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Astrocyte Control of Synaptic Transmission and Neurovascular Coupling

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Haydon, Philip G., and Giorgio Carmignoto. Astrocyte Control of Synaptic Transmission and Neurovascular Coupling. *Physiol Rev* 86: 1009–1031, 2006; doi:10.1152/physrev.00049.2005.—From a structural perspective, the predominant glial cell of the central nervous system, the astrocyte, is positioned to regulate synaptic transmission and neurovascular coupling: the processes of one astrocyte contact tens of thousands of synapses, while other processes of the same cell form endfeet on capillaries and arterioles. The application of subcellular imaging of Ca^{2+} signaling to astrocytes now provides functional data to support this structural notion. Astrocytes express receptors for many neurotransmitters, and their activation leads to oscillations in internal Ca^{2+}. These oscillations induce the accumulation of arachidonic acid and the release of the chemical transmitters glutamate, D-serine, and ATP. Ca^{2+} oscillations in astrocytic endfeet can control cerebral microcirculation through the arachidonic acid metabolites prostaglandin E_2 and epoxyeicosatrienoic acids that induce arteriole dilation, and 20-HETE that induces arteriole constriction. In addition to actions on the vasculature, the release of chemical transmitters from astrocytes regulates neuronal function. Astrocyte-derived glutamate, which preferentially acts on extrasynaptic receptors, can promote neuronal synchrony, enhance neuronal excitability, and modulate synaptic transmission. Astrocyte-derived D-serine, by acting on the glycine-binding site of the *N*-methyl-D-aspartate receptor, can modulate synaptic plasticity. Astrocyte-derived ATP, which is hydrolyzed to adenosine in the extracellular space, has inhibitory actions and mediates synaptic cross-talk underlying heterosynaptic depression. Now that we appreciate this range of actions of astrocytic signaling, some of the immediate challenges are to determine how the astrocyte regulates neuronal integration and how both excitatory (glutamate) and inhibitory signals (adenosine) provided by the same glial cell act in concert to regulate neuronal function.

I. INTRODUCTION

The nervous system consists of two classes of cell, the neuron and glia. Although it is without doubt that neurons are essential for nervous system function, studies over the past decade are raising our awareness about the diversity of roles played by glial cells in nervous system function. In this review we focus on one of the subtypes

of glial cells, the astrocyte, and discuss our current understanding of how these cells operate hand in hand with neurons to regulate integration in the central nervous system. Necessarily, we restrict our focus to the roles of astrocytes served by the release of three transmitters, glutamate, D-serine, and ATP; how these gliotransmitters regulate neuronal function; and how neuronal activity can, through astrocytic signaling cascades, locally regulate vascular tone. We do not attempt to discuss the roles of chemokines released from these glial cells, and instead alert the reader to an excellent recent review on this topic (219).

The discovery that chemical transmitters evoke Ca^{2+} elevations in cultured astrocytes (37) sparked the imagination of a small group of neuroscientists who diverted their attention to the investigation of this class of glial cell. Although these cells play critical roles in supporting neuronal function, astrocytic Ca^{2+} excitability and the consequent induced release of chemical transmitters, which we now term gliotransmitters, has led to an emerging new understanding of the functional roles played by these glial cells; we now appreciate that astrocytes listen and talk to synapses and play roles in synaptic modulation and in mediating synaptic cross-talk (9, 13, 30, 82, 148, 153, 205). In this review we have three goals: to provide a view of nervous system activity from the perspective of the astrocyte, to discuss how as an integrative hub the astrocyte exerts control over cerebrovascular as well as neuronal functions, and to discuss how the astrocyte and gliotransmission play a fundamental role in shaping and dynamically regulating the relative strengths of neighboring synaptic connections.

II. ASTROCYTIC CALCIUM SIGNALING: THE BIOCHEMICAL BASIS OF GLIAL EXCITABILITY

Anyone who has recorded, often by accident, from an astrocyte in a brain slice preparation or in vivo knows that these cells are electrically inexcitable, and offer little to study with electrophysiological approaches. Generally, astrocytes have a high resting K^+ conductance, respond to depolarization with a linear current-voltage relationship, and are coupled by gap junctions. Recent studies have suggested that there may be many types of astrocytes. For example, Steinhauser's group has identified two classes of glial cell with distinct functional properties: one has the linear current-voltage relationship, high resting K^+ conductance, and glutamate transporters expected of astrocytes, while the other expresses voltage-gated conductances together with AMPA receptors (93, 132, 220). We await the results of future studies to determine whether this second class of cell type is a subtype of astrocyte or a distinct glial cell type, and will therefore focus the remainder of our discussion to the more traditional gap junction-coupled astrocyte with a linear current-voltage relationship.

Because of the high resting K^+ conductance and gap junction coupling, the first major function assigned to these cells was in the clearance of extracellular K^+ following elevated periods of neuronal activity (158). Although early studies showed that the application of neurotransmitters can depolarize astrocytes (22, 100), perhaps the most significant discovery that initiated renewed vigor in studies of astrocytes was the discovery that the application of the chemical transmitter glutamate induces Ca^{2+} oscillations and Ca^{2+} waves between cultured hippocampal astrocytes (32, 37, 61).

Glutamate-induced Ca^{2+} oscillations in astrocytes result from the activation of class I metabotropic receptors that induce the phospholipase-dependent accumulation of inositol trisphosphate (IP_3) that stimulates the release of Ca^{2+} from IP_3-sensitive internal stores (99). Consequently, by measuring Ca^{2+} signaling, rather than membrane potential, it was discovered that astrocytes are an excitable system.

Since these initial observations it has been realized that astrocytes express a plethora of metabotropic receptors that can couple to second messenger systems (216, 217). For example, norepinephrine (48, 109), glutamate (48, 165, 174, 175, 190), GABA (96), acetylcholine (11, 190), histamine (190), adenosine (173), and ATP (24, 171, 173) have all been shown to induce Ca^{2+} elevations in glial cells in brain slice preparations. In culture, the list of metabotropic receptors is extensive. However, because culturing astrocytes can lead to the misexpression of proteins, it is not yet clear whether all of these receptors are normally expressed in astrocytes in vivo.

The presence of Ca^{2+} waves that propagate between cultured astrocytes has intrigued several groups who have attempted to identify the mechanism of signal propagation. Although these waves occur in cell culture, emerging evidence suggests that they do not occur under physiological conditions in vivo (85). Nonetheless, understanding the mechanism of wave propagation has provided important insights into signals that can be released from astrocytes. Two prominent hypotheses guided this work: *1*) IP_3 could diffuse through gap junctions to evoke Ca^{2+} signals in neighboring unstimulated astrocytes (87, 115, 183, 193, 214), and *2*) a message, ATP, is released from an astrocyte which, by activating P2Y receptors on adjacent astrocytes, stimulates additional Ca^{2+} signals (38, 51, 72). Although it is likely that both pathways contribute to wave propagation, that the wave can propagate between physically disconnected cells (81) provides compelling evidence for a role for the induced release of ATP signaling to neighboring cells and mediating the propagation of the wave.

Although in cell culture waves of Ca^{2+} elevation are the norm, it is appropriate to ask whether long-range Ca^{2+} waves could provide meaningful information if they were to occur within a nervous system. Several studies have now been performed using brain slice preparations, and these studies indicate that Ca^{2+} signals, under physiological conditions, are less extensive. For example, photorelease of glutamate in hippocampal slices to stimulate individual astrocytes demonstrated that activation of a single cell was able to evoke Ca^{2+} elevations in neighboring astrocytes (200). However, the range of this signal was extremely small compared with those observed in cultures. As will be discussed in section XVII, the extracellular concentration of ATP is tightly regulated by ectonucleotidases. It is likely that the difference in range over which Ca^{2+} signals propagate is in part regulated by the impact of ectonucleotidases that more effectively hydrolyze ATP to adenosine within the confined extracellular space of a brain slice and in vivo (49).

Studies in brain slice preparations have led to the proposal that astrocytes are functionally compartmentalized and Ca^{2+} oscillations are predominantly restricted to local microdomains. Imaging studies performed in hippocampal slices showed that astrocytic Ca^{2+} oscillations occur in portions of a process of individual astrocytes (165). Stimulation of the parallel fibers, which innervate Purkinje cells and Bergmann glia, evoke Ca^{2+} signals restricted to microdomains of the Bergmann glial cell processes (71). Three-dimensional reconstruction of the processes of Bergmann glia shows that microdomains are connected by extremely fine processes, providing a structural basis to support biochemical compartmentalization. Because one hippocampal astrocyte has been calculated to make contact with ~100,000 synapses (29), this local Ca^{2+} signaling provides the opportunity for astrocytes to influence synaptic transmission in response to the glial Ca^{2+} signal while retaining synaptic specificity. The idea of localized Ca^{2+} signaling is supported by in vivo imaging of astrocytes where synchronized Ca^{2+} waves are not generally detected (85).

What is the stimulus for the astrocytic Ca^{2+} signal? Since chemical transmitters can induce Ca^{2+} oscillations in these glial cells, the ability of neuronal activity to stimulate astrocytes was initially tested in studies in brain slice preparations. Trains of activity in the Schaffer collateral pathway of the hippocampus evoke Ca^{2+} signals in area CA1 astrocytes (165, 175). These signals result from synaptically released glutamate acting on subtype 5 of metabotropic glutamate receptors (mGluR5s), as well as a contribution from ATP acting through P2Y receptors (24). More recently, Newman (150) has shown that activation of the retina by light, to stimulate photoreceptors and the associated circuitry, does indeed stimulate Ca^{2+} signals in Müller glial cells. In support of local signaling, Ca^{2+} waves were not detected, but instead Ca^{2+} oscillations were detected in endfeet of these glia. An intriguing observation by McCarthy's group (149) suggests that astrocytes do not rely solely on instructive cues from neurons, but instead can intrinsically oscillate. With the use of a pharmacological cocktail of antagonists and despite blocking activity-dependent synaptic transmission, Ca^{2+} oscillations persisted in hippocampal astrocytes. Thus, although it is clear that neurons can activate astrocytic Ca^{2+} signals, and that this can occur in vivo, it is also possible that astrocytes have intrinsic capabilities of initiating Ca^{2+} signals. However, at this point we are only at the beginning of understanding the regulation of Ca^{2+} signals within astrocytes in vivo. Two-photon imaging in vivo shows that long-range Ca^{2+} signals are not the norm (85). However, we have little idea about how neuronal activity influences the spatial scale of glial Ca^{2+} signals nor how neuronal activity influences frequency encoding of glial Ca^{2+} signals. This is an extremely important area of investigation if we are to develop realistic models of the role of the astrocyte in the control of synaptic transmission, neuronal excitability, as well as the control of the cerebrovasculature.

These studies provide a different picture of Ca^{2+} signaling in the astrocyte than was first described in cultures. Oscillations are restricted to portions of the processes of individual cells, the so-called microdomains. They do not necessarily propagate over large distances, even within one astrocyte. Since, as we will discuss later, such Ca^{2+} signals cause gliotransmitters to be released that have feedback actions on neurons, localized responses to neuronal activity are likely to be of importance in maintaining synaptic specificity, while permitting gliotransmission to modulate neuronal function.

NEURON-TO-ASTROCYTE SIGNALING IN THE CONTROL OF CEREBRAL CIRCULATION

From a purely structural perspective, the astrocyte is situated much like a hub in which it receives inputs from thousands of synapses and at the same time can make contact with the local vasculature (Fig. 1). The combination of this structural relationship together with our newfound appreciation of the presence of dynamic, activity-dependent biochemical signaling between neurons and astrocytes suggests that the astrocyte is an important integrator of neuronal activity and consequently the local control of cerebrovasculature.

The contact of astrocytic endfeet with arterioles and capillaries, that was first described by Golgi at the end 1800s (66), has long been interpreted as an indication that astrocytes can take up nutrients and metabolites from the blood and then distribute them to other brain cells, including neurons (7). Indirect support for such a view derives from a number of more recent studies that de-

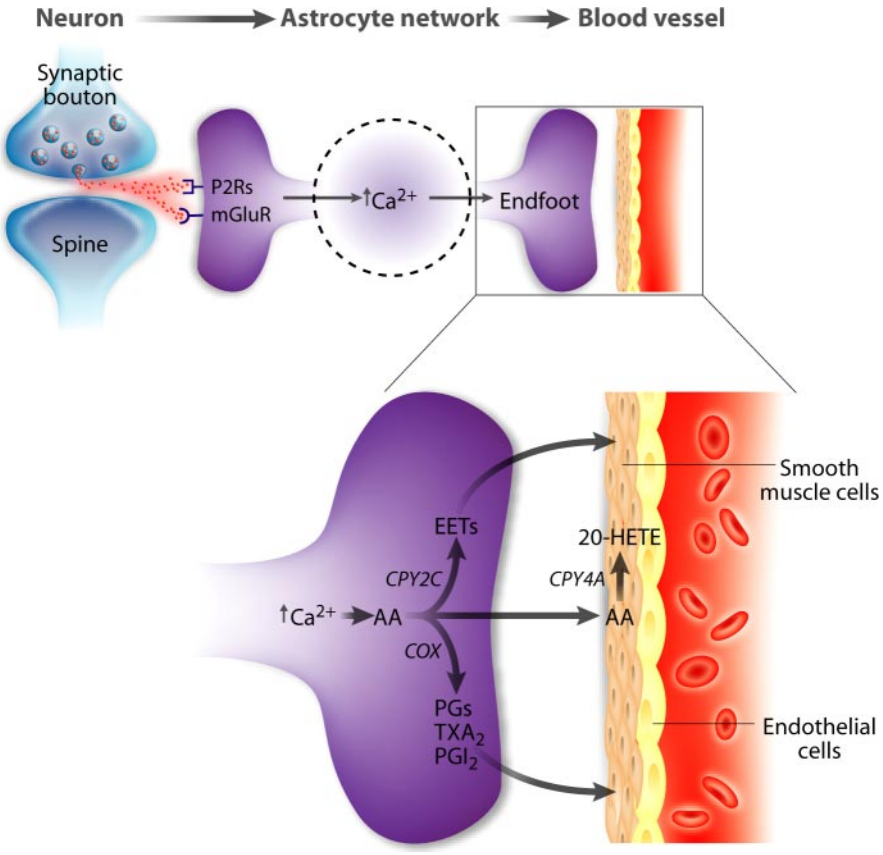

FIG. 1. Neuronal synaptic activity can act through the astrocyte network to regulate the cerebrovasculature. The activity of glutamatergic synapses can regulate astrocytic biochemical signaling through the coactivation of metabotropic glutamate and purinergic receptors to cause a phospholipase C-dependent increase in astrocytic Ca^{2+}, which can propagate to the astrocytic endfoot to exert local actions on the vasculature. Through the activity of Ca^{2+}-sensitive phospholipase A_2, accumulated arachidonic acid can cause vasodilatory and vasoconstrictive actions through at least two of its metabolic pathways. Cyclooxygenase-2 (COX)-dependent accumulation of PGE_2 leads to a vasodilation, while the diffusion of arachidonic acid to the smooth muscle, which contains high levels of CPY4A, leads to the accumulation of 20-HETE that causes vasoconstriction. While these two opposing actions seem in conflict, since both have been seen to occur in vivo, the challenge is to identify the conditions that select for the respective actions.

scribe the structural association of astrocyte processes with both synapses and cerebral vessels (172, 192, 215). Beyond this physical relationship, subsequent studies suggest that synaptic activity regulates astrocytic metabolism, which consequently feeds these active neurons with lactate. According to this view, the transport of glutamate into the astrocyte following synaptic activity leads to the activation of the Na^+-K^+-ATPase to restore the ion gradient that is necessary to drive glutamate transport. Astrocytic ATP is replenished from glucose derived from the vasculature resulting in the accumulation of astrocytic lactate. Monocarboxylate transporters are then believed to shuttle lactate to synaptic terminals as a source of neuronal ATP (170, 208). Though this process can occur, its relative importance in relation to the direct use of glucose as a neuronal energy source is the subject of considerable debate (98, 127).

The increased energy demand of active neurons is also met by local increases in blood flow in the area of elevated neuronal activity. This phenomenon, which was first described by A. Mosso in the late 1800s (142) and later confirmed by Roy and Sherrington (180), is a fundamental event in brain function. Local increases in blood flow result from the rapid dilation of arterioles and capillaries of a restricted area in response to an episode of high neuronal activity. As a consequence, blood flow increases in that region within a few seconds, thereby ensuring that most active neurons receive an adequate supply of oxygen and metabolic substrates for energy consumption. Local accumulation of metabolic products has been initially proposed to directly control blood flow. Although under particular circumstances, such as brain hypoxia or ischemia, this process may indeed affect blood vessels, the time course of the neurovascular coupling argues against this hypothesis (122). Results obtained over the last few years provide conclusive support for the view that blood flow is directly coupled to neuronal activity rather than to local energy needs (16, 184). The present knowledge on the multiple signaling pathways that during activation lead to the production of vasoactive factors suggests that the molecular mechanism at the basis of functional hyperemia is highly complex and may not necessarily be the same in all brain regions. Although various aspects remain to be elucidated, most recent studies highlight a central role of neuron-to-astrocyte signaling in the local control of microcirculation (6, 60, 123, 145, 241, 242).

Because of their polarized anatomical structure and of the vicinity of their endfeet with contractile elements of blood vessels, such as smooth muscle cells in arterioles and pericytes in capillaries, astrocytes have been long proposed to contribute to the regulation of the blood flow during neuronal activity. The ability of astrocytes to remove from the extracellular space around active synapses

potassium ions increasingly concentrated there following high neuronal activity and to redistribute them, through the syncytium, to distal regions, was originally considered a plausible mechanism to couple neuronal activity with dilation of vessels (207). This hypothesis was substantiated in the retina where a high potassium conductance was found in astrocyte endfeet and Müller cell processes in contact with blood vessels (152, 168).

The demonstration that astrocytes produce a plethora of vasoactive substances, such as nitric oxide (NO) (116, 146, 226), cyclooxygenase and epoxygenase activity-derived products (2, 4, 157, 169), and ATP (15, 36, 176), hints at the possibility that the control of microcirculation by astrocytes could not be based simply on the "spatial buffering" of K^+ hypothesis, but rather involves a more complex mechanism and a number of different molecules. Among these, are epoxyeicosatrienoic acids (EETs) that cytochrome *P*-450 epoxygenase forms from arachidonic acid (AA) (179). Support for a distinct role of EETs in neurovascular coupling derives the observations that *1*) by acting on K^+ channels, EETs hyperpolarized smooth muscle cells and trigger dilation of cerebral vessels (5, 65, 88); *2*) pharmacological inhibition of *P*-450 epoxygenase results in reduction of the basal blood flow in the cerebral cortex as measured by laser Doppler flowmetry (2); and *3*) stimulation of astrocytes in culture with glutamate receptor agonists triggers formation of AA that is converted to various AA metabolites including EETs (1, 19). These observations led David Harper to propose a functional role of astrocyte EETs in neuronal activity-dependent regulation of blood flow (74, 75).

IV. NEURONAL ACTIVITY-DEPENDENT CALCIUM ELEVATIONS IN ASTROCYTE ENDFEET

Significant evidence in support of a distinct role of astrocytes in neurovascular coupling was then obtained in a series of experiments performed mainly in brain slice preparations. In hippocampal and cortical slices it was first observed that glutamate released at active synapses triggered Ca^{2+} oscillations in astrocytes that increased in frequency according to increasing levels of neuronal activity (165). These oscillations may represent a digital signal in the control of cell activity as it was originally proposed by Woods et al. (230) and Jacob et al. (94). While this observation demonstrates that astrocytes are sophisticated sensors of neuronal activity (243), it also represents a clue to the possibility that astrocytes transfer to blood vessels information on the level of neuronal activity. Indeed, neuronal activity-dependent Ca^{2+} elevations in astrocytes were observed to propagate to perivascular endfeet (242). Such a signal provides a mechanistic basis for the graded response of the blood flow to different levels of neuronal activity, thereby strengthening the idea of a distinct astrocytic role in neurovascular coupling. Importantly, high-frequency stimulation of neuronal afferents was found to trigger both Ca^{2+} elevations in astrocyte endfeet and dilation of cerebral arterioles. Furthermore, Ca^{2+} elevations triggered in astrocytes by either *t*-ACPD, a mGluR agonist, or direct mechanical stimulation of individual astrocytes by a patch pipette, also evoked dilation of cortical arterioles, while inhibition by mGluR antagonists of Ca^{2+} oscillations evoked in astrocytes by synaptic glutamate, or the incubation with cyclooxygenase (COX) inhibitors that block prostaglandin synthesis, reduced neuronal activity-dependent dilation of cerebral arterioles. Vasodilation appears to be mediated, at least in part, by prostaglandin E_2, since astrocytes in culture were observed to release this powerful dilating agent in a pulsatile manner according to the pattern of *t*-ACPD-mediated Ca^{2+} oscillations (244).

Activation of Ca^{2+} elevations in astrocyte endfeet has been also reported to suppress vasomotion (60), a rhythmic fluctuation in the diameter of cerebral arterioles that accompanies Ca^{2+} oscillations in smooth muscle cells (73, 224). Vasomotion, which is a natural property of cerebral microcirculation in the intact brain, requires some degree of tone that in arterioles from acute brain slice preparations is seriously compromised. Vasomotion could, however, recover upon treatment with agents that induce arteriole constriction (123). Although its precise functional significance and underlying mechanism remain undefined, vasomotion is believed to contribute to microvasculature hemodynamics by enhancing tissue oxygenation especially when perfusion is compromised (209). Interestingly, in arterioles from brain slice preparations, vasomotion and the accompanied Ca^{2+} oscillations in smooth muscle cells are suppressed by stimulation of neuronal afferents (27), suggesting that its inhibition or reduction contributes to neuronal activity-dependent blood flow changes. All together, these observations raise the possibility that the suppression of vasomotion, which accompanies Ca^{2+} elevations in astrocyte endfeet, contributes to the dilating action of astrocytes. In this action of astrocytes, EET release may be involved since blocking EET production with the epoxygenase inhibitor miconazole results in an increase in the frequency of vasomotion (123).

Results from in vivo experiments that used the same mGluR antagonists that in brain slices inhibited astrocyte-mediated vasodilation corroborated the role of astrocytes in functional hyperemia (242). By measuring the blood flow in the somatosensory cortex by laser Doppler flowmetry, the hyperemic response evoked by forepaw stimulation was found to be markedly reduced after the systemic application of mGluR antagonists. The action of the mGluR antagonists was unrelated to unspecific effects on the intensity of neuronal stimulation since the evoked

somatosensory potential was unchanged. This is in agreement with the unchanged amplitude of the Ca^{2+} increase triggered by neuronal afferent stimulation in neurons from brain slices in the presence of the mGluR antagonists.

According to these results, a model is proposed in which astrocytes can encode different levels of neuronal activity into defined Ca^{2+} oscillation frequencies that, at the level of perivascular endfeet, mediate the release of dilating agents, such as EETs (2, 19, 123) and PGE_2 (242, 244) as well as constrictive agents such as 20-HETE (145; see also below). Neuronal activity-dependent Ca^{2+} oscillations may ultimately represent the signaling system that allows blood flow to vary in a manner proportional to the intensity of neuronal activity.

V. PROPAGATING CALCIUM WAVE IN ASTROCYTES MAY CONTRIBUTE TO CONTROL MICROCIRCULATION

During functional hyperemia, the dilation of arterioles in the area of activation will not increase blood flow in that region effectively unless upstream vessels also dilate. How vasodilator and vasoconstrictor responses are conveyed from the initial site of activation to distant locations is unclear. Coordinated vasoactive responses may rely on coupling and communication between cells within the vessel wall. Endothelial cells are indeed extensively coupled (86, 118), and evidence has been also provided for an electronic coupling existing between endothelial and smooth muscle cells (118, 185, 231). Hyperpolarization of an individual smooth muscle cell can thus spread through the endothelial cells to other smooth muscle cells via myoendothelial coupling and evoke a coordinated dilating response along the length of an arteriole (70).

A Ca^{2+} wave propagating between perivascular astrocytes may also be involved. The Ca^{2+} response in an astrocyte in contact with a blood vessel, initially evoked either by neuronal activity (242) or by direct electrical stimulation (192), has been indeed observed to spread to other perivascular astrocytes. Furthermore, connexin43 and purinergic receptors, i.e., the basic elements which mediated the propagation of the Ca^{2+} wave in cultured astrocytes, are highly expressed at astrocyte endfeet (192), and filling single astrocytes that are in the proximity of a blood vessel with Lucifer yellow results in the diffusion of the dye to other astrocyte endfeet. Through the release of vasoactive factors, activation of perivascular astrocytes by the Ca^{2+} wave may affect the tone of upstream and/or downstream blood vessels, thereby regulating the overall conductance of the vascular network in a defined region.

VI. ACTIVATION OF ASTROCYTES CAN ALSO TRIGGER ARTERIOLE CONSTRICTION

In hippocampal slices, Ca^{2+} elevations in astrocyte endfeet triggered by either photolysis of a Ca^{2+} caged compound or *t*-ACPD have been observed to evoke also arteriole constriction (145). Studies in cultured cells show that astrocytes can indeed produce, in addition to various dilating agents, constrictive agents such as the COX products $PGF_{2\alpha}$ (19, 195) and thromboxane A_2 (92, 169), endothelins (126), and 20-hydroxyeicosatetraenoic acid (20-HETE) (154). This latter compound, that derives from ω-hydroxylation of AA by CYP4A, a cytochrome *P*-450 enzyme subtype (179), depolarizes smooth muscle cells by inhibiting the opening of K^+ channels (112), and also enhances Ca^{2+} influx through voltage-dependent Ca^{2+} channels (65). The formation of 20-HETE from AA in smooth muscle cells is proposed to mediate the constrictive action of astrocytes in the hippocampus (145). This action can also account for the constriction of cerebral blood vessels associated with spreading depression and ischemia (45), since Ca^{2+} elevations and Ca^{2+} waves are known to occur in the astrocytes during these pathological brain conditions (17, 110, 113, 114). The release of 20-HETE from astrocytes may, however, have a role also under normal physiological conditions. For example, 20-HETE has been proposed to play a crucial role in the maintenance of myogenic tone in cerebral blood vessels (64). The constrictive action of 20-HETE may also control, together with that of dilating agents, the extent of neuronal activity-dependent increases in blood flow (see also below).

The results reported by Mulligan and MacVicar's study (145) are in conflict with those reported by Zonta et al. (242) in which Ca^{2+} elevations in astrocyte endfeet were observed to trigger dilation of cerebral arterioles. How can these conflicting results be reconciled in a unifying hypothesis? In the latter study, most, although not all, experiments were performed in cortical slices incubated with N^G-nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor that blocks the tonic action of NO on arterioles and thus results in a long-lasting constriction of arterioles. In contrast, this procedure was not applied in most of the experiments described in Mulligan and MacVicar's study in hippocampal slices. Therefore, the different initial state of contraction of cerebral arterioles in the two studies may account, at least in part, for the different results.

The central point here concerns the resting state of pressurized arterioles in vivo. Under normal physiological conditions in the intact brain, cerebral arteries are typically in a state of partial contraction (52). Although the exact mechanisms at the basis of myogenic tone remain uncertain, it is clear that this phenomenon is generated by an interplay of pressure-mediated stretching of the

smooth muscle cell membrane, intraluminal blood flow, and various factors released by neurons, astrocytes, and endothelial cells (43, 44, 84). A change in ion gating in smooth muscle cell membrane, either directly or indirectly via membrane depolarization, results in increased levels of intracellular Ca^{2+} concentration and activation of the contractile process (84). The myogenic tone underlies cerebrovascular autoregulation, i.e., the ability of vessels to respond to changes in transmural pressure with either constriction when pressure increases or dilation when pressure decreases (43, 188, 224). This property has been also proposed to reflect a "regional blood reserve" that various control mechanisms use to produce vasodilation or vasoconstriction (62).

It is the importance of myogenic tone that likely accounts for the discrepancy between the results of these two studies. Accordingly, when the vascular tone is lost, arterioles tend to be in a dilated state, and dilating agents may be ineffective or less effective. Similarly, when smooth muscles are excessively contracted, the inner diameter of arterioles is reduced to such an extent that constrictive agents can hardly induce a further constriction. Therefore, given that in slice preparations the myogenic tone is lost, to detect a dilating effect of vasoactive agents, a certain degree of constriction that mimics the natural occurring myogenic tone of blood vessels in vivo is pharmacologically induced (56, 57, 77, 78, 124, 182). In agreement with this view, constriction of arterioles induced by *t*-ACPD in hippocampal slices changed to dilation when *t*-ACPD was applied after arterioles were preconstricted with L-NAME (145).

All together, these observations hint at the possibility that astrocytes release both dilating and constrictive agents. This ability of astrocytes seems, however, difficult to reconcile with a distinct role of the astrocyte in neurovascular coupling. Indeed, how can a Ca^{2+} signal in astrocyte endfeet lead to diametrically opposite changes in arteriole diameter? The hypothesis can be advanced that the ultimate effect of astrocyte activation may depend on the balance between the action of dilating and constrictive agents, on the one hand, and the resting state of arterioles, on the other. The release of 20-HETE may serve to generate a constrictive action that opposes the powerful action of other dilating agents, released by neurons and/or astrocytes themselves, ultimately modulating the amplitude of the neuronal activity-dependent increase in blood flow.

Certainly, to define the astrocyte's role in the control of cerebral blood flow, first of all, it will be important to provide conclusive evidence for the release from astrocytes of both dilating and constrictive agents. In such a case, are dilating and constrictive factors released simultaneously? If this corelease event does not occur, could it be possible that a dilating, or a constrictive, agent might be preferentially released according to a distinct pattern, or amplitude or compartmentalization, of the Ca^{2+} rise in the astrocyte? An additional interesting issue would be that of clarifying whether the various signaling pathways that rely on diverse enzymes to metabolize AA, i.e., COXs, lipoxygenases, epoxygenases, and ω-hydroxylases, can be differently regulated by modulatory factors. For example, NO that is produced by neurons as well as glia has been reported to downregulate the formation of the cytochrome *P*-450 subtypes CYP2 that produce EETs (121, 211) as well as to inhibit 20-HETE formation (3).

A recent in vivo study provides further support for the distinct role of astrocytes in neurovascular coupling (204). After loading of astrocytes from the somatosensory cortex of adult rats with both the Ca^{2+} indicator rhod 2 and the Ca^{2+} caged compound 1-(4,5-dimethoxy-2-nitrophenyl)-EDTA (DMNP-EDTA), and after labeling of blood vessels with dextran-conjugated fluorescein, a Ca^{2+} elevation evoked in astrocyte endfeet by either photolysis of DMNP-EDTA or stimulation of neuronal activity was followed by a rapid (~1 s delay) and marked dilation of the arteriole. Vasodilation and increase in blood flow, as measured by laser Doppler flowmetry, were sensitive to COX inhibitors (204), thereby confirming the previous finding that COX products are likely released from activated astrocyte endfeet to trigger arteriole dilation (242). When the activation of astrocytes, which accompanies neuronal activity, was blocked with the mGluR5 antagonist MPEP, the vascular response induced by stimulation of neuronal activity was significantly reduced, providing further evidence for the central role of neuron-to-astrocyte signaling pathway in the neurovascular coupling.

It should be noted in this in vivo study (204), as well as in Mulligan and MacVicar's brain slice investigation (145), that a membrane-permeant EDTA-based caged compound was used for the photolytic control of internal Ca^{2+}. As discussed by Ellis-Davies (50), this will result in a compound that is 97% loaded with Mg^{2+} rather than Ca^{2+}. Thus, at this time, it is not clear how photolysis raised internal Ca^{2+}. Nonetheless, Mulligan and MacVicar (145) did perform an important control in which they loaded DMNP-EDTA with Ca^{2+}, then, after dialysis into a single astrocyte, they showed that photolytic release of Ca^{2+} did replicate their results obtained using DMNP-EDTA acetoxymethyl ester.

It is important to note that in these in vivo experiments, Ca^{2+} elevations in astrocyte endfeet occasionally resulted in a significant arteriole constriction (204). Although it was detected only in a few arterioles, this response validates the hypothesis advanced above that activation of astrocytes can release both dilating and constrictive agents.

The ability of astrocytes to trigger also arteriole constriction has been further confirmed in whole-mounted retina preparations (135). Light stimulation as well as photolysis of caged Ca^{2+} that triggered a Ca^{2+} rise in the

stimulated glial cell, i.e., an astrocyte or a Müller cell, were indeed found to evoke both dilation and constriction of retinal arterioles, mediated by different AA metabolites, EETs, and 20-HETE, respectively. Additional evidence for a role of glial cells as mediators of light-induced response of arterioles was the observation that the interruption of neuron-to-glia signaling also blocked the response of arterioles to light stimulation. Based on a series of experimental observations, the authors suggest that NO may play a modulatory role on arteriole responsiveness, favoring a vasodilating and vasoconstrictive response to light when its level is low and high, respectively (135).

While these observations confirm that the mechanism that governs the blood flow response to neuronal activity is complex and relies probably on different vasoactive agents in different brain regions, they underline the central role of astrocytes in functional hyperemia.

The important action of astrocytes in the control of microvasculature raises also the possibility that an astrocyte dysfunction could be implicated in the dysregulation of cerebral circulation in brain pathologies, for example, in the defective neurovascular coupling that is associated with Alzheimer's disease (90, 155), as well as in the vascular abnormal responses during stroke, trauma, and spreading depression (89). The full characterization of the molecular mechanisms that are at the basis of the astrocyte control on cerebral blood vessels in the normal and pathological brain certainly represents one of the most interesting challenges in neurobiological research in years to come.

VII. DISCOVERY OF GLIOTRANSMISSION: ASTROCYTES TALK TO NEURONS

In 1994 two studies (147, 160) provided the first suggestion that the astrocytic Ca^{2+} signals described by Stephen Smith's group do have functional consequences on integration in the nervous system. In these studies it was demonstrated that experimentally evoked Ca^{2+} elevations in astrocytes evoked elevations in the internal Ca^{2+} of adjacent neurons. These breakthrough discoveries, which were later reproduced in independent studies performed by Andrew Charles' (31) and Stan Kater's laboratories (80), provided a new insight into glial-neuron interactions in the nervous system. Although one study suggested an involvement of the gap junction-mediated communication between the astrocyte and neuron (147), the others offered a more compelling mechanistic insight in which the Ca^{2+}-dependent release of the excitatory transmitter glutamate from the astrocyte evoked the depolarization of the neuron through the activation of ionotropic glutamate receptors (160). Indeed, ligands that elevated astrocytic Ca^{2+} led to the release of glutamate from pure cultures of astrocytes (95, 162), and optical assays for glutamate demonstrated external waves of glutamate elevation that followed the internal Ca^{2+} waves in culture (91).

After the demonstration of a glutamate-mediated astrocyte-neuron signaling pathway, it was several years until it was feasible to document a similar pathway in a more intact system to alleviate worries about potential culture artifacts. In 1997, Pasti et al. (165) demonstrated bidirectional signaling between astrocytes and neurons and showed that the activation of astrocytic metabotropic glutamate receptors to evoke glial Ca^{2+} elevations caused delayed neuronal Ca^{2+} signals mediated by ionotropic glutamate receptors. Given the previous culture studies, this result was readily interpreted as being due to the Ca^{2+}-dependent release of glutamate from hippocampal astrocytes, an observation that was later confirmed in slice preparations by Bezzi et al. (19).

VIII. MECHANISMS OF GLUTAMATE RELEASE FROM ASTROCYTES

Several mechanisms of glutamate release have been proposed, and it is likely that more than one does operate within an astrocyte. Evidence has been provided to support roles for the four pathways: exocytosis, hemi-channels, anion transporters, and P2X receptors. However, under the condition of physiological Ca^{2+} elevations, there is a groundswell of support for an exocytotic mechanism. Although release through hemi-channels (234) and $P2X_7$ receptors (46) has been proposed, effective release requires the presence of low divalent saline to promote opening of these channels (67, 101). Since under resting conditions at the membrane potential of an astrocyte and with normal divalent cation concentrations the open probability of hemi-channels and of $P2X_7$ receptors is so low, these pathways are unlikely to be utilized in physiological conditions. However, it should be noted that during neuronal activity, external Ca^{2+} can fall substantially, opening the possibility for these pathways of gliotransmission to become activated under conditions of elevated neuronal activity.

Further doubt has been cast on the potential role of hemi-channel-mediated gliotransmission by the clear demonstration that several antagonists that have been used to block these channels are nonselective and also block $P2X_7$ receptors. Additionally, using genetically modified astrocytoma cells, as well as astrocytes from connexin43$^{-/-}$ and $P2X_7R^{-/-}$ mice, the release of ATP under low divalent cation conditions has been clearly demonstrated to be mediated by $P2X_7R$, not by hemi-channels (199).

Pharmacological evidence has supported a $P2X_7$ mechanism of release (46); however, debate about

whether this receptor is expressed in the nervous system (108, 191), and because it is unlikely to be regulated by elevations of internal Ca^{2+}, limits enthusiasm for this pathway. However, in hippocampal slices, sustained activation by BzATP of a receptor that has features similar to the $P2X_7$ receptor has been recently reported to mediate a sustained glutamate efflux from astrocytes (55). Under pathological conditions, such as ischemia and brain trauma, this $P2X_7$-like receptor in astrocytes might be activated by increasing concentrations of extracellular ATP (47, 59, 125, 130, 134). The consequent glutamate release may contribute to increasing the extracellular concentration of glutamate to the abnormal levels that cause excitotoxic cell death (35). Consistent with this hypothesis are the observations that following an acute spinal cord injury in rats, the functional recovery was enhanced and the death of motoneurons decreased when $P2X_7$ receptors were pharmacologically inhibited (222).

The role of anion transporters/channels is unclear at this time. One of the problems with studying this pathway is the poor selectivity of antagonists. For example, NPPB, which inhibits transporters, can inhibit the filling of vesicles with transmitters (212). Nonetheless, under conditions that promote swelling, it is likely that this pathway could contribute to the release of glutamate from the astrocyte (103, 104). The expression of dominant negative vesicle proteins in astrocytes to inhibit Ca^{2+}-regulated exocytosis leaves a swelling-induced pathway of transmitter release from astrocytes unaffected (236). Thus a volumetric release pathway likely exists in parallel to an exocytotic pathway (203). A future challenge is to identify the conditions that select for each of these two pathways.

There is now compelling evidence supporting an exocytic mechanism of glutamate release from astrocytes. These glial cells express a variety of vesicle proteins that are essential for exocytosis (39, 83, 128, 161, 227, 236). Clostridial toxins, when introduced into the astrocyte to cleave target SNARE proteins, prevent glutamate release (10). Ca^{2+} elevations lead to an increase in membrane surface area synchronous with the release of glutamate (237). Bafilomycin A_1, which inhibits the V-ATPase that pumps protons into the vesicle (23) that are required to drive the transport of glutamate, inhibits the uptake of L-[^{3}H]glutamate in astrocyte vesicles (39) and reduces the release of glutamate (10, 166). Rose Bengal, an inhibitor of vesicular glutamate transporters (VGLUT), similarly blocks Ca^{2+}-dependent glutamate release (140). Finally, immunoelectron microscopy has revealed VGLUT expressing vesicles within the process of astrocytes in vivo (20).

Volterra and colleagues (20) have used total internal reflection fluorescence microscopy to reveal expressed VGLUT-EGFP fusion proteins as well as acridine orange (AO), a dye that accumulates in acidic organelles, and can be used as a marker for exocytosis. Because of an absorbance shift in AO when located within vesicles compared with physiological saline, fusion of an AO-filled vesicle with the plasma membrane leads to a rapid change in AO fluorescence emission, when excited at ~490 nm, as this dye mixes with the extracellular saline. Stimuli that induce Ca^{2+} elevations were shown to cause a brief burst of AO fluorescence events together with a reduction in the numbers of VGLUT-EGFP fluorescent puncta, observations consistent with regulated exocytosis in astrocytes. Furthermore, cocultured glutamate receptor expressing reporter cells simultaneously detected the release of glutamate providing strong evidence supporting exocytotic release of this gliotransmitter from astrocytes. These results have recently been supported by an independent study which has shown Ca^{2+}-dependent fusion of vesicles with the plasma membrane (39).

IX. KISS-AND-RUN RELEASE OF GLUTAMATE FROM ASTROCYTES

Although this evidence is overwhelmingly in support of a exocytic mechanism, there are still detractors who argue that it is possible that the vesicle is inserted into the membrane to provide a channel or transporter to mediate the release of glutamate. Although we feel that the evidence does not support their contention, there is one troubling aspect to the vesicular hypothesis of glutamate release from the astrocyte, since there are few vesicles within astrocytic profiles in vivo. However, one recent observation suggests that the mechanism of transmitter release from the vesicle may be biased towards a kiss-and-run fusion mechanism in which the vesicle does not fully fuse with the plasma membrane but instead forms an ephemeral pore that permits transmitter to be released (33). This possibility is based on the observation that synaptotagmin IV is essential for Ca^{2+}-dependent glutamate release from astrocytes (236). The synaptotagmin gene family is known to play essential roles in the regulation of vesicle fusion in different cell types (105). In nerve terminals, synaptotagmin I plays a dominant role. However, when synaptotagmin IV is expressed in place of synaptotagmin I, fusion events are biased toward kiss-and-run release rather than full fusion (221). An important consequence is that the vesicle reacidifies, a critical step for refilling of the vesicle with transmitter, up to 20 times faster after kiss-and-run release compared with full fusion events (63). Consequently, if synaptotagmin IV does indeed promote kiss-and-run release of glutamate from astrocytes, one can envision that one astrocytic vesicle is equivalent to 20 nerve terminal vesicles.

Studies of exocytosis have been significantly advanced by the electrochemical detection of released transmitters. This approach has now been turned to investigate the Ca^{2+}-dependent release of transmitter from

astrocytes (33). Because glutamate is not directly detected by carbon fiber amperometry, astrocytes were preloaded with dopamine, which, if released, is readily detected electrochemically. Stimuli that elevate astrocytic Ca^{2+} were shown to cause rapid amperometric spikes. Mechanical stimuli, which cause large prolonged Ca^{2+} elevations in astrocytes, caused very large amperometric signals. However, physiological stimuli only led to the release of 1/10th of the vesicle content. During the brief opening of a fusion pore (~2 ms), they showed quantal transmitter release and suggest that relatively large vesicles (~300 nm diameter) normally serve to mediate glutamate release through a kiss-and-run mechanism that only depletes a portion of the vesicular transmitter store.

Such a kiss-and-run mechanism could account for transmission in vivo where there is a paucity of astrocytic vesicles. Though they did demonstrate similar results with acutely isolated astrocytes to answer concerns about culture artifacts, and an independent study supports the potential for large vesicular structures mediating transmitter release from astrocytes in brain slices (97), it is not yet clear where these large vesicles reside within an astrocyte in vivo. Indeed, immunoelectron microscopy has shown the presence of 30-nm vesicles in an independent study (20).

X. THE TRIPARTITE SYNAPSE: ASTROCYTES MODULATE NEURONAL EXCITABILITY AND SYNAPTIC TRANSMISSION

Following the discovery of the regulated release of glutamate from astrocytes, many studies have gone on to demonstrate that this gliotransmitter can modulate synaptic transmission and neuronal excitability. In addition to glutamate, glial-released D-serine and ATP have been discovered to mediate powerful synaptic actions. To maintain some coherence in our discussion, we present information related to each gliotransmitter in independent sections.

XI. RELEASE OF GLUTAMATE FROM ASTROCYTES

Initially cell culture studies showed that astrocytes can use glutamate to modulate neuronal excitability and synaptic transmission. Whole cell recordings from a neuron that was cocultured with astrocytes demonstrated slow glutamate-mediated inward currents (SICs) when a glial Ca^{2+} elevation confronted the recorded neuron (12). These results were later substantiated in thalamus and hippocampus by showing pure *N*-methyl-D-aspartate (NMDA) receptor-dependent neuronal currents following astrocytic Ca^{2+} elevations (54, 55, 163). In addition to direct activation of neuronal currents, glial-released glutamate was also shown to modulate synaptic transmission. Again in culture, astrocytic Ca^{2+} elevations augmented the frequency of miniature excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs), an action that was judged to be due to the gliotransmitter glutamate because effects were blocked by the NMDA receptor antagonist D-AP5 (14). In brain slice preparations, similar actions were observed because GABAergic activation of Ca^{2+} signals in astrocytes caused an NMDA receptor-dependent increase in inhibitory mIPSC frequency detected in pyramidal neurons and a strengthening of certain inhibitory synapses (96). Subsequently, other forms of synaptic modulation have been identified in which metabotropic glutamate receptors and kainate receptors mediate the actions of glial-released glutamate. Single astrocyte photolysis of caged IP_3 increases the frequency of excitatory mEPSCs, an action that is blocked by the metabotropic receptor antagonists LY367385 and 2-methyl-6-(phenylethynyl)-pyridine, suggesting that glutamate release from astrocytes can act through neuronal class I metabotropic glutamate receptors to augment the release of transmitter from nerve terminals (58). Flash photolysis of caged Ca^{2+} also increases spontaneous action potential-driven IPSCs, an action that is mediated by kainate receptors containing the GluR5 subunit (120).

These studies clearly show the potential for astrocytes to integrate neuronal activity and to provide feedback modulatory signals. The roles for these feedback pathways are not yet known in the hippocampus. However, the first critical demonstration of the integrated action of synaptic activity and synaptically associated glial signals was provided by studies performed at the frog neuromuscular junction. Associated with the neuromuscular junction are several perisynaptic Schwann cells that are molecularly and functionally distinct cells from the myelinating Schwann cell. Richard Robitaille (178) performed elegant experiments in which he directly manipulated GTP-binding protein signaling within these glia. Direct microinjection of guanosine 5′-*O*-(3-thiotriphosphate) (GTPγS) into the perisynaptic Schwann cell to activate G protein signaling caused a reduction in the strength of the neuromuscular junction. To ask whether this glial-modulation pathway is recruited during physiological conditions, he studied activity-dependent depression of this synapse. High-frequency stimulation of the motoneuron axon causes a reversible depression of the neuromuscular connection. However, after injection of guanosine 5′-*O*-(2-thiodiphosphate) (GDPβS) into the Schwann cell, to prevent G protein activation, stimulation of the nerve trunk led to a diminished depression. Taken together, these studies led to the proposal of the "tripartite synapse" in which the astrocyte listens to synaptic activity and provides feedback modulation of the strength of the synaptic connection (13).

XII. ASTROCYTES ACTIVATE EXTRASYNAPTIC NMDA RECEPTORS

As discussed above, cell culture studies initially demonstrated that astrocytes, by releasing glutamate, can activate neuronal NMDA receptors. Substantiation of this observation was recently provided in brain slice studies in which glial glutamate was shown to selectively access a specific class of NMDA receptor that contains the NR2B subunit (54). In these studies a variety of stimuli, each of which leads to astrocytic Ca^{2+} oscillations, all caused D-AP5-sensitive, NMDA receptor-mediated SICs in area CA1 pyramidal neurons. Moreover, blockade of synaptic transmission by brief incubation in tetanus toxin did not prevent the detection of these SICs showing that they were from a nonneuronal origin. (It should be noted that while tetanus toxin can prevent glutamate release from astrocytes when applied from the extracellular space, it does so with such a slow time course, due to a paucity of toxin receptors, that it is possible to selectively inactivate nerve terminals with short-term treatment.) Finally, single-cell stimuli such as flash photolysis of caged Ca^{2+}, specifically in the astrocyte, or single astrocyte depolarization all evoked neuronally detected NMDA receptor (NMDAR)-dependent SICs.

Where are the NMDARs located that mediate the SIC? Using MK-801 to allow a use-dependent block of synaptic NMDARs, initial culture studies demonstrated that astrocytes predominantly talk to extrasynaptic NMDARs (14). Measurement of the kinetics of SICs in brain slice studies showed that they are very slow compared with synaptic NMDA currents (8, 54). The rise time of the astrocyte-evoked SIC is ~60 ms, although rise times of 100–200 ms are not rare. The decay time is on the order of 400–500 ms. Different subunit composition of the NMDAR accounts for the different kinetics. Indeed, the NMDAR is normally composed of two subunits: NR1 plus an NR2 subunit and either NR2A, B, C, or D. Studies in which the different NMDAR subunits are expressed in heterologous systems have shown that certain subunit combinations have distinguishing kinetic properties (41, 111, 133, 218). The same distinguishing kinetics are reported from the native NMDARs (137, 141). Once synapses have developed, synaptic NMDARs are primarily comprised of NR1/NR2A subunits, while NR1/NR2B become located at extrasynaptic locales (181, 206). Because NR2B-containing NMDARs exhibit slowly decaying currents of similar kinetics to the SICs we observe, we asked whether the NR2B selective antagonist ifenprodil would attenuate the SICs. In agreement with the kinetic data, ifenprodil selectively blocked the astrocyte-evoked SIC with little or no effect on synaptic NMDA currents (54). These data lend further support to the notion that the glutamate released from astrocytes selectively acts on extrasynaptic NMDARs that contain the NR2B subunit, an observation which is supported by the results of double-label immunoelectron microscopy (20) (Fig. 2).

Can this activation of extrasynaptic NR2B-NMDARs hint at a distinct role of this astrocyte-to-neuron signaling? There is increasing awareness that synaptic and extrasynaptic NMDARs may subserve different, and to some extent opposing, functional roles (42, 213). For example, it has been reported that by shutting down activity of cAMP response element binding protein (CREB), NR2B-

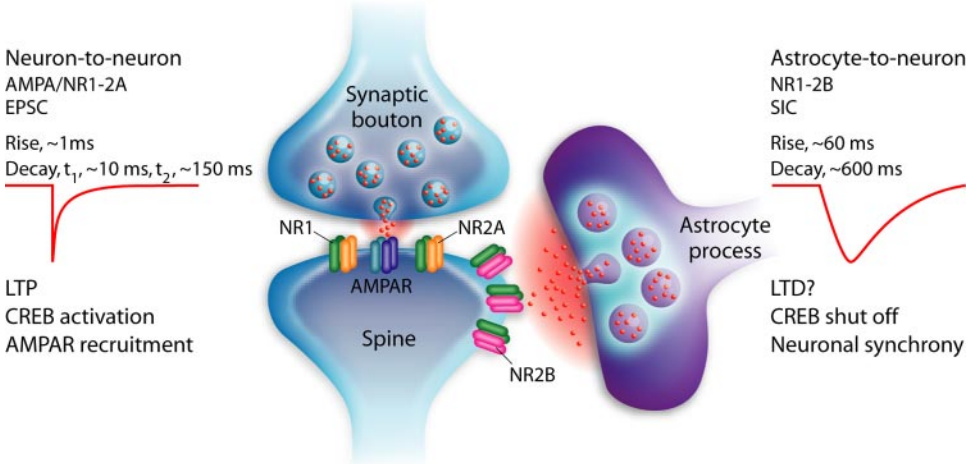

FIG. 2. Glutamate released from presynaptic terminals and from astrocytes acts on distinct NMDA receptors. The application of NMDA receptor subunit-selective pharmacology to mature synapses has shown that synaptic glutamate preferentially acts on NR2A subunit-containing NMDA receptors in addition to AMPA receptors, while astrocytic glutamate activates NR2B subunit-containing, extrasynaptic NMDA receptors. Here we show the NR2B-containing receptors in an extrasynaptic locale of the spine. It should be noted that we provide this location for illustrative purposes as they could equally well be located in the parent dendrite. Activation of NR2A- and NR2B-containing NMDA receptors leads to distinct cellular responses: synaptic NR2A NMDA receptors lead to CREB activation, AMPA receptor recruitment, and LTP, while NR2B-containing receptors have opposing actions potentially being involved in LTD, CREB shut-off, as well as promoting the synchronous activation of neurons.

NMDAR activation promotes neuronal death, while by inducing CREB activity, NR2A-NMDAR activation promotes neuronal survival (76). Activation of the ERK signaling pathway may also depend primarily on NR2B-containing NMDARs (107), although this issue remains controversial (102). A series of studies propose that NR2B-containing NMDA receptors mediate synaptic cross-talk (189), and the induction of long-term synaptic depression (LTD), while NR2A-containing NMDARs seem more important in the induction of long-term potentiation (LTP) (119, 131). As a plausible mechanism of NR2B-NMDAR-mediated LTD, it has been observed that NR2A-NMDARs promote, whereas NR2B-NMDARs inhibit, surface expression of AMPA receptors, essentially by regulating the membrane insertion of GluR1 subunit (102). The conclusions drawn in these studies rely mainly on the use of the specific NR2B subunit antagonist ifenprodil, and the recently developed antagonist of the NR2A subunit NPVAAM077. However, the observations that this latter compound is relatively selective for the NR2A subunit and decreases its selectivity with prolonged exposure cast doubts about its use as a pharmacological tool to dissect out the distinct role of the NR2A-NMDAR in LTP (18, 225).

While we need to improve our understanding of the role of the different NMDAR subunits in the plastic changes of synaptic strength, the available data allow us to advance the hypothesis that NR2B-NMDA receptors, which during early postnatal development are progressively confined to extracellular locations, represent a common, preferential target of either glutamate spilled over from synapses and glutamate released from activated astrocytes (Fig. 2). It will be of extreme interest to determine if and to what extent astrocytic glutamate, while acting on NR2B-NMDARs, may contribute to fundamental events in neuronal transmission such as LTP and LTD.

With the identification of the presence of the astrocyte-evoked NMDA current, it is important to determine under which circumstances this current will be activated, and whether these conditions could provide the synapse with additional information. Specifically designed experiments are needed to clarify these issues.

Given that along with NMDARs, AMPARs are also expressed at extrasynaptic locations, the absence of AMPA-mediated currents came initially with some surprise. However, when cyclothiazide (CTZ) and D-AP5 were included in ACSF to prevent AMPA receptor desensitization (167) and NMDA receptor activation, respectively, AMPA receptor-mediated SICs were detected (54). This result provides two important conclusions. First, it is probably important that the astrocyte only talks to extrasynaptic receptors; otherwise, synaptic access of glial glutamate, by leading to a desensitization of synaptic AMPA receptors, would reduce the fidelity of synaptic transmission. Second, because there is no AMPA component accompanying activation of NR2B-containing NMDA receptors, the astrocyte alone is not able to cause neuronal currents. Instead, a coincidence of an independent depolarizing stimulus together with glutamate release from the astrocyte will be required to admit current through the extrasynaptic NMDA receptor. The exact conditions that support such coincidence are yet to be defined, although astrocyte-evoked SICs can be detected in Mg^{2+}-containing saline albeit at a lower frequency than in Mg^{2+}-free conditions.

Interestingly, the rise time of AMPA-mediated events recorded in the presence of CTZ and D-AP5 is comparable to that of NMDAR-mediated events. Apparently, the concentration of glia glutamate increases relatively slowly in the extracellular space, and this determines the slow activation of NMDARs and AMPARs in the neuronal membrane. While a slow diffusion of glia glutamate in the large extracellular space can reasonably account for the time course of the increase in its extracellular concentration, it cannot be excluded that the process of glutamate release from the astrocyte could be somewhat slower than that from neurons.

XIII. WHY ARE ASTROCYTE-EVOKED NMDA CURRENTS SO LARGE IN AMPLITUDE?

Astrocyte-evoked NMDAR currents can be extremely large in magnitude. The average current detected is ~100 pA (8, 53, 55). In contrast, the NMDA current due to the fusion of a single vesicle in the synapse is of the order of 2–3 pA (156). If both are mediated by exocytosis, why are SICs so large? The size of vesicles within astrocytes have been reported to range from 30 to 300 nm (20, 33, 39). If the vesicles that mediate the release of glutamate from the astrocyte are indeed 300 nm, as suggested by Chen et al. (33), rather than ~40 nm in diameter, then the astrocytic vesicle will contain 422 times as much transmitter as a synaptic vesicle based purely on volumetric arguments. Because extrasynaptic NMDARs are at most 1/10th the density of synaptic receptors (156), the magnitude of an astrocyte-evoked NMDA receptor current could be as large as 85–126 pA (assuming a synaptic NMDA current of 2–3 pA), and an absence of receptor saturation. Thus an average amplitude of the SIC of 100 pA is not out of the question, although it likely involves glutamate diffusion from the site of release to distant NMDARs that are not already occupied by transmitter. Such a requirement for diffusion would also account for the desensitization of AMPA receptors.

These arguments hold for full-fusion of a 300-nm vesicle. However, the studies of Chen et al. (33) show that fusion-pore release of transmitter is the norm under physiological conditions. If, as they identified for the false transmitter dopamine, the vesicle normally only releases

1/10th of its transmitter, then it is likely that astrocyte-evoked NMDA currents are of a much smaller amplitude, ~8–12 pA in magnitude. Although it is possible to detect currents of this magnitude when looking at stimulus evoked events, this is a very difficult task when merely scrolling through ongoing recordings and especially given the slow kinetics of NMDAR-mediated SICs. Based on these arguments, we conclude that the 100 pA SIC detected in recordings from pyramidal neurons likely reflect the full-fusion of a relatively large glutamate-filled vesicle with the astrocytic plasma membrane, which activates distant, extrasynaptic NMDARs. These SICs are rare, occurring within a given pyramidal neuron at a frequency of ~1/min. Because physiological stimuli preferentially cause fusion-pore release of transmitter, we anticipate that these SICs represent large, supranormal events. Instead, higher frequency fusion-pore-related events are likely to be ongoing that have been beneath the resolution of our recording conditions.

XIV. ASTROCYTES SYNCHRONOUSLY ACTIVATE GROUPS OF PYRAMIDAL NEURONS

The quantitative arguments just presented would suggest that the release of a vesicle of glutamate from an astrocyte would have the potential to act synchronously on several adjacent dendrites, because to achieve the magnitude of NMDA receptor current, the transmitter must diffuse to activate unoccupied receptors. The notion of synchronous activation of neurons has been tested using confocal imaging as well as paired electrophysiological recordings. Activation of class I mGluRs causes Ca^{2+} oscillations on astrocytes and to the delayed, synchronous Ca^{2+} accumulation in groups of pyramidal neurons. When paired recordings are made from pyramidal neurons, synchronous astrocyte-evoked SICs are detected as long as the recorded cell bodies were within ~100 μm of one another (8, 54). We do not feel that this means that glial-released glutamate is diffusing 100 μm to access these neurons. Rather, a dendrite of these paired neurons likely occupies a similar volume within the neuropil, allowing them to both detect the glial-released glutamate. At this time the functional implications of this synchronous NMDAR activation are not clear and await a further understanding of whether the 100-pA SICs are the normal amplitude event, or whether the majority are smaller in amplitude with more spatially confined actions that might not lead to synchronous activation of groups of neurons.

When one considers two distinct functions of the astrocyte, the control of the cerebrovasculature and the release of gliotransmitters, it becomes clear that an understanding of the spatiotemporal constraints on glial Ca^{2+} signaling is essential to develop an integrated view of the function of these glial cells. On the one hand, we have discussed the importance of the astrocyte in responding to synaptic activity and causing Ca^{2+} elevations in perivascular endfeet. Yet, on the other hand, we have discussed the equal importance of local microdomain Ca^{2+} signals to maintain a degree of synaptic specificity on glial glutamate signaling. How can these two conflicting issues be resolved?

XV. D-SERINE: SELECTIVE SYNTHESIS IN AND RELEASE FROM ASTROCYTES

It has been appreciated for a considerable time that the NMDAR requires not only glutamate for its activation, but also the coagonist glycine. More recent data suggest that D-serine may actually be the endogenous ligand for this modulatory glycine-binding site of the NMDAR (143). Astrocytes express an enzyme, serine racemase, which converts L- to D-serine (229). Since this enzyme is only expressed in astrocytes (186, 187, 228), these glial cells are the source of this endogeous ligand for the glycine-binding site of the NMDAR.

Although little evidence is available concerning the mechanism of release of this amino acid, one study provides strong evidence for a Ca^{2+}-regulated exocytotic release pathway. Similar to glutamate release, Ca^{2+} elevations are both necessary and sufficient for the release of D-serine (144). Subcellular fractionation on sucrose gradients revealed D-serine in the same fractions as synaptobrevin II and as glutamate raising the possibility of the coloading of glutamate and D-serine in the same vesicles. Further support for an exocytotic mechanism of release was provided by a punctate immunolocalization and that treatment of cultures with the clostridial toxin tetanus toxin, which cleaved synaptobrevin II, caused a significant attenuation of the Ca^{2+}-dependent release of this amino acid.

Because of this evidence supporting an exocytotic release pathway for this transmitter, it is essential that in studies of glial glutamate release, which have been shown to act selectively on NMDARs, controls for actions mediated by D-serine are included. Thus, in several of the studies discussed concerning astrocyte-evoked NMDAR-mediated SICs, glycine was added to slice preparations at saturating concentrations to ensure that results could be assigned to effects of glutamate rather than D-serine (8, 54).

Because D-serine is selectively synthesized in astrocytes, it has been possible to ask whether this gliotransmitter impacts synaptic transmission and plasticity (40). In the retina, addition of exogenous D-serine augments NMDA receptor currents, while addition of D-amino acid oxygenase, which degrades D-serine, reduces the magnitude of these currents (196). In hippocampal cultures devoid of astrocytes, addition of D-serine has been shown

to be critical to enable the induction of synaptic plasticity, while in brain slices and in mixed cultures of astrocytes and neurons, degradation of D-serine blocks LTP induction (233). The crucial role of D-serine in LTP is strengthened by the finding that the impairment of LTP observed in CA1 neurons from slices of 12-mo-old rats is overcome by addition of D-serine (232).

In the hypothalamic supraoptic nucleus (SON), the astrocytic coverage of glutamatergic synapses changes as animals enter lactation, allowing the unique opportunity to determine the role of astrocyte-derived D-serine in the regulation of synaptic transmission and plasticity. As animals begin lactation, the astrocytic coverage of these synapses is drastically reduced. By comparing the NMDA component of the synaptic current in virgin and lactating rats, Oliet and colleagues (159) demonstrated a reduced NMDA component of the synaptic connection that correlates with the reduced glial coverage of the synapse. Because exogenous addition of D-serine enhances the NMDA component in lactating animals compared with that seen in virgin rats, the authors conclude that glial D-serine acts as the endogenous coagonist of the NMDAR and locally regulates the degree to which synaptic glutamate can activate these receptors. In addition to studying synaptic transmission, they also ask whether glial-derived D-serine regulates plasticity. Their results provide the exciting observation that the astrocyte controls a form of metaplasticity: whether a synapse exhibits LTP or LTD is controlled by the glial coverage of the synapse and the extent to which D-serine is provided to synaptic NMDARs. When the astrocytic processes retract and the level of synaptic D-serine is reduced, LTD is induced, whereas in virgin rats that have a high degree of synaptic coverage, the same stimulus induces LTP.

The degree to which D-serine is released in a tonic manner, versus in an activity-dependent fashion, and thus can dynamically regulate NMDAR function remains to be demonstrated. However, that exogenous D-serine can augment NMDAR activity indicates that the glycine-binding site is not saturated under resting conditions, and opens the potential for dynamic regulation of this site by Ca^{2+} signaling in astrocytes and the associated release of D-serine. If glutamate and D-serine are copackaged in the same vesicle, an interesting possibility is raised in which the regulated release of agonist and coagonist will ensure maximal activation of the NR2B-containing NMDAR (Fig. 3).

XVI. RELEASE OF ATP FROM ASTROCYTES

In addition to glutamate and D-serine, astrocytes also release the chemical transmitter ATP. Though many fewer studies have been performed in which this nucleotide has been studied, significant advances have been made in understanding its role in the regulation of synaptic transmission.

Because the studies are at such an early stage, the mechanisms controlling the release of ATP are far from

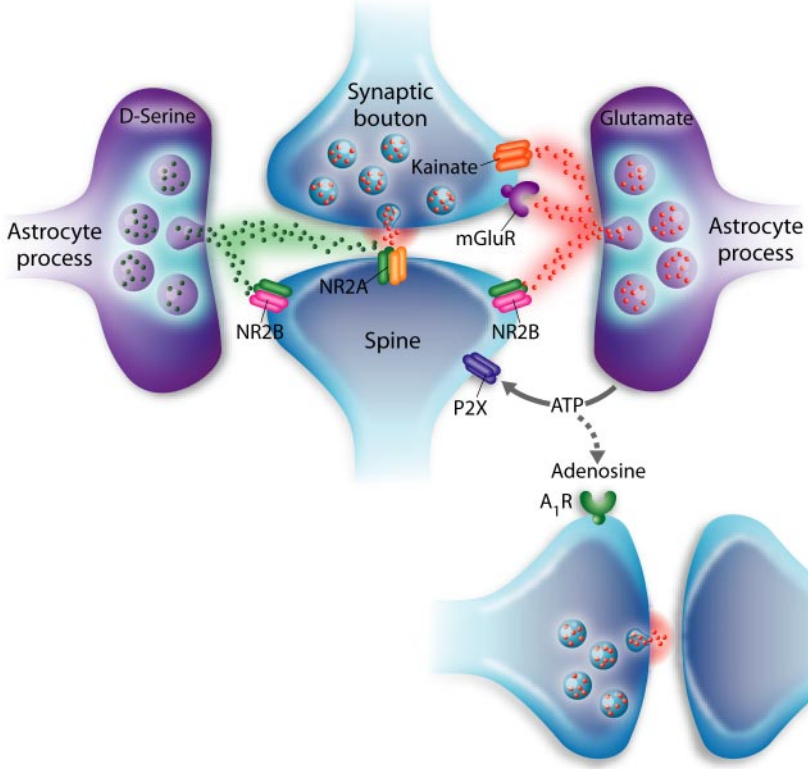

FIG. 3. Astrocyte-derived signals act both presynaptically and postsynaptically to regulate synaptic transmission. The release of glutamate, D-serine, and ATP from astrocytes has a diversity of synaptic actions. Presynaptically, glutamate can access metabotropic glutamate receptors (58) and kainate receptors (120) to enhance synaptic transmission. Postsynaptically, glutamate can act on extrasynaptic NMDA receptors to depolarize the neuronal membrane and promotes neuronal synchrony (54), while D-serine acts on the glycine-binding site of NMDA receptors and can regulate synaptic plasticity (159). ATP may also act postsynaptically on P2X receptors to depolarize the neuronal membrane and regulate the insertion of postsynaptic AMPA receptors (68). After hydrolysis by ectonucleotidases to adenosine, ATP can have distant action on presynaptic A1 receptors to cause heterosynaptic depression of excitatory synaptic transmission (164).

resolved. Indeed, there is even debate about whether the ATP is released through a Ca^{2+}-dependent mechanism. Until the proximate stimulus is understood, it will be difficult to make headway in understanding the details of this purinergic pathway.

The identification of ATP being a messenger of the astrocyte was beautifully demonstrated in studies concerning Ca^{2+} waves that propagate between astrocytes in culture. When waves confronted a cell-free region, they could jump the gap due to the release of a diffusible message (81). Later lucerifin/luciferase studies demonstrated that ATP was released during Ca^{2+} waves. However, chelation of Ca^{2+} with BAPTA, a manipulation which blocks glutamate release, did not affect the release of ATP (223). Although it is clear that both ATP and glutamate release require phospholipase C activity (223), it is not certain whether the diacylglycerol or IP_3 arms of this pathway are critically involved in regulating ATP release. In support of a role for the diacylglycerol pathway is the ability of phorbol esters to stimulate ATP and to a lesser extent glutamate release from cultured astrocytes (36). The action of phorbol esters is, however, very intriguing as it raises the potential for independent regulation of the two primary transmitters that are released from astrocytes. Whether the release of ATP is protein kinase C (PKC) dependent is not clear because it is now appreciated that phorbol esters stimulate a variety of diacylglycerol effectors including PKC, RasGRP, protein kinase D, and the vesicle-associated protein munc-13 (26, 177, 194). Of particular interest is munc-13. This vesicle-associated protein is a homolog of the *Caenorhabditis elegans* uncoordinated (unc)-13 protein that has been shown to be essential for synaptic transmission. Munc-13 contains C1 diacylglycerol binding sites and acts by unfolding the SNARE protein syntaxin so that it is able to form the SNARE complex that is essential for vesicle priming before exocytosis (26). Since the release of ATP from astrocytes exhibits sensitivity to tetanus toxin and is blocked by expression of a cytosolic SNARE domain (164), an exocytic mechanism may underlie the release of this nucleotide. Thus a candidate regulatory pathway for ATP release is through diacylglycerol-dependent activation of munc-13.

XVII. GLIAL-DERIVED ATP MODULATES NEURONAL EXCITABILITY

Although details of the regulatory pathway controlling ATP release are sketchy, what is clear is that following its release it has powerful actions on adjacent neurons. In the retina Eric Newman (151) has demonstrated that activation of astrocytes and Müller glial cells causes ATP to be released. However, after ATP release, which has been measured with luciferin/luciferase imaging in the retina, neuronal actions are mediated by adenosine. Once ATP is released into the extracellular space, it is well known that there are a variety of ectonucleotidases that are responsible for the hydrolysis of ATP to AMP, and then a 5′-nucleotidase that is responsible for the final hydrolysis of AMP to adenosine (240). This nucleoside has potent actions mediated by A1, A2, and A3 receptors. Of relevance to this discussion, however, are A1 receptors that are activated by concentrations of adenosine of the order of tens of nanomolar. Thus ATP can be released in concentrations that might be subthreshold for the activation of neuronal P2 receptors (micromolar) yet sufficiently high to allow an accumulation of adenosine that will activate A1 receptors. In the retina, ATP that is released from glial cells is hydrolyzed to adenosine, where it causes the activation of K^+ currents that hyperpolarize retinal ganglion neurons (151). In hippocampal cultures, the release of ATP from stimulated astrocytes was also found to depress glutamatergic neuronal transmission (106). The authors suggest that both ATP and the ATP metabolite adenosine contribute by acting on presynaptic P2 and A1 receptors, respectively. Activation of $P2Y_1$ receptors on CA1 interneurons and astrocytes from hippocampal slices by ATP, probably released by both neurons and astrocytes, was also found to cause an interneuron excitation that leads to increased GABAergic synaptic inhibition onto pyramidal neurons (24).

It is certainly worth mentioning that the control of the relative levels of ATP, ADP, AMP, and adenosine is a complex process in which several enzymes are involved (28, 49). Of particular interest is the observation that the 5′-nucleotidase responsible for the hydrolysis of AMP to adenosine is inhibited by nucleotides (239). Thus one could envision that under low rates of release of ATP, this nucleotide is effectively hydrolyzed to adenosine. However, with elevated levels of release it is possible that nucleotides will accumulate since the 5′-nucleotidase will be inhibited by their elevated levels.

XVIII. PURINERGIC MODULATION OF SYNAPTIC TRANSMISSION

Since adenosine is known to cause a presynaptic inhibition of transmitter release, it is possible that astrocytes by releasing ATP could exert a purine-dependent regulation of synaptic transmission. Over a decade ago it was realized that activity in a synaptic pathway acts laterally to regulate the relative strength of neighboring synapses (129). Stimulation of one pathway with a high-frequency train (100 Hz for 1 s) caused a depression of the neighboring unstimulated pathway. How did these two pathways talk to one another? Through pharmacological studies it was determined that the heterosynaptic suppression was mediated by the accumulation of adenosine

that acted through A1 receptors. However, the source was unclear. In 2003, it was suggested that the adenosine was derived from the astrocyte (235). These experiments were based on the use of a metabolic poison, fluorocitrate, which due to selective accumulation in astrocytes is alleged to selectively inhibit the tricarboxylic acid cycle of the astrocyte (201, 202). Notwithstanding the concerns about this poison, this study raised the intriguing possibility that the astrocyte mediates heterosynaptic suppression of synaptic transmission (Fig. 3).

It has been unclear how adenosine accumulates in the extracellular space. Several mechanisms have been proposed including the direct release through equilibrative transporters as well as through the hydrolysis of released nucleotides (28, 49). These studies, as well as the study of Newman (151), indicate the importance of the hydrolysis of released ATP. In the presence of a P2 receptor antagonist to block direct actions of ATP, the inhibition of ectonucleotidases reduced the adenosine-dependent heterosynaptic suppression, as well as the glial-evoked activation of neuronal K^+ currents (151).

In contrast to the suppressive effects of astrocytic purines in the hippocampus, ATP has been shown to potentiate the amplitude of glutamate-mediated mEPSCs in the paraventricular nucleus (68). Although details still remain to be determined, this study is consistent with the idea that an extrinsic input to the astrocyte causes the release of ATP from these glial cells which, by acting through postsynaptic P2X receptors, causes the insertion of postsynaptic AMPA receptors. Again, as discussed for the studies by Zhang et al. (235), this study utilized fluorocitrate as a glial metabolic poison. Following treatment purinergic actions were lost. Importantly, however, an independent analysis of stressed animals, in which dehydration causes a retraction of the glial coverage of the synapse, confirmed the importance of the glial cell in mediating the purinergic signal (68). In this condition, and in agreement with fluorocitrate treatment, actions of ATP were lost. Although it is not clear how a persistent elevation of ATP is achieved in the face of the activity of ectonucleotidases, these results point to a widespread role for an astrocytic source of ATP in the regulation of synaptic transmission.

XIX. INTRODUCTION OF MOLECULAR GENETICS TO ADDRESS THE ROLES OF THE ASTROCYTE IN NEURONAL FUNCTION

A challenge in studying the role of gliotransmission in the regulation of synaptic integration is that receptors expressed on astrocytes are similarly expressed on neurons, making cell-selective pharmacological manipulations a challenge. Consequently, the use of a metabolic poison, fluorocitrate, in brain slice studies, in combination with an analysis of purified cultures of astrocytes, has been used to identify roles for gliotransmission in the nervous system (235). The ideal way to overcome this problem is to use cell type-specific molecular genetic approaches to manipulate the astrocyte and then determine consequences for neuronal function. Several laboratories have been making great advances in this direction, which has resulted in the first direct demonstration of the roles of astrocyte-derived ATP in the control of synaptic transmission, plasticity, and heterosynaptic cross-talk (164). Before discussing the results of this study, the general strategy that has been utilized is discussed.

To use molecular genetics, an astrocyte-specific promoter is needed to direct gene expression. Michael Brenner's group has isolated the human glial fibrillary acidic protein (GFAP) promoter and used it extensively in studies of the astrocyte (25, 198, 238). Initially, this promoter was used to drive the expression of reporters, and they were shown to be selective for the astrocyte. However, further analyses of different transgenic animals have shown that this cell type specificity is not absolute and great caution must be used in characterizing each line of animal that is produced (198).

Nonetheless, under stringent conditions astrocyte specificity can be achieved. One powerful way to study the astrocyte would be to employ the Cre recombinase system in combination with the GFAP promoter to excise specific genes selectively in astrocytes. Although initial tests of this approach have been performed, there are grave concerns about this strategy because neuronal progenitors express GFAP. Consequently, the floxed allele is excised in cells that become neurons as well as astrocytes (238).

Perhaps the most powerful approach to be used is the combination of cell type specificity together with inducibility of transgenes. Gossen and Bujard (69) have developed such an inducible transgenic system that can be applied in a cell type-specific manner. This tetracycline-regulated system requires the expression of two transgenes. A cell type-specific promoter is used to express a tetracycline transactivator protein (tTA) which binds to a tetracycline operator (tetO) that drives the expression of the transgene of choice. In cells that do not express tTA, tetO does not drive transgene expression. Additionally, tTA binds doxicycline (dox), a tetracycline analog that can be introduced into mice by addition to the food or drinking water. In the presence of dox, tTA is unable to bind to tetO preventing transgene expression. Thus, in this system, one achieves both cell type specificity and inducibility of transgene expression. Ken McCarthy and co-workers (117, 164) have now developed this approach for the astrocyte and demonstrated astrocyte specificity of inducible transgene expression.

Although the mechanism of release of ATP is not definitively resolved, because its release is reduced by tetanus toxin (36), the expression of a SNARE domain of the SNARE protein synaptobrevin II, which we know is expressed in astrocytes (237), should block the formation of the endogenous SNARE complex and thus prevent vesicle fusion with the plasma membrane and thus release of ATP. Therefore, transgenic mice have been generated in which tetO drives the expression of the dominant negative SNARE domain (dnSNARE) that has been crossed with an astrocyte-specific mouse in which the human GFAP promoter controlled the expression of tTA. Animals were reared on dox, to prevent transgene expression until weaning whereupon transgene was expressed by removal of dox from the drinking water. Extensive characterization of these animals shows astrocyte-selective transgene expression. Moreover, the GFAP-tTA animal has been crossed with at least eight different tetO transgenic animals, and each transgene was only expressed in astrocytes (164).

With the use of these mice, which are referred to as dnSNARE mice, three fundamental observations were made. First, expression of the transgene resulted in an enhancement of the amplitude of basal synaptic transmission at the Schaffer collateral CA1 synapse. This effect was determined to be due to a reduction in the extracellular levels of adenosine because in slices from wild-type animals A1 receptor antagonists mimicked the effect of transgene, and in dnSNARE slices, antagonist had no effect, while an A1 agonist fully reversed the effect of transgene expression. Second, as a consequence of reducing extracellular adenosine levels, transgene expression reduced the magnitude of theta-burst-induced LTP. This reduction in LTP that was observed with dnSNARE expression resulted from a change in the range available for plasticity. Under normal conditions, the persistent astrocyte-dependent suppression of synaptic transmission allows a greater range for the synapse to be potentiated. Third, in agreement with the results of Zhang et al. (235), adenosine-dependent heterosynaptic suppression of unstimulated synaptic pathways was abolished (Fig. 3).

Through a series of pharmacological experiments it was demonstrated that these actions were due to a blockade of ATP release from the astrocyte and that, under normal circumstances, released ATP was hydrolyzed to adenosine. This caused an A1 receptor-mediated presynaptic inhibition of excitatory synaptic transmission. Expression of the dnSNARE by blocking ATP release relieved this suppression of synaptic transmission.

These results were quite surprising as it is clear that neurons can release adenosine through equilibrative transporters. However, the astrocyte-selective expression of dnSNARE shows that the astrocyte is the cell type with the predominant control over extracellular adenosine, and we conclude that this adenosine arises from the hydrolysis of released ATP. As adenosine accumulates we then propose it reenters the astrocyte through equilibrative transporters where it is phosphorylated to replenish intracellular ATP.

XX. GLIOTRANSMISSION REGULATES SYNAPTIC CROSS-TALK

From the preceding discussion it should be clear that although we are still at an early stage in our understanding of the impact of gliotransmission in the nervous system, some principles are emerging (Fig. 3). First, gliotransmitters can have both excitatory and inhibitory actions. Excitation is provided by glial-derived glutamate and D-serine, while inhibition is provided by the release of ATP and the resulting accumulation of adenosine. There is no evidence for GABA being released from astrocytes. Second, gliotransmission mediates heterosynaptic crosstalk. With glutamate this action is likely to be relatively local because glutamate transporters will rapidly bind to and take up this transmitter. With adenosine the distance over which inhibition can be exerted is likely to be considerably greater, since adenosine is cleared from the extracellular space by equilibrate transporters. At this point one transgenic system has been developed to identify the actions of gliotransmitters, and with surprising results. As we develop a further understanding of the pathways that differentially regulate the release of the individual gliotransmitters, it should be possible to pinpoint how these transmitter systems contribute to brain function.

In addition to their roles in human health, there is the potential for the astrocyte and gliotransmission to have a significant impact on human disease. In several neurological conditions as well as psychiatric disorders, alterations in astrocyte structure have been identified (136). In depression, there are fewer astrocytes, whereas astrocytes are reactive in the brains of patients suffering from epilepsy, Parkinson's disease, and Alzheimer's disease. Are there corresponding consequences for neuronal function? The negative symptoms of schizophrenia have been identified to result, at least in part, from hypofunction of the NMDA receptor (138, 139). Consequently, D-serine is receiving considerable attention in the development of a therapeutic approach to treat these negative symptoms (79, 197, 210). Adenosine and A1 receptors have been shown to be critical for mediating the acute effects of alcohol, and perturbation of this pathway leads to alcohol preference (34). Do the astrocyte and the gliotransmitter ATP, which we now recognize play a critical role in the control of extracellular adenosine, serve any functions in alcohol addiction? Adenosine is also known to have powerful anticonvulsant actions (21). Because there is a significant percentage of the epileptic population that do not

respond well to current anticonvulsants, efforts are underway to manipulate this nucleoside. However, because of a diversity of peripheral actions of adenosine, systemic administration is not a treatment of choice. Perhaps targeting the astrocyte with therapeutics would offer an alternative treatment strategy for these patients.

XXI. SUMMARY AND THE FUTURE

Since the discovery that a gliotransmitter glutamate can be released from cultured astrocytes, we have over the subsequent decade made many observations that demonstrate that gliotransmission, either mediated by glutamate, D-serine, or ATP/adenosine, is intimately linked with neuronal function. However, a true understanding of how the astrocyte interacts with neurons is still missing. Are these interactions critical for brain function? Although it is clear that the ratio of glia to neurons increases through phylogeny (148), is this a natural process required to accompany the growing brain or does it provide the system with higher level functions that we currently fail to appreciate? Now that a general strategy to apply molecular genetics to the study of gliotransmission has been elucidated, the next several years should provide the opportunity to begin to address these questions. We now have the tools at hand to determine how gliotransmission contributes to behavior, and whether gliotransmission could contribute to neuropathological conditions in which astrocytes become reactive.

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