suprachiasmatic nucleus; some neurons in the arcuate, supraoptic, and dorsomedial nuclei; the magnocellular neurons of the paraventricular nucleus; and the lateral hypothalamic area (505). Bradykinin-like immunoreactive neuronal cells are found throughout the hypothalamus with especially dense clusters in the periventricular and dorsomedial nucleus (117). B₁ and B₂ kinin receptors are present throughout the brain including the hypothalamus (492).

B. Bradykinin Interaction With Catecholamines, Prostaglandins, and NO

In the paraventricular nucleus bradykinin mitigates the action of angiotensin II, an effect which is blocked by the bradykinin antagonist HOE-140 (181). The rise in arterial pressure induced by the intracerebroventricular injection of relatively large amounts of bradykinin (5 nmol) can be reduced by indomethacin given by the same route, and the injection of prostaglandin E centrally raises the blood pressure (297). This suggests that the central action of bradykinin may be mediated by one or more prostaglandin-related systems. A suggestion that is supported by another experiment in which the central action of bradykinin was studied by recording the blood pressure and sympathetic nerve activity of a recipient rat, the head of which was vascularly isolated and perfused by a donor rat. Intracarotid injection of bradykinin produced an initial fall in pressure and diminution in sympathetic nerve activity followed by a prolonged rise in pressure and increase in sympathetic nerve activity (599). The initial inhibitory effect disappeared after the intracerebroventricular injection of 6-hydroxydopamine, suggesting that bradykinin in large amounts causes a transient release of norepinephrine. The subsequent stimulating effect was reduced after suppressing prostaglandin synthesis by indomethacin and augmented by the prostaglandin precursor arachidonic acid. These results indicate that bradykinin has at least two centrally induced actions on blood pressure, the pressor effect being mediated by the release of prostaglandin.

There does not appear to be any information on the effect of bradykinin on brain NOS, but the studies on isolated endothelial cells (43, 445) and isolated organs (307), which demonstrate that bradykinin activates endothelial NOS, suggests that it could have a similar effect centrally.

C. Hypothalamic Bradykinin and Normal Blood Pressure

There are several studies in the normal rat which suggest that hypothalamic bradykinin either plays no part in the control of normal blood pressure or has a depressor role. The introduction of a kinin receptor antagonist intracerebroventricularly into a normal rat has no effect on the blood pressure (376, 377, 693). Neither has the intracerebroventricular introduction of captopril, a kininase II inhibitor (376), which should increase the local concentration of kinin. In another study in mice, however, in which bradykinin B₂ receptor gene was disrupted, there was a rise in blood pressure (378). There was also a rise in blood pressure in rats given intracerebroventricular antisense oligodeoxynucleotides targeted to kininogen mRNA or bradykinin receptor B₂ mRNA.

These findings which suggest that hypothalamic bradykinin lowers the blood pressure are not in line with many others that it has a pressor role. In these experiments the introduction of bradykinin (75, 118, 144, 146, 297, 362, 376, 377, 693), kallikrein, or mellitin, which activates kallikrein (616, 694) intracerebroventricularly into conscious or anesthetized rats, increases the blood...
pressure and sympathetic nervous activity (75). Furthermore, the experiments in which the entry of CSF into the fourth ventricle was occluded and yet there was a rise in pressure suggest that the pressor effect of the bradykinin is likely to be due to its presence in the hypothalamus (350). This conclusion is supported by the finding that bradykinin introduced into the dorsomedial and posterior hypothalamic nuclei increases the blood pressure and heart rate. Microinjections into the paraventricular nucleus, however, have no effect on the blood pressure (208) and into the anterior hypothalamic nuclei they cause a fall in blood pressure (144).

Overall these results suggest that the normal concentration of endogenous hypothalamic bradykinin either has little effect on the blood pressure or reduces it, whereas the large rise in concentration that occurs when bradykinin is introduced experimentally from without induces a pressor effect. Possibly the pressor contribution of prostaglandin discussed earlier only occurs at high concentrations of bradykinin (297).

D. Hypothalamic Bradykinin and Hypertension

Whereas, in the WKY, CSF kinins decrease with age, they do not do so in the SHR (309). The CSF concentration of kinins, kallikrein, and kininase, in the adult SHR, are raised. At 5–6 wk, the CSF kinin level of the SHR is greater than that of the WKY, but there is no difference in the kallikrein and kininogenase levels. In the adult SHR, however, the CSF concentration of kinin, kallikrein, and kininogenase are all greater than in the WKY (6, 309, 310).

This probably accounts for the exaggerated effect, in the SHR, of an acute intracerebroventricular injection of 500 μg and 1 mg of the kininogenase II inhibitor captopril (376, 638). There is an initial rise in blood pressure followed by a prolonged fall. Alvarez et al. (6) also found that the bradykinin content of the SHR hypothalamus was increased.

The effect on the blood pressure of the SHR of the administration of a bradykinin receptor antagonist intracerebroventricularly has been studied on three occasions under varying conditions. In two experiments the antagonist was given in 3–60 min. In one (6) in which des-Arg^{2-}[Leu^{5}]-bradykinin (0.1–10 μg) was infused in 3 min there was a dose-dependent rise in blood pressure, whereas in the other (376) in which [d-Arg]Arg-Pro-Hyp-Gly-Thi-Ser[d-Phe][Hyp]-TFA (Hyp, l-4-hydroxyproline; Thi, P-2-thienyl-L-alanine; TFA, trifluoroacetic acid) was given, 20 μg over 1 h, there was no change in blood pressure. In a third experiment (377) in which 70 pmol of Hoe-140 were injected per hour for 2 wk there was no significant change in blood pressure. The dose of 70 pmol/h was considered sufficient inasmuch as this amount was able to suppress the cardiovascular effects of an intracerebroventricular injection of 380 pmol bradykinin. The finding that in SHR that were given the highest dose of bradykinin antagonist (0.1–10 μg in 3 min) into the cerebroventricles the blood pressure rise suggests that in the SHR brain bradykinin is acting as a compensatory depressor, but it does not locate this effect to the hypothalamus. There is another study that supports this conclusion, but unfortunately it has only been performed in the SHR. Wang et al. (657) delivered, by intracerebroventricular injection into the SHR, either plasma DNA vector containing the human tissue kallikrein expression unit or adenovirus containing human tissue kallikrein gene. Each induced a fall in blood pressure that began 1 day after the injection and lasted for more than 7 days. Cellular localization was observed in the thalamus, hypothalamus, and the third ventricle.

E. Summary

Kinins that are generated from kallikrein by kininogenase contain bradykinin, which is destroyed by kininases including the angiotensin converting enzyme (kininase II). Most of the effects of bradykinin are mediated by B_{2} receptors, synthetic antagonists of which include Hoe-140. Immunoreactive kallikrein, kininogen, and bradykinin are found throughout the hypothalamus, particularly in the periventricular and dorsomedial nucleus, and to a lesser extent in the paraventricular, ventromedial, and arcuate nuclei.

The effect of hypothalamic bradykinin on the blood pressure appears to be both depressor, possibly mediated by an increased release of catecholamines, and pressor by suppressing the action of angiotensin II in the paraventricular nucleus. The pressor effect appears to be mediated by prostaglandins. The depressor effect is exposed by genetic studies that either interfere with B_{2} receptors or prevent the formation of bradykinin; both cause the blood pressure to rise. The pressor effect is brought out by the experimental introduction of what are probably relatively large amounts of bradykinin intracerebroventricularly.

In the adult SHR, the raised cerebrospinal concentrations of kinins, kallikrein, and kininase and the increased hypothalamic content of bradykinin suggest that there may be an increased release of bradykinin, possibly from the hypothalamus. The hypertensive effect of B_{2} receptor antagonist and the hypotensive effect of the intracerebroventricular injection of plasma DNA vector containing human tissue kallikrein expression unit or adenovirus containing human tissue kallikrein gene indicate that in the SHR hypothalamic bradykinin may have a compensatory depressor role for it is likely that the effects on the blood pressure take place predominantly in the hypothalamus which, in the normal rat, is the site from which most of the bradykinin is secreted.
XVI. THYROTROPIN-RELEASING HORMONE

Thyrotropin-releasing hormone (TRH) is widely distributed throughout the central nervous system where, apart from its endocrine effects, it is involved in the control of a wide variety of other physiological functions. The G protein complex of the TRH receptor affects phosphoinositide-specific phospholipase that increases intracellular cytoplasmic free calcium.

A. Distribution of TRH in the Hypothalamus

Although the thalamus and cerebellum contain greater overall quantities, the highest concentration of TRH in the rat brain, as determined by radioimmunoassay, is found in the hypothalamus (433). Within the hypothalamus TRH is present in all the 17 nuclei in which it has been measured (66). The greatest concentrations are in the median eminence (38 ng/mg) and the medial part of the ventromedial nucleus (9 ng/mg), while the periventricular arcuate, paraventricular, the lateral part of the ventromedial and the dorsomedial nuclei, contain between 3 and 4 ng/mg. The medial preoptic nucleus also contains a small amount (1.95 ng/mg).

B. TRH Interaction with Cholinergic, Angiotensin, 5-HT, and Opiate Mechanisms

There is evidence that TRH interacts with muscarinic mechanisms. The highest concentrations of choline acetyltransferase occur in those areas that contain the highest concentration of TRH and TRH increases the pressor response to intracerebroventricular acetylcholine by increasing the density of muscarinic receptors (192, 472). Conversely, atropine given subcutaneously for 2 wk increases the TRH content of the preoptic and septal areas and of the CSF while it reduces the content of TRH in the hypothalamus. Perfusion of slices of the preoptic area, in the presence of pilocarpine, a muscarinic agonist, reduces the increase in heart rate. The pressor response of a central infusion of TRH is inhibited by electrolytic lesions of the dorsal raphe nucleus (395).

The injection of a plasmid vector encoding pre-TRH into the third ventricle of normal rats induces an overproduction of pre-TRH and TRH in the neurons around the third ventricle (particularly in the paraventricular nucleus) and increases the blood pressure (193). The increase in hypothalamic content of TRH and the rise in blood pressure are dependent on the dose of plasmid vector that is injected. To confirm the conclusion that the rise in arterial pressure is due to the rise in hypothalamic content of TRH an antisense oligodeoxynucleotide was injected centrally together with the plasmid vector encoding pre-TRH. The increase in hypothalamic content of TRH and the rise in blood pressure did not take place.

C. Hypothalamic TRH and Normal Blood Pressure

Bolus intracerebroventricular injections of 3 ng to 60 μg of TRH given to anesthetized rats and of 125–4,000 ng to conscious goats cause dose-dependent rises in blood pressure (161, 191, 315, 628). In the conscious rat the chronic intracerebroventricular infusion of TRH for 7 days causes a transient rise in blood pressure (675). Microinjections of 1.4 pM to 80 μM TRH into the medial preoptic, suprachiasmatic, and posterior hypothalamic nuclei raise the blood pressure and heart rate (145, 176). The rise in pressure appears to be due to an increase in sympathetic nervous activity in that it does not occur in adrenalectomized rats pretreated with bretylium (176). TRH injections into the anterior and dorsomedial nuclei do not raise the blood pressure, although they do increase heart rate (145). Mattila and Bunag (395) observed that intracerebroventricularly infused TRH increases blood pressure, heart rate, and splanchnic sympathetic nerve activity and that these effects are not suppressed by bilateral electrolytic lesions of the medial preoptic, paraventricular, and posterior nuclei (395). Neither is the rise in blood pressure and sympathetic nervous activity induced by a central infusion of TRH, influenced by destruction of catecholaminergic or serotonergic neurons with the central administration, respectively, of 6-hydroxydopamine and 5,7-dihydroxytryptamine, although the latter reduces the increase in heart rate. The pressor response of a central infusion of TRH is inhibited by electrolytic lesions of the dorsal raphe nucleus (395).

The injection of a plasmid vector encoding pre-TRH into the third ventricle of normal rats induces an overproduction of pre-TRH and TRH in the neurons around the third ventricle (particularly in the paraventricular nucleus) and increases the blood pressure (193). The increase in hypothalamic content of TRH and the rise in blood pressure are dependent on the dose of plasmid vector that is injected. To confirm the conclusion that the rise in arterial pressure is due to the rise in hypothalamic content of TRH an antisense oligodeoxynucleotide was injected centrally together with the plasmid vector encoding pre-TRH. The increase in hypothalamic content of TRH and the rise in blood pressure did not take place.

D. Hypothalamic TRH and Hypertension

The content of TRH in the SHR preoptic area defined as “including the AV3V region” is almost twice that in the WKY with a threefold increase in TRH precursor mRNA (191). These changes are accompanied by an increase in TRH concentration in the CSF. The rise in preoptic TRH does not appear to be due to the rise in arterial pressure in that lowering the pressure with diltiazem failed to reduce the content of TRH (191). There is also an increase...
in the density of TRH receptors without a change in affinity in the whole hypothalamus and selectively in the preoptic area (36, 191). Although the blood pressure does not rise until the sixth week of age, the rise in receptor density is present at 3 and 4 wk (36). The intracerebroventricular infusion of a polyclonal antibody raised against TRH lowers the blood pressure of the SHR (191, 426). The intracerebroventricular injection of antisense inhibitors of TRH into SHR diminishes the raised hypothalamic content of angiotensin II and lowers the blood pressure; it does not affect the WKY (190a).

E. Summary

TRH is a tripeptide that has a multiplicity of functions including some control of the blood pressure. The highest concentrations of TRH in the brain are in the hypothalamus where it is widely present and in particularly high concentrations in the median eminence and the ventromedial nucleus. Its action in the hypothalamus is closely connected to hypothalamic cholinergic and angiotensinogen activity.

Acute and prolonged injections of TRH intracerebroventricularly raise the blood pressure and increase sympathetic nervous activity as do microinjections into the preoptic, suprachiasmatic, and posterior hypothalamic nuclei. The blood pressure also rises after the injection of plasmid vector encoding pre-TRH into the third ventricle, which increases the production of TRH in the neurons of the nuclei surrounding the ventricle. In the SHR the evidence suggests that hypothalamic TRH has a pressor role. There is an increased content of TRH in the anterior part of the hypothalamus with an increase in TRH precursor mRNA and an increase in TRH receptors throughout the hypothalamus. The central administration of antisense inhibitors of TRH reduces the blood pressure of the SHR but has no effect on the WKY.

XVII. VASOACTIVE INTESTINAL POLYPEPTIDE

Vasoactive intestinal polypeptide (VIP), a potent peripheral vasodilator, is a 28-amino acid residue peptide that appears to have a role in synaptic function either as a neuromodulator or a neurotransmitter. It is present in high concentrations in the brain within VIP containing neurons (352, 366) found in the stria terminalis, the lateral septum, and the suprachiasmatic nucleus which has the highest concentration of neurons producing VIP mRNA in the brain (14). The highest concentration of cells producing VIP mRNA in the suprachiasmatic nucleus (14, 212) have close synaptic connections with many other hypothalamic nuclei including, particularly, the paraventricular nucleus (14, 366).

A. Hypothalamic VIP and Normal Blood Pressure

The intracerebroventricular injection of VIP produces a dose-dependent rise in blood pressure in the conscious normal rat (90).

B. Hypothalamic VIP and Hypertension

In situ VIP hybridization histochemistry and RNA blot hybridization in rats of unstated age show an overall increase of VIP mRNA in the SHR brain which, in the suprachiasmatic nucleus, is statistically significant and correlates well with the blood pressure (14, 453).

C. Summary

The evidence is limited. The pressor effect of VIP when injected into the cerebral ventricles and the increased expression of VIP in the SHR suprachiasmatic nucleus suggest that hypothalamic VIP may have a pressor role in the SHR.

XVIII. TACHYKININS

Tachykinins are a family of small excitatory neurotransmitter peptides. It includes substance P, neurokinin A (NRA), neurokinin B (NRB), and neurokinin K, which bind onto three G protein-coupled receptors NK1, NK2, and NK3. Substance P binds principally onto NK1, NRA onto NK2, and NRB onto NK3 (121).

The distribution of immunoreactive-like substance P and neurokinin B in the hypothalamus is sparse and mainly in the supraoptic nucleus (413). Injection into conscious and anesthetized rats of substance P into the lateral ventricle (626, 639), the paraventricular nucleus, the ventromedial nucleus, the lateral hypothalamus, and the perifornical area raise the blood pressure (329). The rise in pressure appears to involve acetylcholine and oxytocin for it is substantially reduced by a prior intracerebroventricular injection of hemicholinium-3 (626) and by the prior administration for oxytocin antisense oligodeoxynucleotide (379). The rise in arterial pressure induced by the intracerebroventricular injection of substance P and NRA involves an increase in sympathetic nervous activity in that it is blocked by either pentolinium or phentolamine (602). Opiates also have a suppressive effect on substance P release. The rise in arterial pressure induced by a NRB analog is accompanied by a rise in plasma AVP. It is partially blocked by pentolinium and inhibited by a AVP 1 receptor agonist (421, 602).
There is some evidence that the release of AVP induced by neurokinin B is mediated by angiotensin II (387). Conversely, there is evidence from in vitro studies of the hypothalamus that angiotensin II stimulates the efflux of substance P (147).

A. Hypothalamic Substance P and Neurokinin B and Hypertension

In the SHR there is an increase in substance P and NRB-like immunoreactivity in the hypothalamus and supraoptic nucleus (102, 413). In addition, the rise in blood pressure that is provoked by an intracerebroventricular injection of substance P in the SHR is two to three times greater than in the WKY (639). There is no significant difference in substance P content between the hypothalamus of the 2K1C renal hypertensive rat and that of control rats (101).

B. Summary

The small amount of substance P and NRB in the hypothalamus is mainly in the supraoptic nucleus. When introduced into lateral ventricles and some hypothalamic nuclei including the paraventricular nucleus, there is an increase in blood pressure and sympathetic nervous activity. The increased content of substance P and neurokinin B in the supraoptic nucleus of the SHR and the exaggerated rise in blood pressure that occurs in the SHR following the intracerebroventricular injection of substance P suggests that in the SHR tachykinins might have a pressor role.

XIX. HISTAMINE

Histamine is a basic amine 2-(4-imidazolyl-ethylamine) formed from histidine by histidine decarboxylase and metabolized by histamine-N-methyltransferase (497). It is present in much smaller amounts in the brain than in other tissues. A great deal of the brain content of histamine is due to mast cells rather than neurons, but vesicular storage and calcium-dependent release of histamine have been demonstrated in neurons, and the concentration of histidine decarboxylase is considerably greater in neurons than in mast cells, which makes it a better marker than histamine. There are three histamine receptors: H₁ receptors are linked to transduction systems, which increase intracellular calcium; H₂ receptors involve activation of adenylyl cyclase and an increase in cAMP; and H₃ receptors, which are associated, mainly if not entirely, with neural tissues, inhibit the release of a variety of neurotransmitters. Applied iontophoretically to neurons, histamine induces excitatory and inhibitory effects. Usually the excitatory effects are inhibited by H₁ receptor antagonists and inhibitory effects are inhibited by H₂ receptor antagonists (4, 507).

A. Distribution of Histamine in the Hypothalamus and Interaction With Other Substances

Histamine-containing neurons are situated mainly in the floor of the third ventricle in the tuberomammillary nucleus of the posterior hypothalamus, the median eminence, and the arcuate nucleus (4). Electrical stimulation of the tuberomammillary nucleus of conscious rats releases histamine in the supraoptic nucleus and raises the blood pressure and plasma norepinephrine, but there is no change in plasma AVP (4). Histamine injected directly into the third ventricle of the anesthetized dog increases plasma ACTH (519). Further experiments have established that central activation of H₁ receptors increases ACTH secretion and central activation of H₂ receptors decreases ACTH secretion. Histamine administered into the cerebral ventricles also releases AVP (507). There are no consistent effects on growth hormone or prolactin (519).

B. Hypothalamic Histamine and Normal Blood Pressure

The administration of histamine or an inhibitor of histamine-N-methyltransferase intracerebroventricularly raises the blood pressure (313, 624), an effect which is curtailed by intravenous hexamethonium or spinal cord section. The pressor effect is achieved by stimulation of either H₁ or H₂ receptors (246). There is also a rise in arterial pressure when histamine is perfused into the “paraventricular nuclear-anterior-hypothalamic” area by micropipette (25). The changes responsible for this rise appear to be that histamine first stimulates the local release of norepinephrine which stimulates α₁-receptors to release AVP (25). The intravenous administration of a V₁-AVP receptor blocker prevents the rise in arterial pressure (25). In contrast, therefore, to the rise in arterial pressure when histamine is given into the cerebroventricles, the pressor response evoked by histamine introduced into the paraventricular-anterior hypothalamic area does not appear to involve the sympathetic nervous system.

C. Hypothalamic Histamine and Hypertension

Histamine concentration in the anterior and posterior hypothalamus of the 9- and 18-wk-old SHR is greater than in the WKY, and there is a slight but significant decrease in histidine decarboxylase activity in the ante-
rior hypothalamus of the adult SHR (631, 478). Correa and Saavedra (119) reported that there is a large increase in histamine concentration in the median eminence of the young SHR and a general increase in histamine concentration in the hypothalamus of the adult SHR that is highly significant in the median eminence and also significant in the arcuate and ventral premamillary nuclei. It was pointed out that as the median eminence contains a good fraction of the mast cells found in the brain, the increase in histamine in this area could be due to an increase in mast cells.

The prolonged administration of an irreversible inhibitor (α-fluoromethylhistidine) of histidine decarboxylase to 3- and 7-wk-old SHR for 19 and 13 days does not influence the blood pressure of the 7-wk-old SHR. In the 3-wk-old SHR it induces a transient delay in the development of hypertension followed by a fitful tendency for the blood pressure to rise (477, 480).

D. Summary

There are only small amounts of histamine in the brain, most of it in mast cells. Neuronal histamine is found in the posterior hypothalamus, the median eminence, and the arcuate nucleus. Raising the concentration of histamine in the hypothalamus of the normal rat increases the blood pressure and sympathetic nervous activity by increasing the local release of norepinephrine, which stimulates the release of AVP. The rise in pressure can be prevented by the intravenous administration of a V1-AVP blocker.

The content of histamine in the SHR hypothalamus, particularly in the median eminence and the anterior and posterior hypothalamic nuclei, is raised, perhaps due to diminished destruction. But the intracerebroventricular administration of an inhibitor of the enzyme (histidine decarboxylase) which metabolizes histamine has no effect on the blood pressure of the SHR. It is probable that hypothalamic histamine plays no role in the SHR’s hypertension.

XX. CORTICOTROPIN-RELEASING FACTOR

Corticotropin-releasing factor (CRF) is found in most of the major brain areas (239). The highest concentrations are in the hypothalamus, principally in the median eminence and the para- and periventricular nuclei (543). The CRF receptor activates an adjacent intracellular G protein.

In vitro incubation of rat hypothalamus reveals that norepinephrine, acetylcholine, 5-HT, and NPY stimulate secretion of CRF, whereas GABA, substance P, ANP, opiates, and precursors of NO inhibit its secretion (220).

Drinking 2% saline elevates the level of CRF mRNA in the magnocellular neurons in the paraventricular nucleus and the supraoptic nucleus but reduces the level of CRF mRNA in the parvocellular neurons in the paraventricular nucleus (543). The effect of isosmotic saline does not appear to have been studied. CRF has been conclusively documented to be the major regulator of the mammalian stress response (241).

A. CRF and Blood Pressure

There are three studies on CRF and blood pressure (65, 239, 240). In one (65) the administration of CRF intracerebroventricularly in the normal rat was shown to induce a significant rise in arterial pressure and plasma norepinephrine together with an increase in renal sympathetic nerve activity (64). The rise in arterial pressure was due to an increased cardiac output (64). When introduced in the same manner into the SHR, CRF produces a greater rise in plasma epinephrine and glucose than in the WKY. There is no difference in CRF receptor numbers or binding affinity between the brains of the SHR and WKY (65).

In the 3- and 6-wk-old SHR, CRF is present in lower amounts in the hypothalamus, but at 12 wk the amount in the hypothalamus of the SHR is similar to that of the WKY. Hattori et al. (239) pointed out that the following evidence suggests that the decrease in CRF concentration in the SHR hypothalamus may be due to increased secretion. In the SHR the ACTH response to exogenous CRF is blunted; basal plasma corticosterone is higher in the young SHR than in the WKY, while circulating plasma levels of ACTH are similar yet pharmacological inhibition of hypothalamic CRF secretion in the SHR results in a significant decrease in plasma ACTH. Furthermore, a morphological study has demonstrated that the total number of ACTH immunoreactive cells in the anterior pituitary in the 4-wk-old SHR is greater than in the WKY (240).

B. Summary

In the hypothalamus of the normal rat, CRF is found principally in the median eminence and the para- and periventricular nuclei. The intracerebroventricular administration of CRF in the normal rat increases the blood pressure and renal sympathetic nervous activity. In the SHR hypothalamus the concentration of CRF is reduced, and there is no detectable abnormality of the CRF receptors. The limited evidence available suggests that in the young SHR CRF might possibly have a depressor effect.

XXI. COMPENSATORY EFFECTS ON HYPOTHALAMIC FUNCTION INDUCED BY A CHANGE IN BLOOD PRESSURE

Some of the functional changes described above are pressor and others appear to be compensatory depressor
responses. The distinction between the two can often be made by extrapolation from the known effects on the blood pressure of the normal rat of applying the particular substance under study into various sites in the hypothalamus or by measuring the effect on the hypertension of introducing some agent, which blocks the action of the substance. The effect on hypothalamic function in the normal rat of acutely induced changes in blood pressure also helps distinguish which of the hypothalamic functional changes found in hypertensive animals are likely to be pressor or depressor.

With the use of a push-pull cannula to microperfusion and measure the release of endogenous catecholamines in the superfusate from hypothalamic nuclei in the anesthetized and conscious rabbit, cat, and rat, it has been shown that an induced fall in blood pressure with nitroprusside or bleeding causes an increased release of noradrenaline, epinephrine, and dopamine from the posterior hypothalamus (458, 460, 461, 567) and from the paraventricular nucleus of the WKY and the SHR (479, 653). These are pressor responses. It is to be noted that acute induced changes in blood pressure elicit reciprocal changes in locus coeruleus neuronal firing (434) and that the activity of the posterior hypothalamus is closely influenced by that of the locus coeruleus (462). In some, but not all experiments, a fall in pressure also reduces the release of catecholamines from the anterior hypothalamus, a pressor response. The increase in dopamine release from the posterior hypothalamus is immediate, occurring before the blood pressure fall, whereas the increase in the rate of release of norepinephrine and epinephrine is gradual. Transection of the brain caudal to the hypothalamus in these experiments has shown that both the basal spontaneous release from the posterior hypothalamus and the increased release during the fall in arterial pressure are mainly due to impulses originating in the pons and medulla, particularly the locus coeruleus (458).

Conversely, microperfusion of hypothalamic nuclei has shown that a rise in blood pressure elicited by stimulation of the splanchnic nerve or a bolus injection of phenylephrine or tramazoline increases the rate of norepinephrine release from the anterior hypothalamus of the cat (a depressor response) in both the WKY and SHR (462, 450) and decreases the release of norepinephrine from the paraventricular nucleus of both the WKY and SHR (653) (also a depressor response). Raising the blood pressure of a normal rat with intravenous angiotensin II induces an increase in hypothalamic NOS mRNA (696), a depressor response. Lowering the raised blood pressure of an 11- to 12-wk-old SHR with an angiotensin-converting enzyme inhibitor lowers the increase in hypothalamic NOS activity that is present in such rats at that time (486), a pressor response.

In the anesthetized rat, an acute rise in blood pressure with an intravenous injection of phenylephrine decreases neuronal firing in the central part of the anterior hypothalamic nucleus (presumably a diminution of activity of pressor units), whereas there is an increase in neuronal firing throughout this nucleus (presumably an increase in activity of depressor units).

**XXII. DISCUSSION**

In the SHR, the Dahl salt-sensitive rat, the reduced renal mass rat, and the one-kidney one-clip and DOCA plus salt forms of hypertension, there are extensive functional hypothalamic abnormalities. These include increases in neuronal firing, changes in the secretion of many substances and of enzyme activities, and changes in receptor numbers and gene expression. There is an increase in gene expression of nine of the substances discussed and a decrease in activity of a few substances such as oxytocin, NOS, GABA, and hexokinase activity (326). Despite these widespread disturbances, the activity of certain hypothalamic substances does not appear to change, e.g., the calcitonin gene (351), cholecystokinin (559), and gastrin releasing peptide (453).

Studies on the dispersal of an injection of tracer \[^{14}C\]inulin into the lateral ventricles show that at 5 min the tracer is distributed throughout the ventricle and subarachnoid spaces (downstream of the injection) including the cisterna magna. Penetration of the brain from these sites varies, with preferential entry into the hypothalamus and brain stem. By 4 h, the whole brain is labeled (482). Thus a change in blood pressure obtained with an intracerebroventricular injection does not localize the effect to the hypothalamus unless some maneuver has been carried out to prevent the substance injected traveling down the aqueduct of Sylvius. In all of the substances discussed, however, there is some evidence that their endogenous presence within the hypothalamus influences the blood pressure.

The evidence suggests that the type and direction of most of the hypothalamic changes found in the hypertensive animals are pressor and that they are responsible for the rise in arterial pressure. A few changes do not appear to affect the blood pressure (e.g., histamine and oxytocin) or are probably depressor, effectively compensatory adjustments, such as certain changes in catecholamine, NOS, and bradykinin activity at certain times in certain nuclei. Increases in hypothalamic activity are usually pressor, although the decreases in GABA and NOS activity that occur are also pressor. It is possible, moreover, that the pressor effect of these two changes are directly interrelated, for NO increases the release of GABA and thus GABA mediated inhibitory synaptic activity. Therefore, the pressor effect of a diminution of NOS activity may be due to the resultant diminution in GABA inhibitory activity.
The normal functional interdependence of many of the hypothalamic neuronal substances involved is striking and probably accounts, in part, for the multiplicity of the changes that occur in hypertension. The functional interlocking of several of the hypothalamic substances with AVP and with catecholamines is particularly prominent. There are many other examples including the response of the hypothalamus to acetylcholine, which is dependent on the presence of catecholamines but which then feedback negatively to reduce its release. ANPs inhibit the release of catecholamines, whereas catecholamines stimulate the release of natriuretic peptides. Conversely, angiotensin II increases the release of catecholamines that inhibit its release. NPY inhibits the release of norepinephrine from the paraventricular nucleus. Ouabain inhibits monoamine oxidase and increases the release of catecholamines. Other cross-reactions include the increase in muscarinic receptors by TRH, the increase in TRH induced by a diminution of cholinergic activity, and the increase in angiotensin II by bradykinin.

The multiplicity of the hypothalamic changes may also be due, in part, to changes in activity of certain substances that have a wide distribution and/or affect the activity of an extensive range of reactions. For instance though, catecholamines, 5-HT, and histamine are released by relatively few neurons; the arborization of these cells is extensive and diffuse. NO's remarkable pervasiveness and capacity to inhibit a wide range of enzymes, except guanylate cyclase, is another such substance. CO is another, but although the localization of cGMP in the hypothalamus is much closer to heme oxygenase than to NOS, and in neuron cultures CO can regulate the level of cGMP (578), CO in the hypothalamus of hypertensive animals, does not appear to have been investigated. In addition, it is also evident that the changes in neurotransmitter and neuromodulator activity also stem from underlying changes in certain intermediary substances such as interleukin-1β in NOS and catecholamine activity and prosta-glandins in bradykinin activity.

The changes in hypothalamic activity that occur in hypertension are widespread throughout the hypothalamus but are more prominent in the more rostral part of the hypothalamus and in the contiguous forebrain; the principal exceptions are the changes in cholinergic and GABA activity that take place principally in the posterior hypothalamus. Experiments in normal animals have revealed that both the particular site in the hypothalamus where a functional change occurs and the direction of the change determine the direction of the alteration in arterial pressure. Accordingly, in a hypertensive animal, an increase in activity of a substance in one site may be pressor while the same increase occurring simultaneously in another site is depressor. In the SHR, for instance, there is the undoubted pressor effect of an increased norepinephrine activity in the paraventricular nucleus and the probable depressor effect of the same increase on the anterior hypothalamic area.

The effect of an anesthetic is critical. Certain hypothalamic changes in an anesthetized rat can be the opposite to those found in the conscious animal (171). The age of the rat at the time of observation is also of paramount importance, particularly the period when the blood pressure is rising. Some of the substances have only been studied after this crucial time. A few changes can be detected before the rise in pressure, and others may only appear after the hypertension is established.

A certain hypothalamic functional abnormality in a particular nucleus may alter with time, as may its direction, or the change may subside in one nucleus to reappear in another. For instance, the pressor decrease in NOS activity in the hypothalamus of the 2K1C hypertensive rat at 3 wk, when the blood pressure is rising, changes to a depressor compensatory increase at 6 wk when the hypertension is established. This may be relevant to the unexpected finding in the SHR in which the only direct observation on hypothalamic NOS activity is a depressor increase at 11–12 wk. There is a need to find out whether, in the young SHR, when the blood pressure is rising, there is, in line with the early stages of some other forms of hypertension, a decrease in hypothalamic NOS activity.

Overall, the evidence suggests an evolving pattern of changes. Early pressor increases in catecholamine, 5-HT activity, and NPY activity become less pronounced, and as sustained pressor increases in cholinergic activity, and diminution in GABA activity take over, the earlier pressor diminution in NOS activity changes to a depressor increase. The lessening, with age, of the early increase in sympathetic nervous activity makes it likely that overall there is a corresponding decrease in the intensity of the initial pressor hypothalamic changes. It has been pointed out by Julius and Nesbitt (290) that vascular reactivity increases with age, and they have proposed that it is this emerging increase in vascular reactivity that reduces the initial increase in sympathetic nervous activity. Accordingly, a diminution in pressor hypothalamic activity with age in hypertension would be due, at least in part, to an increase in vascular activity. There is also the impairment of the arterial baroreceptor-renal sympathetic reflex in the SHR which only becomes apparent at ~15 wk of age or later (207, 285).

Evidence on hypothalamic activity in hypertension, although wide ranging, is patchy. It is unusual for a change to have been studied in all the four principal forms of hypertension. For instance, some electrolyte parameters in the hypothalamus have been studied on only three occasions, when different parameters were studied in two different forms of hypertension. When a function has been studied in several forms of hypertension, many of the changes are similar, but there are differences that
probably stem, in part, from the way the hypothalamic changes are induced. For instance, in the 5/6 reduced renal mass and one-kidney one-clip forms of hypertension there is the important contribution of the afferent renal nerves (see below). In DOCA plus salt hypertension there is the pressor effect of the circulating DOCA on hypothalamic mineralocorticoid receptors (277). DOCA plus salt hypertension is strikingly different in several other ways; it is the only form of hypertension studied in which there is no increase in catecholamine activity in the paraventricular nucleus, yet the central administration 6-OHD before the DOCA prevents the rise in blood pressure. In the Dahl salt-sensitive rat for instance, there is the greatly increased permeability of the blood-brain barrier with an associated increase in water content of the brain (563) (see sect. xxiiiE).

Many of the pressor hypothalamic substances that are secreted in greater amounts in certain nuclei in hypertensive animals if introduced into the cerebral ventricles or into the relevant hypothalamic nucleus of a normal animal raise the blood pressure. When, however, an opposing agent, such as an antibody, receptor blocker, or an antisense oligodeoxynucleotide to some of these substances such as acetylcholine, angiotensin II, or ANP is introduced centrally into a normal animal, it has no effect on the blood pressure, but when introduced centrally into a hypertensive animal, the arterial pressure falls. These observations suggest that many of the hypothalamic changes that occur in hypertensive animals that raise the blood pressure are blood pressure-setting mechanisms that are only activated when there is a need for a higher blood pressure, which is the position when the kidneys have an impaired ability to excrete sodium. They also seem to be activated by a need to correct acute changes in blood pressure. In other words, it would appear that most of the pressor hypothalamic mechanisms evident in hypertension are either not involved or are of minor importance in setting normal blood pressure [e.g., catecholamines (229) and angiotensin (466)], or their involvement with normal blood pressure is obscured by the plasticity of hypothalamic function. Similarly, the lack of effect on the blood pressure of the WKY of a single intracardiac administration of angiotensin type I receptor antisense in the WKY, whereas it causes a permanent prevention of hypertension in the SHR, has led Wang et al. (659) to suggest that, in the whole animal the “RAS is important in pathophysiological studies rather than in the control of normal blood pressure. . . .”

The proposal that the hypothalamus appears to play only a minor role in setting normal blood pressure, in contrast to its role in hypertension, is in line with the observations that whereas lesions of the paraventricular and suprachiasmatic nuclei prevent the rise in blood pressure in the Dahl salt-sensitive rat given a high-sodium diet, and alternatively lesions of the paraventricular nuclei lower the blood pressure of the figure 8 renal wrap hypertensive rat, these lesions do not significantly alter the blood pressure of the normotensive Dahl salt-resistant rat on a high-sodium diet or of normotensive controls for the figure 8 renal wrap experiments (163, 209, 245).

It is not clear how raising the hypothalamic concentration of only one of the many hypothalamic substances that appear to have a pressor effect in hypertension is sufficient to increase the blood pressure of a normal animal. Or how in a hypertensive animal blocking the pressor effect of only one of the many substances, the activities of which are increased, lowers the blood pressure. Possibly in each case the extent of the experimentally imposed alterations is so gross that it swamps the naturally occurring changes. These findings make it difficult to determine which of the many changes are the most important to the rise in blood pressure. The effect of the individual changes to the hypertension can be assessed roughly by measuring neural activity. For instance, in the posterior hypothalamic nucleus of the adult SHR, there is an uncertain change in norepinephrine release, a pressor increase in cholinergic activity, and a pressor decrease in GABA activity. The overall contribution of these changes, and others also taking place at this site, is probably pressor in that there is an increase in neural activity (558), and it is known that electrical stimulation of this nucleus raises the blood pressure and sympathetic nervous activity (166, 228).

Attempts to gauge the individual contribution of the hypothalamic changes to the rise in arterial pressure are not made easier by the well-documented plasticity of hypothalamic function (22, 41, 442). For instance, in the 2K2C rat, the rise in arterial pressure occurs despite the prior destruction of central catecholaminergic neurons by the central administration of 6-OHD 1 wk before the clips are applied (23). In the figure 8 renal wrap hypertensive rat, electrolyte ablation of the paraventricular nucleus lowers the blood pressure to normal, but the pressure returns to the prelesion value 7 days after the lesion (245).

A. Summary

The widespread pressor changes in the hypothalamus of hypertensive animals appear to be responsible for the increase in sympathetic nervous activity and the rise in blood pressure. These changes evolve and fluctuate and tend to diminish with age. The overall drive of the hypothalamus to raise the blood pressure in a hypertensive animal survives, after an interval, the destruction of some of the areas, such as the paraventricular nucleus, in which several of the pressor functional changes take place. Many of the substances, the functional alterations of which, in the hypothalamus of the hypertensive animal, appear to raise the blood pressure, do not, in the hypo-
thalamus of the normotensive animal, appear to be involved in setting the level of normal blood pressure.

XXIII. COMMENTS ON POSSIBLE INITIATING MECHANISMS FOR THE HYPOTHALAMIC CHANGES ASSOCIATED WITH HYPERTENSION

Remarkably little work has been carried out to investigate the origin, or to integrate the relevance, of the hypothalamic changes in hypertension. The reluctance of those who have studied the hypertensive hypothalamic changes to put forward suggestions has been matched by the reluctance of most investigators into other aspects of hypertension to acknowledge the need to incorporate the hypothalamic changes into an overall view of hypertension. It is probable that the origin of the hypothalamic changes is related in part to afferent stimulation either from renal or more commonly from cardiovascular receptors and sometimes from both. In the normal rat, renal afferent stimulation induces hypothalamic functional changes (82, 85, 92, 109, 110, 129, 298, 697), and in two forms of hypertension [the reduced renal mass (86) and the one kidney one clip hypertensive rat (446)], the blood pressure can be lowered by section of renal afferents. This suggests that in these relatively rare forms of hypertension the rise in pressure is determined, at least in part, by hypothalamic changes induced by renal afferent stimulation. However, this remains to be demonstrated for the hypothalamic changes that accompany renal afferent section in reduced renal mass, and one-kidney one clip hypertension has not been examined. Renal afferents do not appear to be involved in the rise in arterial pressure in the SHR because dorsal root section does not prevent the onset and development of hypertension (278).

It is suggested that the contribution of cardiopulmonary afferents to the hypothalamic changes in hypertension occur in the following manner. In most forms of hypertension, including the SHR, there is an impaired ability to excrete sodium which is responsible for the rise in arterial pressure (122, 674), but it is not known how this renal impairment causes the blood pressure to rise. It is proposed that collectively the hypothalamic changes associated with most forms of hypertension are one link in an integrated compensatory natriuretic response to the kidney’s impaired ability to excrete sodium (Fig. 1). It is suggested that the impaired ability to excrete sodium gives rise to 1) the early transient retention of sodium (674) and 2) the ensuing rise in intrathoracic vascular pressure from which afferent stimuli to the hypothalamus are responsible for the hypothalamic changes described above. These changes, possibly aided by a change in sodium transport or in sodium concentration in the hypothalamus, are responsible for an increase in sympathetic nervous activity that constricts the arteries and raises the blood pressure. The natriuretic effect of the raised blood pressure then helps correct the kidney’s impaired ability to excrete sodium (Fig. 1). This hypothesis is in line with the findings of Guyton et al. (225) and the suggestion by Borst and Borst de Gues that “the arterial pressure has to rise to correct the kidney’s unwillingness to excrete sodium. Thus a seemingly normal sodium output is maintained at the expense of the hypertension” (46).

A. The Kidney’s Impaired Ability to Excrete Sodium and the Hypothalamic Changes

In the reduced renal mass hypertensive rat and DOCA hypertension it is self-evident that the kidney is involved and that there is an impaired ability to excrete sodium. In the SHR and Dahl salt-sensitive rat, this impairment was discovered after first finding, by cross-transplantation experiments, that the hypertension was determined by the kidney. A graft from a normotensive strain rat donated to a young normotensive hypertensive strain rat prevents the rise in arterial pressure, whereas a graft from a normotensive hypertensive strain rat donated to a young normotensive strain rat raises the blood pressure (128, 504). Similarly, the high blood pressure of patients with essential hypertension and terminal nephrosclerosis may become normal when, following bilateral nephrectomy, they are transplanted with a kidney from a normotensive donor (125). It follows that in hereditary forms of hypertension the rise in arterial pressure stems from one or more genetic abnormalities of the kidney and that whatever associated genetic defects may be expressed elsewhere they do not per se raise the blood pressure, for it does not rise in the presence of a normal kidney.

The hypothalamic changes that occur in rat cross-transplantation experiments have not been studied. It is highly likely that the changes which usually take place in the hypothalamus of a “nongrafted” hypertensive strain rat developing hypertension also occur in the hypothalamus of a normotensive rat which develops hypertension following a renal graft from a hypertensive rat, and that conversely, they are not present in the hypothalamus of a young hypertensive strain rat which, after receiving a graft from a normotensive strain rat, does not develop hypertension. If this is true, the hypothalamic changes that occur in nongrafted hypertensive strain rats are secondary to the abnormal kidney (and independent of renal innervation) (218). It also follows that the hypothalamic changes are not due to the expression of multiple genetic abnormalities in the hypothalamus.

The nature of the renal abnormality that results in pressor hypothalamic changes appears to be related to the kidney’s impaired ability to excrete sodium (513, 674). In Liddle’s syndrome in humans, a single genetic abnor-
mality that selectively impairs sodium excretion by increasing the activity of epithelial sodium channels in the distal tubule can raise the blood pressure (568). In the hereditary forms of hypertension in the rat, and in essential hypertension, there are multiple renal abnormalities capable of impairing sodium excretion (674). In the SHR, the hypothalamic changes and the rise in blood pressure occur on a normal sodium intake, but they are exacerbated by an increased intake. In the Dahl salt-sensitive rat, the hypothalamic changes follow the administration of a high-salt diet, and in the DOCA plus salt form of hypertension, the rise in pressure is also dependent on increasing the sodium intake (325).

1. Effect on the hypothalamus of isosmotic changes in volume

If it is accepted that the effect of a normal sodium intake on the hypothalamus of a hypertensive strain rat with an impaired ability to excrete sodium is likely to be equivalent to the effect of a raised isosmotic sodium intake on the hypothalamus of a normal animal, it should be possible to mimic the effect, on the hypothalamus of a hypertensive strain rat, of an impaired ability to excrete sodium by studying the effect, on the hypothalamus of a normal animal, of a prolonged isosmotic increase in sodium intake. There are no such studies. There are relatively acute experiments, usually with hyperosmotic salt solutions by mouth or intravenously, in highly bred WKY and Dahl salt-resistant rats that have made it difficult to distinguish whether the effects produced in the hypothalamus are due to an overall increase in sodium with an increase in volume or to a rise in plasma sodium or osmolality, and whether they have been influenced by the rat’s origin.

There is one study in the Sprague-Dawley rat in which salt intake was increased isosmotically by chang-
ing the drinking water to 1% saline (611) for 2 wk. In the whole hypothalamus there was a significant fall in immunoreactive ANP.

There are a few studies of the effect on the hypothalamus of a normal animal of acute isotonic changes in volume, but their relevance to the effect of a prolonged change is uncertain. A rapid intravenous infusion of isotonic saline 0.6 ml/min up to 1.2–4.2 ml inhibited the activity of 12/20 paraventricular spinal neurons in anesthetized rats, four neurons became excited and four were unaffected (368). Experiments performed on the conscious rat and rabbit have shown that an acute rapid isotonic volume expansion with saline or hemacel increases the presence of immunologically detectable fos in the parvo cellular portion of the paraventricular nucleus, the supraoptic nucleus, and OVLT (15, 496). Central hypervolemia induced by intravenous isotonic modified gelatin, however, increases the number of fos immunoreactive neurons in the supraoptic, paraventricular, arcuate, suprachiasmatic, and median preoptic nuclei (476). Volume expansion with dextran, however, which induced a substantial increase in arterial and right auricular pressure, did not have these effects on the hypothalamus (15).

A rapid acute intravenous injection of 1.5 ml of 0.9% saline/100 g body wt at a speed of 2 ml/min in the anesthetized rat increases the concentration of ANP in the OVLT, the periventricular and medial preoptic nuclei, and the SFO (443). Volume expansion with 2 ml of isotonic saline rapidly injected within 1 min into the right auricle of the conscious rat increases plasma oxytocin and reduces plasma AVP (226), but with hypertonic saline, the increase in plasma oxytocin was accompanied by a rise in plasma AVP (226).

The effect on the hypothalamus of acute isotonic reduction in volume has also been studied. Volume depletion of an anesthetized rat by removal of 1 ml blood/100 g body wt in 1 min decreases the content of ANP in the OVLT, the periventricular and medial preoptic nuclei, and the SFO (443). Isotonic volume contraction by a 2-ml hemorrhage that does not lower the arterial pressure has no effect on hypothalamic fos activity, whereas a bleed of 4 ml that does lower the blood pressure increases fos activity in the OVLT, the supraoptic and the paraventricular nuclei, and the medulla (16). Studies in normal conscious rats have demonstrated that both isotonic hypovolemia induced by an intraperitoneal injection of polyethylene glycol (635) and hypotension induced by a hemorrhage or an intravenous injection of sodium nitroprusside (456) increase NOS activity in the parvo- and magnocellular portions of the paraventricular nucleus and the supraoptic nucleus. The blood pressure changes that occurred in the polyethylene glycol experiments were not given.

2. Summary

In most forms of hypertension, the kidney is responsible for the rise in arterial pressure, and the relevant functional abnormality is an impaired ability to excrete sodium. In these forms of hypertension it is probable that the kidney is responsible for the hypothalamic changes. Nevertheless, there is little evidence that the kidney, particularly an impaired ability to excrete sodium, can alter hypothalamic function. A few experiments have been carried out in normal animals and normotensive strains that show that acute isosmotic changes in sodium balance can alter hypothalamic function, but there is insufficient evidence of the effect of a prolonged change.

B. Venous Compliance and Central Venous Pressures in Hypertension

There is some evidence that needs confirmation and expansion, that in genetically hypertensive rats and renal ischemic forms of hypertension, although there is no rise in blood volume there is a decrease in venous compliance (213, 234, 439, 564, 627, 683), accompanied in the SHR by an increase in left auricular pressure (425). Similar changes occur in essential hypertension in which there is a decrease in venous compliance of both the systemic and pulmonary venous beds (13, 365, 432), with no detectable increase in central blood volume (160, 289, 636). These changes are accompanied by a rise in both right and left (pulmonary wedge) auricular pressures. In the study of London et al. (365) of 49 men with essential hypertension, there is a correlation between systolic and diastolic pressures and the pulmonary wedge pressure (13). These changes were not due to cardiac decompensation because the response to acute volume expansion was either similar to or better than in normal subjects (365, 432).

Venous tone is controlled by the paraventricular nucleus (382) and sympathetic nervous activity (24, 516, 552). Systemic venous tone in Long Evans rats can be increased by electrical stimulation of the paraventricular nucleus (382), and in the Sprague-Dawley rat, it is reduced by an AV3V lesion (24). It is possible therefore that the diminished venous compliance in established hypertension may be related, at least in part, to the increased paraventricular nucleus and sympathetic nervous activity that occurs in hypertension. However, the increase in auricular pressure that accompanies the reduction in venous compliance will stimulate auricular hydrostatic vagal receptors and increase ANP secretion within the walls of the auricle (508), which will stimulate chemical vagal receptors (2, 369). Such stimulation of hydrostatic and chemical vagal receptors can cause changes in hypothalamic function similar to those found in hypertension (see below). There is a possibility, therefore, that a decrease in venous compliance may tend to be self-perpetuating.
1. Summary

In the SHR and renal ischemic forms of hypertension in the rat, and in essential hypertension, there is a decrease in venous compliance and a rise in central venous pressure.

C. Cardiopulmonary Afferents and the Hypothalamus

An acute increase in right auricular pressure in the conscious rat, by means of an implanted, indwelling intracardiac balloon, increases fos deposition in the paraventricular nucleus, the medial preoptic area, and the lateral septum (135). The increase in the number of fos immunoreactive neurons which follows acute central hypervolemia induced by isotonic modified gelatin is unaffected by arterial baroreceptor denervation, indicating that it is primarily a consequence of afferent input from cardiac receptors, apart from any contribution induced by changes in the circulating concentration of ANP, AVP, or angiotensin II (476). This is in line with the inhibitory effects of volume loading on neuronal activity in paraventricular nuclear spinal neurons that are abolished by bilateral vagotomy (368).

There is also the increase in the mural concentration of ANP which accompanies a rise in auricular pressure (508) for this stimulates ANP receptors to stimulate ad-joining vagal afferents (2, 369, 618) to paraventricular neurons with axons that connect either with the medulla or spinal preganglionic sympathetic neurons (369). The effect of ANP on the hypothalamus via atrial vagal receptors varies with its concentration. A rapid intravenous injection of 250 ng ANP stimulates parvocellular spinal neurons, but they are inhibited by an intravenous injection of 3–5 μg (369). Both effects are abolished by bilateral vagotomy. Atrial receptors, which participate in the release of ACTH in response to a small hemorrhage (187) and right atrial stretch (219), send afferents to the medial dorsal hypothalamus via the vagus (219).

AVP secretion is sensitive to volume changes mediated by neural pathways activated by distension of the left auricle. The afferent impulses exert a tonic inhibition so that stretching of the left atrium of a dog or a cat decreases neuronal firing in the paraventricular and supraoptic nuclei (316). Therefore, an increase in volume that increases this inhibition results in a diminution in AVP secretion (656). These effects are abolished by bilateral vagotomy.

The vagus also appears to control the secretion of kinins and norepinephrine from nervous tissue adjacent to the third and fourth ventricle in that ventriculocisternal perfusion during electrical stimulation of the central ends of the cut vagi increases the concentration of kinins and norepinephrine in the perfusate (617).

1. The salt-sensitive Dahl rat, a possible exception

There is increasing evidence that although hypertension in the Dahl salt-sensitive rat is related to an abnormal kidney, the rise in arterial pressure induced by the customary sudden increase in hyperosmotic salt intake can occur independently of a change in volume and therefore probably of a change in central venous pressure (214, 487). Qi et al. (487) have found that on an increased hypertonic salt intake the plasma sodium and blood pressure of the Dahl salt-sensitive rat rises, although the animal’s weight is prevented from rising. In contrast, the Dahl salt-resistant rat on the increased hypertonic sodium intake has no rise in plasma sodium or arterial pressure. A linear regression between the arterial pressure and plasma sodium in the Dahl salt-sensitive rat whether body weight is controlled or not controlled is highly significant.

These observations suggest that the abnormal kidney in the Dahl salt-sensitive rat is probably responsible for the rise in plasma sodium and that it is this rise which induces some of the hypothalamic changes that increase sympathetic nervous activity and the blood pressure. This suggestion is in line with some of the known direct effects of changing CSP sodium concentration on hypothalamic function (see below) and the recent finding that the permeability of the blood-brain barrier of the Dahl salt-sensitive rat given an increase in hypertonic sodium intake is due to the expression of a genetic abnormality in the hypothalamus that is precipitated by a sudden rise in plasma sodium concentration.

2. Summary

Acute changes in vascular volume, central venous pressure, and stretch of the auricles are accompanied by changes in hypothalamic function. This suggests that although there is no direct evidence, cardiopulmonary afferents are probably involved in the hypothalamic changes that occur in some forms of sustained hypertension. There is a need for the development of autonomic telemetric facilities that would enable long-term measurement of neural afferent traffic from intrathoracic receptors in various forms of hypertension, particularly in the SHR. It would also be helpful to study the effect of bilateral section of cardiopulmonary afferents on hypothalamic function and the blood pressure, particularly during the initiation of the hypertension.

The Dahl salt-sensitive rat is an exception in that the rise in arterial pressure induced by a rise in salt intake is related to a rise in plasma sodium and appears to be independent of changes in volume.
D. Sympathetic Nervous Activity in Hypertension

Direct evidence for an increase in sympathetic nervous activity in nearly all forms of hypertension in animals including the spontaneously hypertensive rat, the Dahl salt-sensitive rat, renovascular hypertension, chronic renal failure from reduction of renal mass, and mineralocorticoid hypertension, has been obtained by measurement of electrical activity in sympathetic nerves. An increase in splanchnic and renal nerve activity has been observed in the SHR (286, 287, 373, 428) in which the rise in sympathetic nervous activity can be inferred pharmacologically as early as 5 days of age (575). Subsequently, the rise in renal nerve sympathetic nervous activity in the SHR follows the same course as the rise in arterial pressure increasing rapidly up to 24 wk and slowly thereafter (286). Splanchnic nerve activity is increased in the DOCA plus salt hypertensive rat (604, 605) and in the salt-fed Dahl salt-sensitive rat (73) in which it is evident at 5 wk of age, before the rise in blood pressure. In the Dahl salt-sensitive rat the diminution in hindquarter vascular resistance after denervation is greater than in the Dahl salt-resistant rat (609), suggesting that sympathetic nervous activity to vessels is increased. Similarly in hypertensive rats with experimental coarctation of the aorta, the fall in hindlimb vascular resistance after local nerve section is greater than in control normotensive rats (27). In the Dahl salt-sensitive rat (73), as in the normal dog (610), splanchnic and renal nerve sympathetic nervous activity is directly related to salt intake.

There is also evidence of increased sympathetic nerve activity in human forms of hypertension particularly in the early stages. In patients with essential hypertension below 40 yr of age (164), normotensive progeny of hypertensive parents, and subjects with a family history of hypertension (174), total norepinephrine spillover into plasma is increased, and ~50% of the total increase is due to an increased spillover from the kidneys and heart. By measuring neuronal activity in the posterior tibial or peroneal nerve, an increase in sympathetic nerve activity of muscle has been demonstrated in patients with essential hypertension between the ages of 22 and 75 yr (7, 215, 682), in the middle aged (682) (including one group with accelerated hypertension, Ref. 391), and in another group more than 51 yr old (682). In the first study of neuronal activity in essential hypertension, however, Wallin and Sundlof (655) found no difference in sympathetic nerve activity between 12 severely hypertensive patients, only 2 of whom were below the age of 31 yr, and 33 normotensive subjects with a mean age of 32 yr (655).

Evidence of increased sympathetic nerve activity in the peroneal nerve has also been obtained in humans with hypertension associated with chronic renal failure (87, 112, 357) on maintenance hemodialysis and also following the administration of cyclosporin (536). In patients on hemodialysis, the increase in peroneal nerve sympathetic activity is no longer present after bilateral nephrectomy, which suggests that chronic renal failure is accompanied by reversible sympathetic nerve activity that appears to be mediated by an afferent signal in the failing kidney (112).

1. Summary

In both animal and human forms of hypertension, there is an increase in sympathetic nervous activity that is most prominent in the early stages. That there is such an increase in several models of experimental hypertension, in inherited hypertension in rats, and in essential hypertension “suggest that a common central circuitry exists that may become activated, regardless of the etiological factors of the disease, and results in a chronic elevation of arterial pressure” (108).

E. Other Influences on Hypothalamic Function in Hypertension

It is unlikely that hypothalamic pressor changes are only brought about by afferent stimulation. Changes in hypothalamic sodium transport for certain, and perhaps sodium concentration, may also be involved, particularly in inherited forms of hypertension. There are some interesting observations that there may be some intrinsic although subsidiary genetic abnormalities in the SHR that may have a pressor effect. It would appear, however, that in inherited hypertension the pressor activity of such additional mechanisms are subservient to the state of the kidney because the blood pressure of a young hypertensive strain rat does not rise in the presence of a renal graft from a normal rat.

1. Changes in sodium transport on hypothalamic function and blood pressure

In the Dahl salt-sensitive rat, the uptake into the whole brain of $^{22}$Na given intravenously is up to five times greater than in the Dahl salt-resistant rat (563), revealing that the permeability of the blood-brain barrier of the Dahl salt-sensitive rat is five to eight times greater in the salt-resistant rat. These experiments also revealed that on the 8% NaCl diet, in contrast to the Dahl-resistant rat, the volume of CSF and content of water in the brain of the salt-sensitive rat is significantly greater. There do not appear to be any reports on sodium transport across the blood-brain barrier in the SHR and renal ischemic forms of hypertension.

There are mineralocorticoid receptors in the periventricular area of the third ventricle (133, 491), and a chronic infusion of aldosterone or DOCA intracerebroventricularly raises the blood pressure (202). Stimulation
of mineralocorticoid receptors enhances transcription of mRNAs for proteins that promote ion transport through tight epithelia including the amiloride-sensitive sodium channels. DOCA also upregulates central AVP receptors (596). The central administration of an aldosterone receptor blocker (204) or of benzamil (205), an amiloride analog which inhibits the sodium channel, prevents the rise in blood pressure in both the normal rat given aldosterone (203) and the Dahl salt-sensitive rat. It appears therefore that DOCA, in addition to the central pressor effect of its sodium-retaining property on the kidney, also has a more direct central hypertensive effect and that the Dahl salt-sensitive rat has an increase in hypothalamic sodium channel activity that presumably accounts for the increase in permeability. There is no convincing evidence that there is a significant increase in circulating mineralocorticoid activity in the Dahl salt-sensitive rat, nor are there reports of increased mineralocorticoid activity in the SHR and renal ischemic forms of hypertension.

The contribution to the rise in arterial pressure of the presence of mineralocorticoid receptors in rats given DOCA and the increased blood-brain barrier permeability in the Dahl salt-sensitive rat would appear to be subsidiary, in both instances, to the pressor effect of the kidney’s impaired ability to excrete sodium, for in the DOCA rat the arterial pressure will not rise in the presence of a low salt intake, and in the Dahl salt-sensitive rat on a high salt intake renal cross-transplantation decides which rat develops hypertension.

In summary, in the Dahl salt-sensitive rat, the rise in arterial pressure with the administration of 8% of salt is associated with disturbance of cerebral sodium transport that increases the sodium and water content of the whole brain. Studies limited to the hypothalamus have not been performed. In DOCA plus salt hypertension, it is probable that the change in hypothalamic sodium transport induced by DOCA’s stimulation of hypothalamic mineralocorticoid receptors contribute to the rise in blood pressure.

2. Changes in sodium concentration on hypothalamic function and the blood pressure

There is a possibility that an impaired ability to excrete sodium might raise plasma sodium and thus CSF sodium, but information on the concentration of sodium in the CSF in hypertension is scarce. An increase in the sodium concentration of culture fluid from 142 to 150 mM of neuronal cultures taken from 1-day-old SHR and WKY brains induces a 260% increase in angiotensin II binding in WKY neurons but a 410% increase in SHR neurons, due to an increase in angiotensin II specific receptors (168).

In the Dahl rat, an increase in dietary sodium to 4% was followed by an increase in CSF sodium from 152.3 to 155.2 mM on the third to fourth day (415). The blood pressure, however, had begun to rise on the first day. It was concluded, therefore, that the change in CSF sodium concentration was not a stimulus for the initial rise in pressure. Huang et al. (267) pointed out that the delay might have been due to the way the CSF was collected and the nocturnal feeding habits of rats.

There is one experiment in conscious Wistar rats in which the CSF sodium concentration was raised only from 146 to 152 mM for 2 wk, with a continuous intracerebroventricular infusion at a rate 50 times slower than the lowest previously used which had barely affected the cerebrospinal pressure (267). A small prolonged rise in sodium concentration occurred that was accompanied by a rise in arterial pressure and an increase in hypothalamic ouabain and renal sympathetic nervous activity. These effects were largely prevented by concomitant infusions of antibody fab fragments which bound ouabain (Diggibind). It was also prevented by an intracerebroventricular infusion of an AT1 receptor blocker, losartan, which suggests that the small prolonged physiological rise in CSF sodium stimulated the hypothalamic production of both ouabain and angiotensin II, the concentrations of which rise in most forms of hypertension.

Raising the intracerebroventricular sodium concentration with an acute introduction of hypertonic saline into the cerebral ventricles causes a brisk increase in arterial pressure accompanied by an increase in sympathetic nervous activity (11, 302, 416). Infusion of sucrose and urea do not raise the blood pressure (74). It is unfortunate that many of these experiments were performed with very high concentrations of sodium chloride at infusion rates that are now known to increase the CSF pressure because Nakamura and Cowley (416) have since shown that a rise in CSF pressure may be pressor per se. This is reminiscent of the proposal of Lee et al. (342) that it may be the forceful apposition of the two sides of the third ventricle by paraventricular swelling that triggers the onset of hypertension induced by a high-sodium diet. They had noted that normal rats on a high-sodium diet have an increased concentration of sodium and potassium in the paraventricular tissues (620). To prevent the two sides of the third ventricle from touching in Dahl salt-sensitive rats on a high-sodium diet, and DOCA plus salt hypertensive rats, Lee et al. (342) blocked the aqueduct of Sylvius with inert silicone. Rats with a block sufficient to cause hydrocephalus had a substantially lower rise in arterial pressure (341, 342). The recent discovery of aquaporin-4 mRNA preferentially in numerous periventricular sites may be relevant to the initiation of periventricular swelling (649).

CSF sodium concentration in patients with essential hypertension has been recorded in three studies (211, 303, 304). Kawano et al. (303) found that the CSF sodium concentration of 10 patients with essential hypertension was not different from that of 10 normal controls. In two
other hypertensive groups, 1 of 13 salt-sensitive and the other of 15 undefined hypertensive patients, CSF sodium concentration rose significantly on a high-sodium diet, 2–25 g/day for 7 days (211, 304).

In one extensive study, serum sodium in essential hypertension was found to be significantly greater than in normals (318). The mean serum sodium concentration was significantly higher in the 1,226 hypertensive patients for each decade of age compared with 3,222 controls, but whereas the serum sodium of the controls rose with age, in the hypertensive patients, it was equally high in all age groups. In patients with essential hypertension, the peak of the serum sodium distribution curve was ~2 mmol higher. The prevalence of sodium concentration above 147 mmol was 4.4% in the normals, whereas it was 24–29% in the hypertensive patients.

In summary, blood pressure and hypothalamic function are sensitive to local changes in sodium concentration within the hypothalamus. There is some evidence that hypertension may be associated with a rise in sodium concentration in the plasma, particularly in the Dahl salt-sensitive rat, and in the paraventricular tissues, possibly due to the kidney’s impaired ability to excrete sodium. There is little evidence on the relation of the sodium concentration in the CSF in sustained hypertension.

3. Other intrinsic hypothalamic disturbances in hereditary forms of hypertension

Tissue from the rostral end of the hypothalamus of an SHR embryo transplanted into the hypothalamus of a young normotensive WKY causes a 25% increase in systemic and a selective degeneration of many paraventricular neurons (138, 155, 156). Tissue obtained from the caudal hypothalamus, however, has no effect on the blood pressure. It has also been found that hypothalamic cultures from 1-day-old hypertensive strain rats have some of the functional abnormalities of hypothalamic cells from mature hypertensive rats (168, 493).

XXIV. CONCLUSION

Hypertension in animals is associated with multiple evolving functional abnormalities in the hypothalamus. There are both pressor and compensatory depressor changes. They are responsible for the increase in sympathetic nervous activity that initiates and thereafter diminishingly sustains the rise in arterial pressure. The origin of the hypothalamic changes in hypertension is not known. It is unlikely that they are due to the expression in the hypothalamus of multiple genetic abnormalities. It is suggested that the pressor hypothalamic changes in hypertension are part of a compensatory natriuretic response to the kidney’s impaired ability to excrete sodium. Many of the substances, functional alterations of which in the hypothalamus of hypertensive animals appear to raise the blood pressure, do not, in the hypothalamus of the normotensive animal, appear to be involved in setting the level of normal blood pressure.

I thank the Blood Pressure Research Trust for its support and Joy Lyall and Dr. Robin Woolfson for their generous help. Address for reprint requests and other correspondence: H. E. de Wardener, Dept. of Clinical Chemistry, Imperial College School of Medicine, Charing Cross Campus, St. Dunstan’s Road, London W6 8RP, UK (E-mail: h.dewardener@ic.ac.uk).

REFERENCES


HYPERTENSION AND THE HYPOTHALAMUS


sympathetic nerve activity of the spontaneously hypertensive rat.  


287. JUDY WV, WATANABE AM, MURPHY WR, APRISON BS, AND YU PL. Sympathetic nerve activity and blood pressure in normotensive backcross rats genetically related to the spontaneously hypertensive rat.  


290. JULIUS S AND NESBITT S. Sympathetic overactivity in hypertension.  


291. JULIUS S AND NESBITT S. Sympathetic overactivity in hypertension.  


292. JULIUS S AND NESBITT S. Sympathetic overactivity in hypertension.  


293. JULIUS S AND NESBITT S. Sympathetic overactivity in hypertension.  


294. JULIUS S AND NESBITT S. Sympathetic overactivity in hypertension.  


295. JULIUS S AND NESBITT S. Sympathetic overactivity in hypertension.  


296. JULIUS S AND NESBITT S. Sympathetic overactivity in hypertension.  


370. Lutten PG, Ter Horst GJ, Karst H, and Steffens NA. The course of...


680. Wong M, Samson WK, Dudley CA, and Moss RL. Direct, neuronal 


