MOLECULAR ASPECTS OF STRUCTURE, GATING, AND PHYSIOLOGY OF pH-SENSITIVE BACKGROUND K_{2P} AND Kir K^{+}-TRANSPORT CHANNELS

Francisco V. Sepúlveda, L. Pablo Cid, Jacques Teulon, and María Isabel Niemeyer

Centro de Estudios Científicos, Valdivia, Chile; UPMC Université Paris 06, Team 3, Paris, France; and Institut National de la Santé et de la Recherche Médicale, UMR_S 1138, Paris, France

I. INTRODUCTION

The inwardly rectifying Kir K^{+} channels are the simplest from the point of view of topology within the ion channel superfamily. As will be discussed below, they form a K^{+}-selective pore by assembling as tetramers of two transmembrane domains with a membrane reentrant loop that contains the elements for ion selectivity. K_{2P} channels are like two Kir pore structures linked together and assemble in pairs in a pseudotetrameric arrangement. Both types of channels are central in the control of the membrane potential of cells, but recent work has brought to light a much more sophisticated contribution to cell and organ function. The present review will look at two subsets of Kir and K_{2P} channels: the so-called K^{+}-transport channels among the Kir channels and those of the K_{2P} channels that are prominently regulated by changes in pH. In addition to sharing structural simplicity with K_{2P} channels, Kir K^{+}-transport channels are crucially dependent on pH for their function. As will be seen below, their apparent simplicity in basic blueprint hides sophisticated regulation, originally unsuspected structural complexity, and central importance in physiological processes.

This work is not exhaustive and need not be, as excellent reviews touching on different aspects of the subject matter have been published recently and will be cited below. It is also biased by the penchant of the authors for certain physiological functions adjudged more thoroughly investigated, controversial, or both. Others, like the conspicuous absence of an analysis of the function of K_{2P} channels in the central nervous system (CNS), are arbitrarily not touched upon, but the reader is directed to previous works covering this area.

A. Tandem Two-Pore Domain K_{2P} Channels

Channels belonging to the K_{2P} family of proteins underlie the background conductance essential to the maintenance of the interior negative membrane potential of all cells. Leak
The currents carried by K⁺ ions had long been known to be key players in setting the membrane potential in excitable cells (158–160), but the nature of these membrane leaks remained mysterious until relatively recently, and their identification with bona fide ion channels had to await the discovery of their molecular counterparts. In 1995, a K⁺ channel was identified in yeast with a predicted topology of eight transmembrane domains and two pore domains in tandem (191). Shortly afterwards, the first K₂P channels (see FIGURE 1A) were obtained: human TWIK-1 (219), Drosophila melanogaster ORK1, later called KCNK0 (120) and mouse TREK-1 (99). These channels differed from those cloned thus far in having two pore domains (P) in tandem, rather than the usual single P-domain, and four transmembrane domains (TM1-TM4). As we will discuss in more detail below, they form homodimers, and sometimes heterodimers, where each monomer contributes two P-domains and transmembrane domains 2 and 4 to the conductance pore (FIGURE 1A). Functional study of TWIK-1 [K₂P1] in the International Union of Pharmacological Sciences nomenclature (118)] led the authors to postulate it as responsible for a leak or background conductance of a type present in all cells (219). Indeed, TWIK-1 was found to be open at rest and therefore is capable of driving the membrane potential towards the equilibrium potential for K⁺. TWIK-1 lacked time or voltage dependence and approached open channel rectification properties. A small deviation from perfect Goldman-Hodgkin-Katz rectification of the TWIK-dependent current was given by a weak inward rectification at depolarized voltages due to intracellular Mg²⁺ inhibition. The name given to the channel is an acronym for tandem of P-domains in a weak inwardly rectifying K⁺ channel. TWIK-1 was the first in a family of proteins comprising 15 mammalian members all sharing the property of having four transmembrane domains and two P-domains in tandem that rapidly emerged in the scientific literature and are now termed K₂P channels (219). The biology, physiology, and biophysics of K₂P channels have been widely reviewed recently (119, 221, 236), including a recent in-depth comprehensive review by Enyedi and Czirják (93) and one dealing specifically with pH-gated K₂P channels, the focus of the present work, by Lesage and Barhanin (218).

Eight of the 15 mammalian K₂P channels respond prominently to changes in extracellular or intracellular pH concentration (218). TWIK-related acid-sensitive K⁺ channel 1,
exposure to 100% CO₂, which is expected to acidify the extracellular pH. Changes in the number of channels expressed at the surface membrane (63, 186, 235, 335) result in changes such as the number of channels expressed at the surface membrane (63, 186, 235, 335).

The inhibition of TREK-2 by extracellular alkalinization tails off at pHₐ 8.0. Together with TREK-1, TREK-2, and TWIK-2 are also gated open by increases in pHᵢ (281). TASK-2 is also activated by increases in cell volume (280) and inhibited by a direct effect of G protein βγ subunit (1).

Extracellular pH affects TASK-1, which is inhibited by acidification (325). TWIK-1 and TWIK-2 are inhibited by extracellular pH changes such as the number of channels expressed at the surface membrane (63, 186, 235, 335).

The inhibition of TREK-2 by extracellular alkalinization tails off at pHᵢ 8.5. pHᵢ gates TREK-1 and TREK-2, both of which are activated by acidification (163, 247). TRAAK, on the other hand, is activated by pHᵢ > 7.8 (193).

Extracellular pH affects TWIK-1, which is inhibited by acidification (325). TWIK-1 and TWIK-2 are inhibited by exposure to 100% CO₂, which is expected to acidify the intracellular milieu, but there is no evidence that the inhibition is due to a direct effect of pHᵢ (50, 219).

These effects of extracellular pH on the gating behavior of Kᵢ channels seem likely to take place through changes in the probability of the channels to be in the open state, without any change in single-channel conductance or other changes such as the number of channels expressed at the surface membrane (63, 186, 235, 335).

B. Kir K⁺-Transport Channels

Kir channels have only two transmembrane segments and form tetramers where P-domain and TM2 contribute to the permeation pore structure (FIGURE 1B). The Kir potassium channel family comprises 15 members in mammals. It emerged in 1993 with the expression cloning of ROMK1 (Kir1.1) (157) and IKR1 (Kir2.1) (205). The general properties of Kir channels have been presented elsewhere in several reviews (145, 153, 278) and will not be dealt with in detail here. Kir channels are formed by proteins possessing two transmembrane domains with intracellular NH₂ and COOH termini connected by a reentrant P-domain loop organized in a homo- or heterotetrameric arrangement (FIGURE 1B).

The inward rectification results from an interaction between magnesium and polyamines with the pore (153, 278). We should be reminded that inward rectification was discovered in the skeletal muscle by Bernard Katz long before the cloning of Kir channels (188; see Ref. 153) and was originally designated as anomalous rectification, i.e., not following the Goldman-Hodgkin-Katz equation.

The elementary conductance of all Kir channels is dependent on extracellular K⁺ concentration over a large range (344; see Ref. 153). Some of these channels are also slowly activated (minutes) when exposed to increasing extracellular K⁺ concentration (86, 91, 92, 312, 343, 354). The membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PIP₂) is necessary to sustain the activity of most Kir channels (169, 170, 224, 225, 238, 330, 355, 427). Kir channels are readily blocked by low concentrations of Ba²⁺ and Cs⁺ (153), a feature that distinguishes them from Kᵢ₂P channels. Kir7.1 is an exception as it requires millimolar concentrations of Ba²⁺ and Cs⁺ for blockade (204).

The Kir K⁺ channel family has been divided into four distinct subsets according to their biophysical properties and physiological roles (153): classical Kir (Kir2.x), G protein-gated Kir (Kir3.x), KATP channels (Kir6.x/SURx), and K⁺-transport channels (Kir1.1, Kir4.x, Kir5.1, and Kir7.1). The present review focuses on the latter subgroup, composed of channels involved mainly in epithelial transport or Kir homoeostasis rather than simply controlling excitability.

Kir 1.1 was identified by expression cloning using a library from the renal outer medulla of the rat (hence the original name of this channel, ROMK) (157). Several isoforms have been identified, three in the rat, which are distinguished by short truncations at the NH₂ terminal of the protein (409). Kir1.1 is expressed mainly in the kidney. Kir4.1 and Kir4.2 were cloned independently by several groups (35, 123, 312, 362, 377). Kir5.1 was cloned by Bond et al. (35) together with Kir4.1 and Kir4.2. Kir4.1 is expressed in the brain, sensory organs, kidney, and stomach (35, 104, 260, 362); Kir4.2 in the brain, liver, pancreas, kidney, and lung (35, 315, 362, 381); and Kir5.1 in the kidney, sensory organs, and brain (151, 176, 315, 381, 394). The homotetrameric Kir5.1 channel is not functional in heterologous expression systems (35, 315, 316, 381), but surprisingly it can be found alone in different parts of the cochlea (151). It has also been reported that Kir5.1 can be driven to the plasma membrane in the presence of PSD95 anchoring protein (380). Kir5.1 is
presently considered as an associated, modulatory subunit of Kir4.1 and Kir4.2. Kir7.1, identified in 1998 (87, 204, 305), differs markedly in sequence from other Kir channels and has not been reported to form heteromers. Functional properties of Kir7.1 include low sensitivity to Ba$^{2+}$ and Cs$^+$ compared with other family members as well as a very low single-channel conductance (204). Also unusual for a Kir channel, Kir7.1 inward rectification properties are independent of K$^+$ concentration (87, 204), and its activity is PIP$_2$-dependent though showing rather low affinity for the phospholipid (306, 339). The unusual pore properties of Kir7.1 appear to be due to the presence of a methionine residue at position 125, which is occupied by an arginine in other Kir channels (204).

All these channels are sensitive to pH$_i$ as shown in FIGURE 2. Kir1.1, Kir4.1, and Kir4.2 are inhibited by intracellular acidification with pK$_o$ values of 6.5, 6.0, and 7.1, respectively (94, 216, 256, 315, 422, 423, 427). The association of Kir4.1 or Kir4.2 with Kir5.1 modifies the pK$_o$ of the heterotetramers, making these channels highly sensitive to pH$_i$ (Kir4.1-Kir5.1: 7.5; Kir4.2/Kir5.1: 7.6) (315, 421, 423, 427). At pH 7.3, a plausible figure for physiological pH$_i$, the open probability ($P_o$) of Kir1.1, Kir4.1, and Kir4.2 is nearly maximal; these homotetramers will be more sensitive to intracellular acidification than to alkalinization. The $P_o$ of the heterotetramers Kir4.1/Kir5.1 and Kir4.2/Kir5.1 is largely lower, around 0.3–0.4, implying high sensitivity to pH changes in both directions. Kir7.1 exhibits a bell-shaped dependence on pH (171) and appears rather insensitive to pH changes around pH 7.3. It is important to underscore that there are multiple interactions between sensitivity to pH and other regulatory processes (153, 169, 409, 420).

C. Gating Processes of K$^+$ Channels

The process by which ion channels fluctuate between open and closed states has been termed gating. As the name suggests, the pore of channels is regulated through the action of gates that can be opened to reach an active state or closed in a deactivated or inactivated state (154). The gating processes of K$^+$ channels have long been explored in functional experiments using ion channels in native cells or in heterologous expression systems utilizing cloned ion channels. The vision of channel morphology and the possible location of these gates garnered from early functional experiments has received reassuring confirmation with the more recent availability of high-resolution structures of K$^+$ channels (6, 429).

A key element in what were later to become known as K$^+$ channels was the description by Hodgkin and Keynes of the passive permeation of K$^+$ across the squid axon membrane as though “in a system in which ions move through the membrane in single file” (161). These findings presented a view of permeation through structures made of narrow tubes crossing at least part of the membrane thickness. Other experiments addressing the function of voltage-dependent K$^+$ channels in squid giant axons exploited the effect of intracellular injection of tetraethylammonium (TEA) to block the currents. It was observed that fast blockade of K$^+$ channels by TEA required the channels to be opened by depolarization and that gate closure could actually trap the blocker in what was deduced to be a pore cavity accessed from the intracellular space. These pioneering experiments by C. M. Armstrong provided the first, as it turned out remarkably accurate, idea of K$^+$ channel structure and its main gating process, as he concluded that “a K$^+$ pore has two distinct parts: a wide inner mouth that can accept a hydrated K$^+$ ion or a TEA$^+$-like ion, and a narrower portion that can accept a dehydrated or partially dehydrated K$^+$ ion, but not TEA$^+$” (4). The channel was therefore envisioned as a (proteinaceous) structure spanning the membrane and constituted of a narrow tube affording single file migration of at least partially dehydrated ions and an intracellular large vestibule accepting hydrated ions and larger molecules such as TEA derivatives. Gating was proposed to occur via the action of a sort of flap that would close or open the intracellular entrance of the vestibule (5).

Soon after cloning the nicotinic acetylcholine receptor, the first ion channel to be thus isolated, the same group led by Shosaku Numa cloned the Na$^+$ channel of Electrophorus electricus electroplax responsible for the depolarization of excitable membranes (288). The channel was a membrane
protein with four repeats of six transmembrane α-helices with several positively charged residues in helix number four, which immediately was suggested to play a role in sensing membrane potential. Cloning of K+ channels followed soon, the first being reported in 1987 and corresponding to a channel that when mutated in Drosophila flies gives rise to the phenotype known as Shaker (184, 302, 387). The Shaker K+ channel was predicted to form a protein with a single six-transmembrane domain structure with no repeats, and was later suggested to organize as a tetramer to form a K+ channel pore. Subsequently cloned K+ channels uncovered a high conservation in the linker joining transmembrane segments 5 and 6 (S5 and S6), suggesting that it might contribute to the formation of a selective pore, a concept that was supported by mutagenesis followed by functional expression in heterologous systems. Examples of these advances are papers that demonstrated that point-mutations in the S5-S6 linker affected the action of inhibitors known to interact with the pore (430) or producing marked changes in ion selectivity (431). It was also shown that exchanging the S5-S6 segment between channels with differing permeation properties also exchanged those properties (143). These advances identified the segment between putative transmembrane domains 5 and 6 as the pore-forming region, or P-domain, that confers its conductance and selectivity properties to this six transmembrane domain protein (373).

The identification of Shaker K+ channel brought about a flurry of activity in the field of molecular identification of K+ channels that now constitute a large superfamily of 88 human membrane proteins that share the highly conserved P-domain (434). They include voltage-gated Kv1–9 channels, Ca2+-activated KCa channels of similar general topology, and CNG/HCN channels that together with Kv10–12 are concerned with the operation of what has been termed the activation gate. A great deal is known, and debated, about the voltage-sensing mechanism (27, 228), but this will not concern us here as conventional voltage dependence does not appear to exist in the Kir and K2p channel families we are discussing. Although described in voltage-gated channels, such as for example the Na+ and K+ channels involved in the generation of the action potential in excitable cells, the mechanisms of channel opening and closing are thought to apply to gating processes obeying other stimuli such as membrane tension, phospholipid signaling, pH, intracellular Ca2+, etc.

A clue as to what must be the mechanism that opens and closes K+ channels arose from the discovery of the structure of another prokaryotic channel, the MthK K+ channel (180, 181). Comparison of KcsA and MthK structures revealed that whilst inner helices in the first bundle together at the intracellular COOH-terminal end, they were splayed apart in MthK. Evident in the MthK structure, there was a bend of ∼30° in the inner helices at what was called a “gating hinge” so the inner helices instead of bundling to-

D. K+ Channel Gating in the Era of Atomic Scale Structural Studies

The publication of the first atomic structure of a K+ channel obtained by the laboratory of Roderick MacKinnon initiated a new era in the understanding of ion channel function influencing the views about how these proteins select and conduct ions, how the pore is gated, and how the gating is coupled to relevant signals such as membrane voltage, second messengers, and the interaction with lipid or protein cell constituents. The emerging structural data came to confirm many of the general principles about channel structure deduced from early functional studies (245). The first K+ channel structure to emerge was that of a Streptomyces lividans KcsA channel which has only two transmembrane helices in addition to the P-domain (88). The protein was seen to organize as four identical subunits surrounding the permeation pore in a fourfold symmetrical way. As shown schematically in FIGURE 1C, the NH2 terminus of KcsA is intracellular, with TM1 as an outer helix that becomes a turret domain as it emerges extracellularly. A so-called pore helix enters about one-third of the membrane, and turns outwardly to form the selectivity filter (SF) with its highly conserved TVGYG signature sequence. TM2 is a pore-lining, inner helix that forms the COOH terminus of the protein at its intracellular end. The SF at the outer end of the pore ensures selectivity and conductance at rates approaching the diffusion limit. The section of the pore towards the intracellular end of the permeation pathway is wider with a large water-filled cavity lined by the TM2 transmembrane helices. The TM2 helices come together to form a packed bundle at their intracellular ends. This packing would become interesting in the context of the gating behavior of K+ channels.

Three different types of gating processes had been identified in functional studies of K+ channels. A first gating process was associated with the opening of voltage-gated channels selective for K+, but also for channels selective to other ions, brought about by changes in membrane potential and corresponding to the operation of what has been termed the activation gate. A great deal is known, and debated, about the voltage-sensing mechanism (27, 228), but this will not concern us here as conventional voltage dependence does not appear to exist in the Kir and K2p channel families we are discussing. Although described in voltage-gated channels, such as for example the Na+ and K+ channels involved in the generation of the action potential in excitable cells, the mechanisms of channel opening and closing are thought to apply to gating processes obeying other stimuli such as membrane tension, phospholipid signaling, pH, intracellular Ca2+, etc.

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gether are separate, leaving a wide intracellular entry pathway of ~12 Å. The gating hinge corresponds to a glycine residue located roughly halfway down the helix, which is highly conserved throughout the K⁺ channel superfamily. The gating suggested by the structures of KcsA and MthK beautifully explains experimental results using the Shaker K⁺ channel showing that the inner helix bundle coincides with a point where the pore becomes inaccessible to thiol-reactive compounds and metal ions applied at the intracellular side of closed channels (78, 79). Conservation of the glycine hinge in both Kir and K$_{2P}$ channels would suggest that this type of activation or inner gating exists in these channel families, a point that will be discussed in more detail below.

A second type of gating process characterized in many K⁺ channels takes place at the SF and corresponds to what has been termed C-type inactivation (166). The possible structural counterpart of C-type inactivation was again first suggested by structural information obtained with the KcsA channel crystallized in low K⁺ concentrations (443). This type of gating appears to close the channel by a deformation of the SF as it loses some of its coordinated K⁺ ions essential for the structure and partially collapses. This is consistent with the findings that C-type inactivation is dependent on the extracellular concentration of K⁺ or other permeant cations or TEA and is sensitive to mutation of residues in the SF region (56, 195, 231). Although C-type inactivation gating can act independently, there is evidence that it is often coupled to the activation gate whose opening is followed by closure of the SF gate (21, 301). More recent structural work using the KcsA channel reports channels at different degrees of activation gate opening. Strikingly there is correlation between the degree of gate opening and presence of ions in the SF, where the wider this opening the lower the occupancy of the SF by permeant ions and therefore its capacity to conduct (66).

A third mode of gating known as ball-and-chain or N-type inactivation, which unlike C-type inactivation occurs with a fast time course, involves an autoinhibitory open pore block by an NH$_2$-terminal portion of the channel protein (165). This type of gating has not been reported for the Kir and K$_{2P}$ channels that occupy us here.

II. MOLECULAR ASPECTS OF pH-DEPENDENT K$_{2P}$ AND Kir CHANNEL GATING

A. K$_{2P}$ Channel Structure

The study of the relationship between K$_{2P}$ channel structure and function took advantage of the high degree of homology between these channels and those for which structures were available to produce homology models to guide experimental work or simply to gain mechanistic insights into function (202, 284, 319, 374, 435). Recently, the field of K$_{2P}$ channels received with delight the publication of the first two X-ray structures of K$_{2P}$ channels, those of TRAAK and TWIK-1 (42, 259). The structures were remarkably similar, and the features commented upon here about TWIK-1 generally apply to TRAAK. Figure 3 illustrates the general structure of TWIK-1 where the salient features are identified. As predicted, the selectivity filter of these dimeric channels has a quasi fourfold symmetry coordinating K⁺ ions as in their tetrameric relatives despite their differences in amino acid sequences, particularly in TWIK-1. Examination of inner helices shows that the equivalent of the activation gate is wide open. G141 and G256 are positioned in the inner helices at a location equivalent to those residues that form the gating glycine hinge in other channels and are conserved in K$_{2P}$ family. M2 helix is notable for being longer and bending at a proline residue halfway down the membrane (P143, Figure 3), while M4 is shorter and straight.

A remarkable feature of TWIK-1 and TRAAK structures is the presence of a helical cap that sits at the extracellular side of the channel apparently obstructing access to the pore mouth normal to the membrane. This cap corresponds to the large extracellular loop known as self interacting domain (SID) that is stabilized by a disulfide bridge (C69, Figure 3) essential for function in TWIK-1 (222) but not in every K$_{2P}$ channel (282). The extracellular cap is formed by two α-helices from each subunit that are connected to the pore-forming portion of the channel at bend-forming residues G89 and P47 (Figure 3), which are conserved among K$_{2P}$ channels. An amphipathic helix located near the membrane/cytosol interface, the C helix (Figure 3), is speculated to serve a gating purpose (259). This structure is not conserved in TRAAK, where instead an amphipathic helix at the intracellular end of the first inner helix (TM2) is seen to be positioned to interact with both the hydrophobic tails and charged heads of membrane phospholipids (42).

Access to the external mouth of the pore is provided by long, tunnel-like entrances of molecular dimensions, the extracellular ion pathways (EIP). This type of arrangement explains the resistance of K$_{2P}$ channels to inorganic and toxin pore blockers (100, 219). The presence of the EIP also offers the possibility for interaction between ions affecting the state of the SF in a confined space where electrostatic interactions might develop less hindered by solvent screening than in free solution. Both K$_{2P}$ channel structures have unusual prominent fenestrations connecting the inner part of the pore and the lipidic milieu of the membrane. This type of arrangement, which has also been seen in the voltage-gated sodium channel structure, might help in supporting the effect of lipidic signals that affect the gating of several K$_{2P}$ channels (e.g., Refs. 51, 289). Future X-ray work will surely reveal more secrets of the K$_{2P}$ channel structure-function relationship. Already a new, higher resolution TRAAK structure has been generated showing a domain-swapped chain connectivity exchanging outer helices 180°
around the channel, plus an unrelated conformational change of an inner helix sealing a fenestration to the bilayer and associated structure changes around the SF with possible functional consequences (41). Interestingly, the domain-swapped type organization can be observed in the crystal structure of human TREK-2 (K2P10.1) recently deposited by Carpenter et al. (PDB ID: 4BW5). It might turn out that this form of organization could apply to the K2P family at large.

B. Gating of K2P Channels by Extracellular pH Takes Place at the Selectivity Filter

As discussed above, a type of gating occurring in various K+ channels and thought to take place at the SF might involve a profound change in its structure (429) and corresponds to what has been termed C-type inactivation (166). Gating at the SF is characterized by its dependence on extracellular K+ concentration and is particularly sensitive to mutations near or at the external opening of the conduction pore. Opening and closing of various K2P channels by changes in extracellular pH has been demonstrated to entail extracellular K+-dependent C-type inactivation. Other forms of gating, including that modulated by changes in pHo, have been attributed to changes in the bundle crossing or internal gate (see Refs. 64, 253 for reviews). But recent evidence, to be discussed here, has very seriously upset this view.

First inklings for a prominent role of gating at the selectivity filter in K2P channels came from work with the Drosophila KCNK0 channel. KCNK0 channels gate by switching between open and closed states driven by protein kinases C, A, and G acting at the COOH-terminal end of the protein (445). That the gating process occurs through changes at the SF was suggested by the effect of extracellular Zn2+ that inhibits KCNK0 in a state of activation-dependent manner and requires the presence of a histidine residue in P1, demonstrating that gating affects the conformation of the outer side of the pore even if phosphorylation occurs at the intracellular COOH terminus (444). Additional evidence pointing to a C-type inactivation process was that the effects of the external permeant ion, extracellular TEA, and mutagenesis of residues near the SF affecting KCNK0 were as predicted for the analogous phenomenon in voltage-dependent K+ channels (444).

TASK-1 and -3 as well as TWIK-1 are gated closed by extracellular H+. The sensor for this effect is a histidine residue located at the mouth of the SF at a position occupied by negatively charged glutamic acid in most K+ channels (194, 230, 325, 326). It is therefore easy to conceive that the action mediated by the histidine sensor, presumably neutral at physiological pHo, will be to interfere with SF permeant ion occupancy leading to C-type inactivation upon acquiring a H+ with acidification.

The conductance of TASK-1 was found very early on to be dependent on extracellular K+ concentration (90). In addition to this dependence, extracellular K+ also affected

FIGURE 3. Overall structure of TWIK-1 derived from its 3.4-Å-resolution crystal structure. A: tertiary structure of TWIK-1 shown in a ribbon representation viewed from the side. One subunit is colored blue-to-red from the NH2 to the COOH terminus. The other subunit is gray. K+ ions are represented as green spheres. Approximate boundaries of the lipid membrane are shown as horizontal lines. The intersubunit disulfide bond (at C69) at the apex of the extracellular cap is in green. The location of P143 and hinge glycines G141 and G256 is also shown. E1 and E2 are extracellular cap helices. Bends in the structure at the base of the extracellular cap are afforded by G89 and P47. C helix is stretch of a-helix running parallel to the membrane at the COOH terminus of the channel. Loop regions not included in the final model are indicated as dashed lines. The central cavity is located below the K+ ions along the axis of symmetry (behind G141). The intramembrane fenestration is visible above the C-helix. B: a view after a 90° rotation around the axis of symmetry. The extracellular ion pathway is seen above the outermost K+ ion. [From Miller and Long (259). Reprinted with permission from AAAS.]
TASK-1 gating modulation by pHo, a result that is expected from an effect taking place at the SF (229, 230). An idea of the possible structure of TASK-1 was obtained by homology modeling using the crystal structure of KcsA as a template by Yuill et al. (435). This work suggested that histidine-98 (H98) pHo sensor was located behind the SF. Protonation of H98 at acidic pHo was proposed to distort the SF leading to a nonconducting state. Site-directed mutagenesis affecting ion selectivity brought about modifications in agreement with pHo-gating taking place at the SF (435). Later, molecular dynamics studies showed that protonation of H98 is accompanied by its removal from behind the SF and by loss of a coordinated water molecule that contributes to the structural stability of the filter. The flipping away of H98 from behind the selectivity filter is also proposed to create an electrostatic barrier to K+ ions at the outer mouth of the pore (370).

Although closely related, there are differences in the pHo responses between TASK-1 and TASK-3. These include differences in pHo sensitivity and in the cooperative nature of pHo effect and the effect of the permeant ion (122). Some of these differences may be due to an effect of the TM1-P1 extracellular loop on the pHo sensors. Clarke et al. (60) exchanged TM1-P1 domains between TASK-1 and TASK-3 and demonstrated the importance of this loop in pHo dependence and Zn2+ blockade. The effect of pHo on TASK-3 gating is cooperative occurring with a Hill number nH of 2 (194). The same holds true for TASK-1 but only at high extracellular K+ whilst the effect of pHo occurs with an nH of 1 at physiological K+ (230). A study in TASK-3 determined that H98 pHo sensors in both subunits of TASK-3 channel need to be neutral for the channel to open, with neutralization of H98 in one subunit enhancing the propensity of H98 in the second to become neutral. The permeant ion plays a central role in this change of acidity of the histidine sensor, an effect that is enhanced by the electrostatic effect of glutamate E70 (122). This glutamate is part of the wall of the EIP of K2p channels (see above), is known to be close to the H98 sensors (60), and is proposed to influence the electrostatic potential in EIP, presumably becoming a major determinant of EIP K+ concentration and affecting the occupancy of the outermost SF K+ binding sites (122). A similar mechanism might take place in TASK-1 where E70 replaced by a lysine and the K+ effect is only manifest at high concentrations. The importance of electrostatics of the EIP is also highlighted by the effect of TASK-3 blockers Zn2+ and ruthenium red which depend on the presence of the E70 residue (59, 60, 69, 265), requires a neutral H98 (59, 60), is cooperative, and is strongly impeded by increasing extracellular K+ concentration (122).

TWIK-1 pHo gating is a process in which the occurrence of changes at the SF becomes manifest through major alterations in ion selectivity, with TWIK-1 changing from being K+ selective to a generally monovalent cation-permeable channel (49, 244). The study of TWIK-1 has been difficult because of a generally low plasma membrane expression. The low currents observed for TWIK-1 in heterologous expression systems have been interpreted as TWIK-1 K+ channels having a very low Pn, in the cell surface owing to silencing by sumoylation at residue K247 in the intracellular COOH terminus (325). Desumoylation with SUMO isopeptidase is reported to open silent TWIK-1 channels reversibly in isolated membrane patches (321). This mechanism has also been proposed to regulate TWIK-1 activity in cerebellar granule neurons (322). The concept of TWIK-1 regulation by sumoylation has been directly challenged (95), and the alternative view has been advanced that TWIK-1 silencing is due to its internalization to the recycling endosomal compartment by a dynamin-dependent mechanism (96). This mechanism, dependent on a COOH-terminal di-isoleucine motif, is thought to be responsible for the localization of TWIK-1 in native renal epithelial cells and nonpolarized cells in the subapical and pericentriolar recycling compartments, respectively. Independently of the mechanism involved, use of mutated TWIK-1 channels has allowed its detailed functional study in heterologous systems. The change in ion selectivity upon acidification is unique among pH-sensitive K2p channels with significant increase in the permeability to Na+ relative to that to K+ upon acidification to pH 6. Paradoxically, there is a transient increase in current upon acidification before inhibition takes place. Histidine-122 acts as H+ sensor and is necessary for both phases of the effect, but the results suggest that additional, as yet unidentified sites contribute to inhibition by acidification. TWIK-1 differs significantly from other K2p channels in the P1 and P2 domains, the pore helix of the first P1 domain among other changes, which might account for its markedly different functional behavior (49).

TASK-2 is activated by alkalinization with a pK1/2 of around 8 and no evidence for cooperativity (284, 335). Decreasing pHo to 6.0 abolishes TASK-2 channel activity. A group of five charged residues in the TM1-P1 extracellular loop affects pHo-gating sensitivity of TASK-2 (261, 283), but coupling of TASK-2 activity to extracellular alkalinization is mediated by neutralization of R224 near the second pore domain (284). In the protonated form, R224 might decrease occupancy of the SF by K+ leading to a blocked state. Analysis of free-energy profiles delineating ion permeation in the SF studied in silico (446) suggests that protonated R224 electrostatically increases the height of energy barriers between binding sites impeding ion movement. The pK1/2 of the pHo effect on TASK-2 measured under quasi-physiological K+ concentration gradient ([K+]/[K+]o ~140/5 mM) decreases with depolarization (281, 335). Voltage dependence of
the effect by charged compounds on ion channels can be interpreted on the basis of a model where H⁺ interacts with a site within the electric field of the membrane (417), as has been proposed for H⁺ effect on TASK-1 channels (230). This does not seem to be the case for TASK-2, however, as there is no voltage dependence of pH₀ gating measured in symmetrical high K⁺ concentrations ([K⁺]/[K⁺]₀ 140/140 mM) (58). The effect has been interpreted to occur by changes in SF, or EIP, occupancy by K⁺/H₁1001 reversed by depolarization-promoted flux of K⁺ sumed at the outermost coordination sites, a situation as has been proposed for H⁺ with a site within the electric field of the membrane (417), sensing residues. At low [K⁺]₀, a low occupancy is assumed at the outermost coordination sites, a situation reversed by depolarization-promoted flux of K⁺ from the intracellular K⁺-rich compartment. These external sites would already be highly occupied when high K⁺ concentration is used at both sides of the membrane, hence the lack of voltage dependence (58, 281).

Extracellular acidification inhibits TREK-1 but activates TREK-2 within the same pH range. The mechanism for this difference has been explored by Sandor et al. (346). The proton sensor in TREK-1 and -2, conserved histidines H₁26 and H₁51, respectively, is located in the TM1-P1 loop preceding P1 domain. Molecular modeling and mutagenesis indicate that the differential effect of acidification in TREK-1 and TREK-2 resides in differences in residues in the also extracellular P2-TM4 loop leading to attraction or repulsion between the protonated sensing histidine and closely located negatively or positively charged residues impacting the SF gate of the channels. This is supported by effects of extracellular K⁺ concentration on the pH₀ dependence of these channels (63, 346).

C. Gating of K₂p Channels by pHᵢ

pHᵢ prominently affects the gating of TREK-1 and -2 with activity increasing with decreases in pHᵢ (163, 247). The gating of these channels is complex, but at the same time it has been extensively studied (see specific reviews in Refs. 77, 162, 289). TREK-1 is activated by a variety of chemical and mechanical stimuli such as arachidonic acid, stretch, and phosphorylation. Internal acidification converts low-activity TREK channels into high-activity channels. Glutamate E₃₀₆ behaves as the intracellular proton sensor in this activation by acidification process, and its neutralization by mutation converts TREK-1 into a permanently active K⁺ channel. Membrane phospholipids open TREK-1 channels, and a cluster of positively charged residues is the phospholipid-sensing domain. E₃₀₆ is found within this stretch of positively charged residues, and its negative charge counteracts their ability to interact with the membrane. Protonation of E₃₀₆ by acidification or neutralization by mutation is thought markedly to enhance channel-phospholipid interaction thus favoring TREK-1 opening. Although revealing a series of molecular details, these studies did not disclose which gate was put into action to mediate the effect of pHᵢ in modulating opening and closing of TREK-1 channel. Nevertheless, cartoon illustrations of the phenomenon (e.g., FIGURE 3 in Ref. 162) left one with the impression that it might correspond to the activation, bundle crossing gate.

TASK-type K₂p K⁺ channels are insensitive to pHᵢ (93), but TALK-subfamily K₂p K⁺ channels TASK-2 and TASK-2 are activated by increases in pHᵢ (281). The pHᵢ dependence occurs in the same pH range as the pH₀ dependence but is unrelated as pHᵢ-insensitive TASK-2-R224A is not altered in its pHᵢ dependence. A truncation and directed mutagenesis approach identified COOH-terminal K245 as pHᵢ sensor. It was proposed that separate gates mediate pH₀ and pHᵢ gating of TASK-2, as pHᵢ gating lacks voltage and [K⁺]₀ dependence, both typical of pH₀ gating (281). Based on this separateness and on the location of the pHᵢ sensor, it was argued that the gate affected by pHᵢ might correspond to the bundle crossing gate (281).

The existence of separate inner helices bundle crossing opening/closing gating mechanism in K₂p channels has been approached in an examination of the voltage dependence of TASK-3. This study advocated the existence of an activation gate that could be interfered with by mutation of putative hinge glycine residues in TM2 and TM4 (9). Ben-Abu et al. (25) used roughly the TM1-P1-TM2 transmembrane portion of the KCNK0 structure transplanted into a Shaker Kᵥ to supplant the whole of its TM5-TM6 region. This structure possessed an activation gate that could be opened by depolarization. This demonstrated that the bundle crossing machinery can be put into action using a K₂p channel conducting structure and the voltage-sensing portions of a Kᵥ channel, albeit under artificial conditions including the use of a single set of KCNK0 TM-P-TM units in a tetrameric arrangement.

A direct examination of this problem was more recently carried out after the discovery by Piechotta et al. (319) that hydrophobic quaternary ammonium ions are rather efficient intracellular blockers of K₂p channels TRESK, TREK-1, and TASK-3. Experiments exploring the access of inhibitors to the inner vestibule of TREK-1 channel suggest that gating by pHᵢ and pressure do not involve gating transitions taking place at an inner helix bundle-crossing location, as inhibitors reach their site of action freely even when the channel is closed (319, 331). This very clear set of experiments indicates that the primary activation mechanism in TREK-1 channels is close to or at the SF and that the activation gate is locked permanently open. Thermal- and pHᵢ-sensing elements are found at the intracellular COOH terminus of TREK-1, but this sensing is probably translated into changes at the SF. This separates the sensing elements from remotely located action elements of the gating process in K₂p channels (10, 11). In retrospect, this idea was already implicit in the work of Zilberberg and co-workers (444, 445) who demonstrated that phosphor-
ylation of the COOH terminus of KCNK0 channel was translated into marked changes in activity with the hallmark of C-type inactivation at the SF. Nevertheless, even for TREK-1, observations pointing to separate gates for pH₁ or AKAP150 and pH₂₀ (346) will have to be incorporated into an integrated view of channel gating. Whether the hypothesis that the main gating process in TREK-1 is located at the SF, with the inner gate being locked open, can be extended to the rest of the family members will have to await further work.

In view of the paucity of K₂P inhibitors, the pharmacological tools introduced by Piechotta et al. (319) should become useful in probing not only the gating of other K₂P channels but also their function in native cells and tissues.

D. Structure-Function Studies in Kir Channel Gating

The inwardly rectifying K⁺ channels known as Kir are important regulators of excitability but also, and particularly with the group of channels we are concerned with in this paper, they support a variety of transport processes particularly in epithelia. The Kir family of proteins has been comprehensively reviewed (153), and therefore, we will center here on specific aspects relating to their gating by pH and on some new aspects of how molecular structure relates to function. As illustrated in FIGURE 1, Kir channels are made of only two transmembrane domains and lack a voltage sensor. There is, however, evidence for both C-type inactivation gating at the SF and for activation gating that opens the intracellular access to the inner vestibule of the channel by splaying apart/turning the TM2 pore lining transmembrane domains.

None of the Kir channel types we discuss in the present work has been crystallized. As for other classes of K⁺ channels, their prokaryotic counterparts were the first to prove amenable to successful exploration using X-ray diffraction methods. The first of the Kir channels to disclose its structure was KirBac1.1 (206). The structure of KirBac1.1 showed a transmembrane portion of the TMD in general quite reminiscent of that of KcsA, with the selectivity filter at the extracellular entrance connecting to an intramembrane cavity. Flexible linkers connect the TMD with a large cytoplasmic domain (CTD) made of numerous β-sheets and enclosing a large intracellular pore. A similar structure emerged from the analysis of a chimera between the TMD of bacterial KirBac3.1 and mammalian Kir3.1 CTD (287) and later for full-length eukaryotic Kir channels, many of which are Kir2.2 and Kir3.2 (386, 411). These main organizational features are illustrated in FIGURE 4A that reports the structure of Kir3.2 (GIRK2), a Kir channel gated by G protein (411). The CTD is interesting as it prolongs the length of the pore in an unusual way with respect to channels from other subfamilies. The intracellular reaches of the pore are contained within the CTD and harbor the sites responsible for interaction with particles, such as Mg²⁺ or polyamines, responsible for the inward rectification phenomenon. The cytoplasmic domain is also the structure through which the gating of Kir channels can be regulated by a variety of stimuli such as ATP, Na⁺, pH, and signaling proteins or lipids (29, 153). Early structures such as that of KirBac1.1 presented a closed channel as the intracellular exit pathway of the transmembrane pore was occluded by the bundling of TM2 helices (61, 206, 287, 386). In more recent structural studies, Kir channels in various states of activation by physiological modulators such as G protein subunits and PIP₂, or by mutation, have provided a picture of pore opening (22, 142, 411, 412). PIP₂ binding to Kir2.2 was seen to induce a transition as though there was an upward movement of the CTD to make contact with the TMD producing a preopen PIP₂-bound channel (142). Whorton and MacKinnon (411) resolved the molecular structures of wild-type (WT) G protein-gated K⁺ channel Kir3.2 and a single point mutant (R201A) that is constitutively active, both in the absence and presence of the physiological activator PIP₂. The resulting structures were used to deduce the changes taking place in the channel in the transition from the closed to the open state. Unlike other K⁺ channels, GIRK channels possess two activation gates, the conventional inner helix gate and a G-loop gate (FIGURE 4). The channel was found to present the inner helix gate in the closed state in all but in the R201A mutant in the presence of PIP₂, where it appears wide enough to allow conduction of a hydrated K⁺ ion (FIGURE 4B). Mutation R201A is viewed as mimicking the action of G proteins in opening the G loop gate. Complete opening with inner helices splaying open requires the presence of PIP₂. Channel opening occurs through a conformational change in the CTD acting allosterically to control the gates in the pore. Later work has confirmed this view and associates a twisting motion of the CTD in the opening of Kir gates by action of Gβγ subunits and PIP₂ (412). These structural studies suggest that an inner or activation gate is functional in Kir channels. In addition, analysis of the structure reveals a mechanism for channel gating in which TM2 bending at the glycine hinge and bundle crossing splaying is coupled to a twist of the CTD. Channel activation coupled to a CTD conformational change of the nature described could account nicely for the action of various intracellular signals that modulate Kir channels. It is not known whether changes in pH₁ exert their regulatory effects on Kir channels through a similar mechanism.

E. Molecular Mechanism of the Regulation of Kir1.1 by pH₁

All transport type channels of the Kir superfamily are pH₁ sensitive. Among those inhibited by intracellular acidification are Kir1.1 (ROMK), Kir4.1, and Kir4.2, with Kir5.1 acquiring pH₁ sensitivity when expressed together with either Kir4.1 or Kir4.2. Kir7.1 has a biphasic, shallow depen-
dence on pH with a peak of activity at around physiological pH and partial inhibition with either acidification or alkalization. The molecular mechanism of Kir channel pH regulation has been intensively studied but remains rather mysterious.

The gating of ROMK1 channels, the first Kir channel to be molecularly identified (157), by protons has been extensively scrutinized, a body of work whose basis has been ably summarized in recent reviews (145, 153) and will not be rehearsed here. ROMK1 or Kir1.1 channels are closed by intracellular acidification with a \( pK_{1/2} \) of 6.5, and the mechanism for this regulation was ascribed to a trio of basic residues R41, K80, and R311. The TM1 lysine residue was identified as the sensor itself, i.e., the residue whose side chain that becomes protonated at neutral pH drove the gating of ROMK (353). As lysine \( \epsilon \)-amino group \( pK_a \) in solution is 10.5, a marked shift in titration is necessary for K80 to act as the pH sensor of Kir1.1 channels, and purportedly accomplished by NH2 and COOH terminus R41 and R311 closeness in the tertiary structure of the protein affecting K80 \( pK_a \) electrostatically. More recent studies, however, cast doubt on the role of K80 as a bona fide pH sensor. In 2006, Rapedius et al. (329) examined the issue of K80 as the pH sensor of ROMK channels by looking at its conservation across species, finding that it is generally highly conserved in vertebrates, but a neutral hydrophobic residue was present at the equivalent location in zebrafish and Fugu. Despite this, Fugu Kir1.1 exhibits a healthy pH dependence (329). Homology modeling of Kir1.1 implies indeed that K80 occurs in a lipidic environment compatible with it being permanently protonated and that R41 and R311 are too far to affect K80 electrostatically. Instead, it is considered that K80 participates in a TM1-TM2 H bonding at the bundle crossing influencing the pH sensitivity of Kir1.1 by stabilization of the closed state (328, 329). An independent study by Leng et al. (217) also probed the validity of the K80 pH-sensor hypothesis upon noticing that residues scattered along the NH2 and COOH termini of Kir1.1 modify its pH sensitivity. They proposed that salt bridges forming subunit interactions at the NH2 and COOH termini of the channel, and crucial for stabilizing the open state, are affected by proton concentration and therefore account for pH gating.

Challenging of the hypothesis that K80 acts as a proton sensor in Kir1.1 begs the question of what is the main sensor prompting the closing of Kir1.1 and other transport Kir
channels. A systematic search for such sensor(s) has been done using randomly mutagenized Kir1.1 expressed in a K^+-auxotrophic strain of *S. cerevisae* in a high-throughput assay (311). This unbiased approach failed to identify any pH_sensor candidate residue in Kir1.1. Instead, the idea was put forward that pH modulation takes place by an effect on an intrinsic gating mechanism common to all Kir channels with pH sensitivity obeying the titration of a number of intracellular domain salt-bridges stabilizing the channel in the open state. Differences in Kir channel pH sensitivity can therefore arise by altering the relative stability of the open and closed states rather than the presence of additional pH sensors. The alanine scanning mutagenesis approach coupled to electrophysiological and homology modeling analysis of Kir 1.1 has also been used to study the sensitivity to pH, (34). The results show that a large number of mutations destabilize the open state and that pH gating may operate at the transition between the open and closed conformations previously identified in crystallographic analyses of Kir channel gating (22, 142, 411, 411).

### III. FUNCTIONAL ROLES OF Kir AND K\textsubscript{2p} IN RENAL EPITHELIAL CELLS

Many K^+ channels have been found to be expressed in the kidneys. Here, we will concentrate on the data on Kir1.1, Kir4.1, Kir4.2, Kir5.1, and TASK-2 and refer to other channels only when sound data on the native tissue is available. Indeed, some K^+ channels have been described using the patch-clamp technique, but there is no clue about their molecular identity. Conversely, others have been detected in parts of the renal tubule using in situ hybridization, RT-PCR, or immunohistochemistry, but there is no functional data available, for example, TWIK-1 (62, 220, 279) and KCNA10 (428) in the proximal tubule as well as TWIK-1 (279, 295), TASK-2 (335), and KCNQ1 (KVLQT1) (80) in the distal nephron. Kir7.1 has been shown to be present in the basolateral membranes of rat distal convoluted tubule, connecting tubule, and cortical collecting duct (except for the intercalated cells), and at much lower intensity in the thick ascending limb and outer medullary collecting duct (294); in contrast, Derst et al. (81) found that Kir7.1 was expressed in the basolateral membranes of the guinea pig and human proximal tubule and thick ascending limb.

#### A. Basolateral K^+ Channels: Recycling of Potassium

One chief function of the kidneys concerns sodium balance. The stock of sodium in the organism controls extracellular volume and volemia, and thereby regulates the arterial pressure on the long term according to Guyton’s hypothesis (140). The fine regulation of sodium balance is achieved by the kidneys according to the following steps: glomerular filtration of an enormous quantity of sodium (~25 mol/day), bulk reabsorption in the proximal tubule and thick ascending limb, and finely tuned reabsorption in the so-called aldosterone-sensitive distal nephron, which comprises the distal convoluted tubule, connecting tubule, and collecting duct (97, 128, 372). The various mechanisms governing sodium reabsorption in different parts of the renal tubule are shown in Figure 5 and can be described as follows: entry at the apical membrane via a cohort of coupled transport systems (Na^+/H^+ exchanger, Na^+-K^+-2Cl\textsuperscript{-} cotransporter, Na^+-Cl\textsuperscript{-} cotransporter, etc.) or channels (ENaC) and exit at the basolateral membrane via the Na^+-K^+-ATPase. A bicarbonate/sodium cotransporter participates in this process in the proximal tubule.

Within this context, basolateral K^+ channels have two main functions: they maintain a negative membrane potential (around ~70 mV in renal cells), which is important for electrogenic transport of substances, and recirculate the potassium entered into the cells via the Na^+-K^+-ATPase. This second function is crucial in epithelia in general, and in renal cells in particular, where high-rate transepithelial Na^+ absorption occurs. The “recycling” of potassium was proposed as early as in the 1950s when Koefoed-Johnsen and Ussing (196) produced their seminal scheme of Na^+ absorption in frog skin. Several studies have documented a possible coupling between the pump and the potassium channels in the basolateral membranes of the renal tubule (203, 266, 393, 407). It should be emphasized however that the apical K^+ channels, in addition to their specific function of secreting potassium (see next section), may participate in K^+ recycling together with basolateral K^+ channels. Indeed, this phenomenon can be understood as the necessity of balancing K^+ influxes and effluxes across the cell membranes. The apical or basolateral location of efflux may then be considered as secondary. However, the basolateral K^+ conductance is generally higher than the apical one and plays a dominant role, at least in the proximal tubule (212).

In the past, several groups using microelectrode recording techniques have described the properties of basolateral K^+ conductance in most renal segments of the amphibian, rabbit, rat, and mouse kidneys. In most cases, a large K^+ conductance highly sensitive to barium was detected (320, 347, 349, 371, 432, 433), anticipating the discovery that Kir channels had a major importance in renal function. It was also observed that intracellular acidification depolarizes the cell membrane in the proximal tubule, likely by inhibiting K^+ conductance (24, 28) (see also Ref. 212). With the availability of the patch-clamp technique, a wealth of information on the basolateral channels was then obtained showing that many distinct K^+ channels were present in the renal tubule (23, 155, 156, 210, 237, 241, 254, 255, 310, 336, 337, 385; see also Ref. 143).
B. The Population of Basolateral K\(^+\) Channels in the Proximal Tubule Includes Kir Channels and the K\(_{2p}\) TASK-2 Channel

The proximal tubule was one of the first segments to be searched for K\(^+\) channels. In the amphibian proximal tubule, whole cell recording studies reported a dominant K\(^+\) conductance that was barium sensitive and inwardly rectifying (172, 336, 357). At the single-channel level, a 25- to 30-pS, inwardly rectifying channel was described (172, 190, 254, 255), the properties of which were studied in great detail by Mauerer et al. (254): the channel was inhibited by ATP (>2 mM), blocked by glibenclamide, a classical inhibitor of K\(_{ATP}\) channels, but only at very high concentrations (0.5 mM), and activated by the channel opener diazoxide. The channel also required ATP for maintaining its activity in cell-free patches (254, 255). In the rabbit proximal tubule, several studies also reported the properties of an inwardly rectifying K\(^+\) channel with a conductance of 40–50 pS (23, 116, 173, 303) that was sensitive to ATP (23, 173). The group of Lapointe proposed that this channel belonged to the K\(_{ATP}\) family (40). In contrast, there is no precise description of K\(^+\) channels in the mouse or the rat proximal tubule. Likewise, their molecular identity remains undetermined. Kir4.1 is clearly not present in the proximal

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**FIGURE 5.** Mechanisms of sodium reabsorption in the mammalian renal tubule. A: proximal tubule. Many distinct Na\(^+\)-coupled transport systems are present on the apical membrane of the proximal tubule. On the basolateral side, together with the Na\(^+\)/HCO\(_3\) cotransporter, many facilitated transport systems (not represented) allow the exit of glucose, phosphate, etc. The K\(^+\) channels have not been identified with certainty, except for Kir6.1, an ATP-sensitive K\(^+\) channel. B: thick ascending limb. The Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter NKCC2 is the main actor of NaCl reabsorption in the apical membrane. K\(_{Ca}\)4.1 (also named slo2.2) is a high-conductance Na\(^+\) and Cl\(^-\)-sensitive channel; 80pS represents an unidentified channel type. C: distal convoluted tubule. The Na\(^+\) and Cl\(^-\) cotransporter NCC is present on the apical membrane. The chloride channels in the thick ascending limb and the distal convoluted tubule are ClC-K1 and ClC-K2 (ClC-Ka and ClC-Kb in humans) associated with the regulatory subunit Barttin. D: connecting tubule/collecting duct. Sodium enters the cell from the apical side via the epithelial Na\(^+\) channel, ENaC. In this renal segment, part of sodium is exchanged for potassium, while some chloride is absorbed through the intercalated cells or the paracellular pathway. K\(_{Ca}\)1.1 (also named Slo1, maxi K or BK) is a high-conductance calcium-activated channel; 20 & 80pS represent as yet unidentified channel types.
The expression of TASK-2 is more firmly established: using TASK-2 knockout (KO) mice in which the LacZ marker gene was inserted, Barrière et al. (19) observed TASK-2 promoter-driven staining by β-galactosidase all along the proximal tubules. In addition, recordings of whole cell currents from proximal cells in primary culture demonstrated that cell swelling-activated K⁺ currents, inhibited by quinidine and clofilium, were present in WT but not in TASK-2 KO cells. A follow-up study on primary cultures of proximal cells further demonstrated that TASK-2 currents were activated during HCO₃⁻ transport provided that extracellular pH is increased (209, 406). These interesting results led Warth et al. (406) to hypothesize that TASK-2 activity might be linked to extracellular pH variations and help maintaining membrane polarization during HCO₃⁻ reabsorption. However, it is not known whether bulk reabsorption of Na⁺, HCO₃⁻, and water is associated with a measurable local increase in pH at the basolateral side of the proximal tubule cells. In addition, the expected presence of TASK-2 K⁺ channels in the basolateral membrane has not yet been ascertained. Likewise, no patch-clamp study has identified TASK-2 in the native proximal tubule. Nevertheless, the fact that TASK-2 KO mice appeared to have a reduced maximum capacity for HCO₃⁻ transport in vivo and exhibited slight acidosis are a strong argument in favor of Warth et al.’s hypothesis (406).

C. Kir4.1/Kir5.1 Supports a Major Basolateral K⁺ Conductance in the Distal Nephron

The distal nephron comprises the thick ascending limb, early and late distal convoluted tubule, connecting tubule, and collecting duct; of note, the two latter segments are composed of different cell types, i.e., the principal and intercalated cells. As in the proximal tubule, the basolateral K⁺ conductance in the distal nephron is dominated by a Ba²⁺-sensitive conductance (127, 347, 349, 432, 433) that was shown to be inhibited at acid pH in the rat collecting duct (348). In addition to the Kir4.1/Kir5.1 dominant type described below, several additional K⁺ channels have been described: a Na⁺-dependent high-conductance K⁺ channel of the slo2.2 type (Kₛa,4.1) in the mouse thick ascending limb (309) and various channels in the rat and mouse collecting ducts (Ca²⁺-inhibited, 200-pS channel (155); 85-pS channel (402); 20-pS channel stimulated by cGMP-dependent protein kinase (403, 407) probably underlain by Kir2.3 (258, 405, 408)).

However, the predominant channel along the distal nephron is an inwardly rectifying channel, of ~50 pS which was initially reported in the rabbit thick ascending limb and distal convoluted tubule (174, 385). More recent studies have concentrated on the distal nephron of the mouse where a major channel with an inward conductance of 40–45 pS was described in the thick ascending limb, distal convoluted tubule, connecting tubule, and cortical collecting duct (210, 237, 308, 310). This channel is the only one detected in the distal convoluted tubule (237). Inward rectification with a Gₘᵢᵣto-Gₘᵞₒᵤᵣ ratio of 3–5 was due to intracellular Mg²⁺ (210, 237, 310). As is the case for other K⁺ channels, the inward unit conductance was dependent on external K⁺ concentration over a large range of concentrations (Kₘᵢᵣ = 65 mM, Gₘᵞₒᵤᵣ = 48 pS) and the channel was inhibited by spermine, which reduced the Pₒ (210, 237, 310). In outside-out patches, Ba²⁺ at 0.1 mM blocked the channel by 70%. In addition, following excision, channel activity ran down progressively in the presence of Mg²⁺ (237). The channel exhibited great sensitivity to pH (pKₐ = 7.2–7.6) but was not inhibited by ATP (210, 237, 310). All these properties compare well with the general properties of Kir K⁺ channels and more particularly with those of Kir4/Kir5, prompting the proposal that they are Kir4.1/Kir5.1 heterotetramers (210, 237). The renal localization of Kir4.1 and Kir5.1 channels, examined using immunofluorescence in the rat, mouse, and human kidneys (33, 178, 210, 237, 315, 334), is coherent with this hypothesis. The general pattern shows that Kir4.1 and Kir5.1 are present in the basolateral membranes of the distal convoluted tubule and principal cells of the connecting tubule and cortical collecting duct in mouse, rat, and human. Kir4.1/Kir5.1 is also present in the thick ascending limb of humans and CD1 mice (210, 334) but not in the rat (178) or in the C57BL6 mouse strain (33, 334). In addition, it was found that Kir4.1 and Kir5.1 coimmunoprecipitate in rat renal tissue (381). According to these data, it is probable that the two channels exist as heterotetramers in kidney cells. Using Kir5.1 KO mice, Paulais et al. (308) showed that in the absence of Kir5.1, the only K⁺ channel present in the distal convoluted tubule had a conductance of ~25 pS (instead of 45 pS in WT mice) and was independent of pH in the range 6.8–7.8. This is typical of the properties of Kir4.1 described in heterologous expression systems and further documents that the K⁺ channel in the mouse distal convoluted tubule is composed of Kir4.1/Kir5.1. It should also be noted that the number of K⁺ channels was not reduced in the absence of Kir5.1 (308), making evident that Kir5.1 is not necessary for targeting Kir4.1 to the membrane.

Kir4.1/Kir5.1 is therefore a major player in the control of basolateral membrane potential and in recycling potassium entered into the cell via the Na⁺-K⁺-ATPase. This could hold particularly true for the distal convoluted tubule compared with other renal segments because of Kir4.1/Kir5.1 predominance in this segment: this is the only K⁺ channel
described so far in the basolateral membrane of this segment (237).

In conclusion, Kir5.1 provides Kir4.1 channel with great sensitivity to pH. Small intracellular acidification due to lowering of external pH in perturbed acid-base condition (26) could then result in a large decrease of K+ conductance and possibly a reduction in renal reabsorption of NaCl. It should be borne in mind that pH sensitivity can be modulated by PIP2 (328, 330) and via phosphorylation-dephosphorylation (340, 436, 438). Thus channel regulation might not proceed directly via pH variations but indirectly by additional factors modulating the sensitivity to pH. In addition, Kir4.1 interacts with the CaSR calcium receptor (48, 168) and several PDZ-domain proteins (164, 378, 382). All these agents are possible physiological modulators of Kir4.1-Kir5.1 activity.

D. Mutations Affecting Kir4.1 and Renal SeSAME-EAST Disease

Several laboratories have recently reported SeSAME-EAST syndrome, a new multiorgan syndrome, due to mutations in the KCNJ10 gene encoding Kir4.1 K+ channel (33, 350, 351). Patients display tonic-clonic seizures in infancy, speech and motor delay, and ataxia. Sensorineural hearing loss and mental retardation are frequent. The central (33) or peripheral (350) origin of ataxia is a matter of debate (15, 351). About 10 disease-related mutations have been reported, which increase sensitivity to protons or reduce channel surface expression (304, 334, 345, 379, 384, 414). Coexpression of mutant Kir4.1 with WT Kir5.1 does not restore impaired Kir4.1 function, while coexpression with WT Kir4.1 does (334, 345, 384; for a review, see Ref. 15). Recently, a mutation in the KCNJ10 gene has been associated with juvenile-onset spinocerebellar ataxia in terrier dogs, but the functional effect of this predicted single point mutation at the COOH terminus of the channel has not been studied (112).

In addition to their symptoms in the nervous system, SeSAME-EAST patients develop a renal disease characterized by sodium wasting, secondary hypokalemia, and metabolic alkalosis associated with hypomagnesemia and hypercalciuria. It was suggested that Kir4.1 dysfunction induced partial inhibition of NaCl reabsorption in the distal convoluted tubule, likely because of an impaired K+ recycling across the basolateral membrane (15, 33, 334, 350). According to Paulais et al. (308), Kir5.1 KO mice develop a partially converse disease with acidosis, hypokalemia, and hypercalciuria. A functional assay in vivo using thiourea inhibition of distal convoluted tubule transport demonstrated that Kir5.1 KO mice displayed an increased transport activity in the distal convoluted tubule, likely due to an increased K+ conductance. Taken together, these data indicate that modifications in Kir4.1/Kir5.1 channel activity result in altered transport capacity in the distal convoluted tubule, and establish a functional link between Na+-K+-ATPase and Kir4.1/Kir5.1 channel activity in this segment. It can be speculated that modifying Kir4.1/Kir5.1 has less effect on other renal segments because the latter are endowed with additional potassium channels.

E. Apparent Absence of Kir K+ Channels at the Apical Membrane of Proximal Tubules

Most of the studies devoted to the apical K+ channels in the proximal tubule were done in the beginnings of the patch-clamp technique and provided little information on channel molecular identity (115). The general view is that apical K+ channels in the proximal tubule should be able to counteract membrane depolarization induced by the coupled transport of substrates such as glucose, amino acids, or phosphate together with Na+ (145, 212, 269, 396). Notice that the proximal tubule being a leaky epithelium, this role can equally be achieved by basolateral channels. In possible agreement with this requirement, KCNQ1 (396) and regulatory subunit KCNE1 (375), which form a depolarization-activated K+ channel, were found in the apical membrane by immunohistochemistry. However, KCNQ1 KO mice have no renal phenotype (397) in contrast to KCNE1 KO mice, which exhibit a moderate increase in NaCl and water excretion (269, 396). Neal et al. (269) supported the idea that KCNE1 regulated a K+ channel distinct from KCNQ1 in the proximal tubule on the basis of pharmacological inhibition. As far as we currently know, the inwardly rectifying K+ channels do not play any role at the apical side of the proximal tubule. Indeed, studies published so far discard the presence of the following channels at the apical side of the proximal tubule: Kir 1.1 (145, 200, 409), Kir4.1 (33, 178, 210, 334), and Kir7.1 (81, 294).

F. Kir1.1 Is Necessary for Potassium Recycling in the Thick Ascending Limb of the Henle Loop

A small-conductance K+ channel, rapidly ascribed to Kir1.1, was detected in the apical membrane of the thick ascending limb of the rabbit, rat, and mouse, together with a second 80-pS conductance K+ channel (32, 239–241). The identity of the small-conductance K+ channel has been definitely demonstrated by its absence in Kir1.1 KO mice (240). That of the 80-pS K+ channel has not been elucidated yet, but it has been proposed that Kir1.1 participates to its formation since it is no longer observed in Kir1.1 KO mice (240). The 80-pS K+ channel, therefore, is not expected to compensate for the loss of Kir1.1. The pioneering work of Rainer Greger’s laboratory in the 1980s described the scheme for NaCl reabsorption in the thick ascending limb (129–133) based on experiments using the then novel technique of isolated microperfused tubules (201). The model
introduced in a paper by Greger et al. (130) proposes that recycling of potassium across the apical membrane was strictly necessary to the process of reabsorption. At the time, this observation was linked to the necessity of maintaining a sufficiently high K+ concentration in the lumen of the renal tubule to keep the Na+-K+-2Cl− cotransporter working (105). However, it has been contended that the luminal K+ concentration at the beginning of the distal convoluted tubule was increased to ~8 mM in Kir1.1 KO mice (12), and could not therefore be considered limiting for NaCl reabsorption via the Na+-K+-2Cl− cotransporter (129). Likely, Na+ absorption in the thick ascending limb of Kir1.1 KO mice is reduced, not only because recirculation towards the tubule lumen is reduced, but because K+ recycling at both membranes, via basolateral (Kir4.1/Kir5.1, KCa4.1) and apical (80 pS, Kir1.1) K+ channels, is necessary to maintaining steady-state [K+], and normal re-absorption of NaCl in this segment. The thick ascending limb is the only renal segment where NaCl reabsorption is accompanied by massive K+ entry both at the apical (via the Na+-K+-2Cl− cotransporter) and basolateral (via the Na+-K+-ATPase) sides. In summary, it could be concluded that the loss of Kir1.1, by decreasing global K+ conductance, critically impairs the overall capacity of the TAL cells to counterbalance the K+ influx experienced during the process of NaCl absorption.

G. Kir1.1 Is a Key Player in K+ Secretion in the Connecting Tubule and Collecting Duct

The control of kalemia is another crucial function of the kidneys, achieved according to the following scheme. Following glomerular filtration, most of the potassium is reabsorbed passively in the proximal tubule, thick ascending limb, and inner collecting duct. In the usual state of a potassium-rich diet, the final balance of potassium is achieved in the distal nephron via apically located K+ channels (111, 404). Frindt and Palmer (102) were the first to identify a small-conductance inwardly rectifying K+ channel in the apical membrane of the rat collecting duct, which was further studied by Wang et al. (405). Many properties of this channel in rat and mouse collecting duct have been described, including high sensitivity to pH, activation by PKA, and inhibition by PKC (111, 404). After the cloning of Kir1.1, it was rapidly accepted that the so-called small secretory potassium channel was underlain by Kir1.1, due to similar conductance and rectification, as well as cation selectivity sequence and sensitivity to pH (299). This was confirmed by immunofluorescence studies showing the location at the apical membrane of the collecting duct of rat and mouse, but also of the late distal convoluted tubule, connecting tubule, and thick ascending limb (103, 200, 404, 409). Furthermore, patch-clamp studies provided compelling evidence that Kir1.1 was active in the apical membranes of the connecting tubule (103). Aldosterone and kalemia are responsible for the modulation of potassium absorption/secretion in the distal nephron (111, 200, 300, 404, 409). In particular, changes in external K+ concentration are by themselves powerful modulators of Kir1.1 channels, the mechanisms of which have been studied intensively and have been reviewed elsewhere (111, 404).

Although Kir1.1 is the major channel in potassium secretion, evidence has progressively emerged that the high-conductance, calcium-activated K+ channel (KCa3.1, slo1.1) is also involved in potassium secretion (12), probably at the level of the CNT (103). In a very elegant study, Bayley et al. (12) established that K+ secretion was still present in Kir1.1 KO mice and supported by KCa3.1. This channel had been previously detected in the intercalated cells of the collecting duct (298). Other K+ channels have been postulated at this site, where their functional role remains elusive. This includes TWIK-1, reported by Millar et al. (257) as a Ba2+-insensitive, quinidine-sensitive, outwardly rectifying K+ current in principal cells of the collecting duct, which were absent in TWIK-1 KO mice. In agreement with the recent findings on TWIK-1 cation selectivity, this conductance did not have a high K+-to-Na+ selectivity ratio.

In conclusion, Kir1.1 plays a crucial role in the renal tubule, both for K+ recycling (in the thick ascending limb) and K+ secretion (in the collecting duct). On the short term, acidemia reduces while alkalalemia increases K+ secretion in the distal nephron (7). This effect is probably transmitted via variations of pH, inhibiting the Na+-K+-2Cl−cotransporter and the apical K+ channels (7). Thus sensitivity to pH might be a physiological modulator of Kir1.1 activity. It should be noted that the P_o of Kir1.1 is very high at pH 7.4, implying that intracellular acidification rather than alkalinization would modulate channel activity. However, Kir1.1 as other Kir channels is sensitive to a myriad of factors; as noted above for Kir4.1/Kir5.1 channel, the activity of Kir1.1 might be modulated by shifts of the pH sensitivity curve possibly via cAMP-dependent phosphorylation (214, 216). In addition, as far as Kir1.1 is concerned, channel trafficking to the membrane is held to be the most important physiological regulator (409).

H. Kir1.1 in Bartter Syndrome and Effect of Its Inactivation in the Mouse

Bartter syndrome is a hereditary renal disease with autosomal recessive transmission, resulting from mutations in genes encoding ion transporting proteins in the thick ascending limb. There are four variants all characterized by salt wasting, normal to low blood pressure, hypokalemic alkalosis, and secondary hyperaldosteronism (for reviews, see Refs. 39, 318, 360, 395). Bartter syndrome type II resulting from mutations in the KCNJ1 gene encoding Kir1.1 (364) has a severe, antenatal presentation, the major symptoms including prematurity, polyuria, hyperprostaglandin-
uria, hypercalciuria, and frequent nephrocalcinosis. A large number of KCNJ1 mutations have been described, affecting protein abundance, targeting to the apical membrane, or channel regulation (317, 353, 356, 364).

Lorenz et al. (233) created a Kir1.1 KO mouse model for Bartter syndrome type II. The phenotype is very severe, with 95% of the mice dying within 21 days. A disorganization of the medulla, indicative of hydronephrosis, was apparent in young and adult mice. Adult Kir1.1 KO mice exhibited polyuria, polydipsia, volume depletion, low blood pressure, and huge urinary concentrating defect, as expected for salt wasting disease targeting the thick ascending limb (46, 233). Unexpectedly however, homozygous mice were acidotic and showed normal kalemia. As glomerular filtration rate was reduced by a factor of 10, a likely consequence of a reduced number of nephrons (233), renal insufficiency may explain metabolic acidosis (179). Lu et al. (240) analyzed in detail inbred Kir1.1 KO mice having a high survival rate and showed that they recapitulate most of the features of Bartter syndrome type II, except for their normal kalemia.

IV. Kir4.1 IN PARIELT CELLS OF THE STOMACH AS A COMPONENT OF THE ACID SECRETION MACHINERY

The parietal cells in the oxyntic glands of the stomach are able to secrete protons at high rate when stimulated by hormones and mediators that include gastrin, histamine, and acetylcholine (148, 149). The scheme of proton secretion by parietal cells is shown on Figure 6. The main actor in the secretory process, the H⁺-K⁺-ATPase, is inserted in the apical membrane upon gland stimulation by fusion of tubulovesicles containing the H⁺-K⁺-ATPase, which largely expand secretory canaliculi of the apical membrane. Stimulation also activates Cl⁻ and K⁺ conductances in the luminal membrane, which are silent in resting cells. The initial secretion of K⁺ and Cl⁻ ions drives secondarily the onset of the H⁺-K⁺-ATPase, which needs exchanging K⁺ for H⁺ for its functioning. The protons originate from the hydration of CO₂ within the cell in the presence of carbonic anhydrase producing H⁺ and HCO₃⁻. The HCO₃⁻ anion exits the cell at the opposite, basolateral membrane side via HCO₃⁻/Cl⁻ exchangers. Also present in the basolateral membrane, Na⁺-K⁺-2Cl⁻ cotransporter NKCC1 supplies the cell with chloride together with the anion exchanger. As in most epithelia, the Na⁺-K⁺-ATPase is present in the basolateral membrane where it coexists with K⁺ channels. Several K⁺ channels have been described in the basolateral membrane using the patch-clamp technique on isolated parietal cells from different species, but their molecular identity has not been elucidated (148, 149).

The channels on the apical membrane cannot be studied directly by electrophysiological methods. A number of studies have tried identifying K⁺ channels in the apical membrane by biochemical, immunofluorescence, and physiological assays. A large body of evidence favors the implication of KCNQ1 (148, 149, 189); the channel and accessory subunit are found at the apical membrane and intracellular tubulovesicles but not in the basolateral membrane, and a
Kir channels are also present in the apical membrane of parietal cells. One early finding (332) was that proton secretion could be inhibited by application of barium, a non-specific blocker of inwardly rectifying K⁺ channels. More recently, the presence of Kir4.1, Kir4.2, and Kir7.1 was detected in rat parietal cells (104, 189). According to Fujita et al. (104), Kir4.1 was present solely at the apical membrane but not in the intracellular tubulovesicles or at the basolateral membrane. In contrast, work from the group of Seidler and colleagues (189) showed that Kir4.1 and KCNQ1 colocalized with the H⁺-K⁺-ATPase, although in distinct tubulovesicular pools. Such localization suggested that Kir4.1 might participate in the process of gastric acid secretion. Indeed, it was shown that blocking KCNQ1 completely did not totally inhibit acid secretion but rather delayed it (189). In addition, Kir4.1 fully translocated to the apical membrane upon stimulation, whereas KCNQ1 remained mostly cytoplasmic. The same workers succeeded in examining the gastric acid secretion in Kir4.1 KO mice, which die very early (366). They report that, unexpectedly, rather than being impaired acid secretion is faster in KO mice than WT animals. This might be due to the contrasting morphologies of the parietal cells in the two types of mice: in the resting state, membranes of the tubulovesicular system are clearly detected close to the canaliculi in WT mice but are absent in KO mice. In addition, the microvilli of the canaliculi were more elaborate (without the lumen being expanded). This was explained by the absence of Kir4.1 impairing the endocytic machinery. As a result, there was a permanent high expression of the H⁺-K⁺-ATPase in the apical membrane, resulting in a rapid onset of acid secretion. In summary, KCNQ1/KCNE2 and Kir4.1 K⁺ channels appear to have complementary roles in gastric acid secretion. Kir4.1 is well suited for participating in the process of proton secretion since its activity is independent of extracellular pH (104) and does not change much around the expected physiological pHr (see FIGURE 2). It is likely that additional K⁺ channels participate in the proton secretion process since there are reports that Kir4.2 and Kir2.1 channels are present on the apical membrane of parietal cells (144, 249).

V. Kir4.1 IS THE DOMINANT K⁺ CHANNEL OF GLIAL CELLS: ROLE IN K⁺ BUFFERING

A. Müller Cells and K⁺ Siphoning in the Retina

Müller cells, the major glial cells in the retina, extend through the whole thickness of the tissue from proximal aspect, where endfoot processes envelop blood vessels and the vitreal endfoot is adjacent to the vitreous body, to the distal portion which forms villi adjacent to the pigment epithelium. As in other neuronal tissues, K⁺ ion tends to accumulate during cell activity within the confined external space and needs to be removed. Eric Newman was the first to recognize that Müller cells had an essential function in this respect; he elaborated the so-called “K⁺ siphoning” concept also recognized for glial cells of the CNS (K⁺ clearance) (272). This paradigm proposes that K⁺ excess is not removed by simple extracellular diffusion but involves longitudinal K⁺ transport within the Müller cells. As demonstrated in the nonvascularized retina of the salamander (272), Müller cells take up K⁺ where its concentration is elevated and release it for clearance at the endfeet, close to the large reservoir of vitreous humor, via K⁺ channels. Accordingly, the K⁺ conductance along the Müller cell was highest at the endfeet close to vitreous body and declined steadily in the soma and the distal end, where it was very low (274). Rather similar profiles were found in other nonvascularized retinas (274). Newman (275, 276) also showed that the major part of the K⁺ conductance in the amphibian Müller cell was inwardly rectifying, depended on external K⁺, and was blocked by Ba²⁺. The single-channel variant of the patch-clamp technique allowed describing an inwardly rectifying K⁺ channel with a conductance of 28 pS present in all parts of the Müller cell but with highest density at the endfeet (38, 276). Additional types of K⁺ channels have also been recorded at low to very low frequency in various species (38, 273, 274, 285, 333, 365).

The K⁺ conductance shows a different pattern in vascularized retinas, being maximal at the endfeet (close to the vitreous body) and blood vessels, but not negligible at the distal end (close to the pigment epithelium), while showing a secondary peak in the soma close to capillaries (274, 333). Newman (274) concluded that “in vascularized retinas, excess of K⁺ is transferred into retinal capillaries as well as into the vitreous.” One type of inwardly rectifying potassium channel has been described in rabbit and rat Müller cells (177, 208, 376), and it seems to correspond to the activity of Kir4.1: the properties of the native channel were quite similar to those of Kir4.1 homotetramer (177, 376); immunofluorescence established the presence of Kir4.1 protein in the plasma membrane of Müller cells but not that of neurons (176, 197); and Kofuji et al. (197) indirectly demonstrated that channel activity disappeared in Kir4.1 KO
mice. As expected, the distribution of the channel along the Müller cell was highest close to the capillaries and endfeet (176, 197).

Overall, the major role of Kir4.1 in the second step of K⁺ clearance, that of evacuating K⁺ in excess from the Müller cells towards capillaries or vitreous body, is well documented (45, 198, 199, 293). The mechanism of K⁺ flux into Müller cells for removing K⁺ from the regions where external K⁺ concentration is most elevated is far less well established. The influx may theoretically be due to two contrasting mechanisms, active uptake via Na⁺-K⁺-ATPase and Na⁺-K⁺-2Cl⁻ cotransporter on the one hand, and K⁺ channels on the other. The latter mechanism can only operate if K⁺ accumulation in a limited extracellular region between neurons and Müller cells locally reverses the K⁺ driving force while membrane potential remains comparatively stable. This is generally considered as plausible in the setting of the retina (45, 198, 199, 293). The localization of Kir4.1 is not incompatible with this channel mediating influx as well as efflux: Kir4.1 is also present in the inner and outer plexiform layers where external K⁺ is dramatically increased by light (177).

It should also be mentioned that Kir5.1 has been detected in the cell body and distal parts of the Müller cells where it could form heterotetrameric channels with Kir4.1 (176) while homomeric channels would be concentrated adjacent to capillaries and vitreous body (176, 177). Ishii et al. (176) proposed that pH sensitivity of Kir4.1-Kir5.1 channels might be important in K⁺ uptake: an increase of external K⁺ would depolarize the membrane and stimulate HCO₃⁻ uptake by the electrogenic Na⁺-HCO₃⁻ cotransporter, inducing alkalinization and promoting positive feedback for the activity of the channel. Additional K⁺ channels including Kir2.1 are thought to mediate K⁺ influx into Müller cells (see Refs. 198, 293).

**B. K⁺ Spatial Buffering: A Role for Astrocytes in the CNS**

Astrocytes help stabilize the extracellular environment around neuronal cells. In particular, they are involved in removing K⁺ accumulated in the narrow CNS extracellular space and glutamate released at the synapses (198, 199, 293). According to the K⁺ spatial buffering mechanism postulated by Orkand et al. (296), glial cells transfer K⁺ ions from high to low K⁺ regions adjacent to capillaries by means of K⁺ channels. This mechanism is very similar to the one described above for Müller cells, considering that astrocytes form a syncytium network communicating through gap junctions and maintaining an ensemble membrane potential. In regions with elevated K⁺, the locally less negative K⁺ Nernst potential will reverse the driving force for K⁺, allowing inward K⁺ current (via Kir K⁺ channels), K⁺ flow along the glial network, and K⁺ exit in regions of low external K⁺ (also via Kir K⁺ channels). Nevertheless, as further discussed below, whether the uptake of K⁺ depends on Kir K⁺ channels or proceeds by means of other K⁺ transport systems (Na⁺-K⁺-ATPase, Na⁺-K⁺-2Cl⁻ cotransporter) remains a matter of debate.

The electrophysiological profile of astrocytes is heterogeneous in terms of resting membrane potential and K⁺ currents (73), including the so-called complex and passive, or mature astrocytes. Only the passive astrocytes seem to be connected through gap junctions. However, it is largely accepted that Ba²⁺-sensitive inwardly rectifying currents predominate at least in a subpopulation of astrocytes (13, 292, 327; see also reviews in Refs. 199, 293). Other types of glial K⁺ channels, nevertheless, have been described in different CNS regions (45, 109, 114, 141, 367, 439), including TWIK-1 and TREK-1 K₂P channels (401, 442). Kir4.1, which is highly expressed in glial cells (35, 45, 183, 377) while its presence in neurons is restricted to specific brain regions (418, 424), is emerging as a key component of glial K⁺ conductance (see Refs. 198, 199, 293). At the single-channel level, Zhang and Verkman (439) reported a Kir4.1-like inwardly rectifying K⁺ channel displaying weak inward rectification, 20-pS conductance, high Pₒ, and inhibition by external Ba²⁺ in hippocampal glial cells. In line with this report, it is generally considered that Kir4.1 homotetramers underlie K⁺ conductance in most glial cells even if Kir4.1/ Kir5.1 heterotetramers might be functional in specific brain regions (151). Taking advantage of the existence of total and glia-specific Kir4.1 KO mice, two groups showed that the resting membrane potential was less negative and input resistance increased in Kir4.1 KO glial cells (83, 270). The same observation was done in spinal cord astrocytes where Kir4.1 expression was knocked down with shRNA (292). In addition, inwardly rectifying K⁺ currents were no longer visible when Kir4.1 was absent, verifying that these currents were supported by Kir4.1 (83, 270, 292, 384).

The possible implication of Kir4.1 channels in K⁺ spatial buffering has been evaluated using glia-specific Kir4.1 KO mice. Trains of electrical stimulation in the hippocampus induced a comparable fast increase in external K⁺ in WT and KO mice, which was followed by exponential decay. Although slower, this decay was still present in glia-specific Kir4.1 KO mice (55), which implies that Kir4.1 is not strictly necessary for K⁺ clearance. After repetitive long-lasting stimulations, a phenomenon called [K⁺]ₒ reaches a plateau that is lower than the one before stimulation (72). The undershoot is observed, which is attributed to stimulated activity of the Na⁺-K⁺-ATPase: [K⁺]ₒ reaches a plateau that is lower than the one before stimulation (72). The undershoot was magnified in glia-specific Kir4.1 KO mice (55). This observation is in accordance with the hypothesis of D’amabrosio et al. (72) that Kir channels might be crucial for K⁺ recirculation towards the extracellular compartment following K⁺ uptake by the Na⁺-K⁺-ATPase. Similar results were obtained by Neusch et al. (270) in the ventral...
respiratory group and by Haj-Yaein et al. (139) in the hippocampus. As a whole, these studies disclose that Kir4.1 is not strictly necessary for $K^+$ clearance, but rather that it plays a major part in setting the membrane potential of glial cells and the level of resting $[K^+]_o$. Compensation via alternative mechanisms to $K^+$ spatial buffering probably occurs. Such an alternative pathway involves $K^+$ uptake through the Na+-K+-ATPase and Na+-K+-2Cl- cotransporter (13, 72, 246; see also Refs. 199, 293).

Tong et al. (390) studying mouse models of Huntington’s disease (HD) have provided evidence that alteration in astrocyte maintenance of extracellular $K^+$ due to downregulation of Kir 4.1 channels correlates with depolarization of striatal astrocytes and the onset of motor symptoms. HD is an autosomal dominant disease and characterized by chorea, cognitive, and neuropsychiatric defects caused by polyglutamine expansion at the NH$_2$ terminus of the protein huntingtin. The mechanism by which the mutated huntingtin (mHTT) leads to degeneration of striatal medium spiny neurons (MSN) is not completely understood. Striatal but not hippocampal astrocytes were depolarized by 5 mV and exhibited lower membrane conductances by up to 20% at age P60–80. These findings were associated with electrophysiological and Western blot analysis evidence of loss of Kir4.1 channels in striatal astrocytes. The spatial $K^+$ buffering impairment elicited a twofold increase in extracellular $K^+$ in the striatum in vivo, and its relationship with MSN hyperexcitability was established in vitro. Viral-based delivery of Kir4.1 channels to striatal astrocytes rescued the electrophysiological defects, ameliorated the motor deficits, and extended the survival of the mutant mice (390). This interesting study provides a new explanation for MSN hyperexcitability in current mouse models of HD and a potential therapeutic approach. How mHTT impairs astrocyte Kir4.1 function is not known.

Additionally, Kir4.1 expression in glial cells is developmentally regulated. Borday and Sontheimer (36, 37) observed that the percentage of astrocytes showing Kir currents increased from day 5 after birth to day 20 in rat hippocampal astrocytes in situ. Kir4.1 immunoreactivity was also developmentally regulated in fine processes of astrocytes from the optic nerve or ventral respiratory group (183, 271). Likewise, several studies demonstrated that Kir4.1 expression and current increased when progenitor oligodendrocyte cells differentiated into mature oligodendrocytes (248, 271). A similar observation had also been made for Kir currents in Schwann cells (415). The increasing expression of Kir4.1 after birth correlates quite well with the increasingly tighter regulation of $[K^+]_o$, which becomes optimal around day 20–30 after birth when Kir4.1 expression is maximal (293). It can be concluded that Kir4.1 dysfunction might prevent normal maturation of astrocytes, oligodendrocytes, and Schwann cells, a feature indeed observed in Kir4.1 KO mice and implying that the presence of Kir4.1 is essential for motoneuron myelination (271).

C. Heterotetrameric Kir4.1/Kir5.1 Channel in Glial Cells

$K^+$ clearance should be quite independent of pH, variations in all regions where the homotetramer Kir4.1 is the predominant $K^+$ channel, i.e., in most glial cells. However, there is a clear suggestion that Kir5.1 is also present in glial cells. Hibino et al. (151), investigating the expression of Kir 5.1 in the mouse brain by immunolabeling, showed that this channel was expressed in astrocytes but not in neurons of the neocortex and olfactory bulb, where their expression overlapped with that of Kir4.1. The authors contend that heteromeric Kir4.1/Kir5.1 channels were functional in processes around blood vessels and pia mater. In agreement with these findings, pH-sensitive $K^+$ currents that could possibly be attributed to Kir4.1/Kir5.1 were reported in the astrocytes of the retrotrapezoid nucleus (410). Other parts of the mouse brain, such as the hypothalamus and thalamus, only expressed homomeric Kir4.1 channels (151). Kir5.1 has also been detected in the cell body and distal parts of the Müller cells where it could form heterotetrameric channels with Kir4.1 while homomeric channels would be concentrated adjacent to capillaries and vitreous body (176, 177). Thus Kir4.1/Kir5.1 channels seem to be located at the site of $K^+$ efflux in astrocytes while they are at the site of $K^+$ uptake in Müller cells. Whatever the exact meaning of these distinct locations, the presence of Kir5.1 in astrocytes in several parts of the brain and in Müller cells obviously makes the Kir4.1-dominated $K^+$ conductance highly sensitive to pH. As mentioned above, the pH sensitivity of Kir4.1/Kir5.1 channels might contribute to spatial $K^+$ buffering through their positive feedback resulting from the electrogenic activation of the Na+-HCO$_3^-$ cotransporter (151, 176).

VI. Kir4.1 Genetic Ablation in Mice Reveals Its Role in Inner Ear Function and Disease-Causing Mutations Suggest Novel Physiological Roles

Two Kir4.1-deficient mouse lineages have been generated, the first using a standard gene-targeting approach for total ablation and the second using the Cre/loxP system for specific deletion in glial cells. At birth, total and glia-specific Kir4.1 KO mice showed no obvious difference with WT animals, but both rapidly stopped gaining weight around 10–15 days after birth and could subsequently be easily identified by their small size. They displayed severe defects in posture, balance, and movement control and were unable to perform some simple motor tasks. There was 100% mortality at 4 wk age (83, 197, 271). In addition, Kir4.1 KO mice had a dehydrated appearance. The investigation of
renal function revealed polyuria, renal salt loss, and reduced calcium excretion, as observed in patients with SeSAME-EAST syndrome (33) (see sect. III D).

Analyzing the consequences of the deletion of Kir4.1 in glial cells, Djukic and co-workers (55, 83) observed that spontaneous neuronal activity was reduced and synaptic potentiation enhanced in hippocampus slices, possibly because of impaired glutamate uptake and K⁺ clearance by astrocytes. Neusch et al. (271) described hypomyelination, spongiform vacuolation, and degeneration events in the spinal cord in Kir4.1 KO mice, which they considered as possible causes of motor dysfunction. Kir4.1 is present in oligodendrocytes and oligodendrocyte precursors where it is important for membrane potential setting (67, 183, 248, 271, 323). Kir4.1 appears to be necessary for maturation of cultured oligodendrocytes as only a small proportion of cultured Kir4.1 KO oligodendrocytes produces myelin compared with cells of WT origin (271).

Patients affected by EAST syndrome (see sect. III D) showed altered electroretinogram response to light flashes (389). The retina function has been investigated at weeks 2–3 after birth in Kir4.1 KO mice. The general organization of the retina as well as the morphology of Müller cells, in which Kir4.1 expression is predominant, seemed normal despite large membrane depolarization and high input resistance (197). Kofuji et al. (197) observed that a component of the electroretinogram, the so-called slow PIII response, was absent in Kir4.1 KO mice, indicating that this response was due specifically to Kir4.1. Overall, despite the proposed role of Kir4.1 in the regulation of [K⁺]o in the retina, Kir4.1 KO mice do not seem to present any important visual alteration.

In contrast, the impact of Kir4.1 deletion on hearing is dramatic. Kir4.1 is mainly expressed in the satellite glial cells of cochlear ganglia (152) and in the intermediate cells of the stria vascularis (2), underscoring two distinct functions for Kir4.1 at this site: [K⁺]o control around ganglion neurons and generation of endocochlear potential (see Ref. 52). K⁺ accumulation within the endolymph (up to 140 mM) and generation of endocochlear potential (up to about +80 mV) are due to the stria vascularis (see FIGURE 7). The stria vascularis in the inner ear is an elaborate structure formed by fibrocytes, basal cells, and intermediate cells connected by gap junctions on the perilymphatic side, and by marginal cells on the endolymphatic side. The fibrocytes take up K⁺ from the perilymphatic compartment via the Na⁺-K⁺-ATPase and Na⁺-K⁺-2Cl⁻ cotransporter while intermediate cells secrete it into the atrial compartment via

**FIGURE 7.** Mechanism of K⁺ accumulation in endolymph. The stria vascularis in the inner ear is formed on the perilymphatic side by fibrocytes, basal cells, and intermediate cells connected by gap junctions and on the endolymphatic side by marginal cells. The fibrocytes take up K⁺ from the perilymphatic compartment via the Na⁺-K⁺-ATPase and Na⁺-K⁺-2Cl⁻ cotransporter. K⁺ is secreted via Kir4.1 K⁺ channel into the atrial compartment and taken by Na⁺-K⁺-ATPase and Na⁺-K⁺-2Cl⁻ present on the marginal cells. K⁺ is finally secreted into the endolymph by KCNQ1/KCNE1 K⁺ channel. The process is only possible because of the polarized organization of K⁺ channels. The largely positive (around +80 mV) endolymphatic potential is necessary to the transduction of sound. [Adapted from Nin et al. (286). Original figure copyright (2008) National Acadeny of Sciences, USA.]
Kir4.1 channels. Marginal cells then remove K\(^+\) by the operation of Na\(^+\)-K\(^-\)-ATPase and Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter and finally secrete K\(^+\) into the endolymph through KCNQ1/KCNE1 K\(^+\) channel (437). Marcus et al. (251) observed that Kir4.1 KO mice did not generate the highly positive endocochlear potential (32) that is necessary to hearing function and showed a reduced [K\(^+\)]\(_i\) in the endolymph. This interesting result highlights the major role of Kir4.1 in the apical membrane of intermediate cells of the stria vascularis. In addition, a large alteration of ear anatomy was apparent in Kir4.1 KO mice, including collapse of the scala media, swelling of the tectorial membrane, as well as their central processes (341). Whether the loss of endocochlear potential is due solely to the absence of Kir4.1 or it is aggravated by the associated ear degeneration remains an open question (242, 251).

A totally novel and unexpected implication of Kir4.1 in pathology came to light recently: in the course of screening serum IgG from patients with multiple sclerosis, Srivastava et al. (369) observed that levels of antibodies to Kir4.1 were higher in affected individuals. When injected into the cisternae magna of the mouse brain, human serum antibodies specific for Kir4.1 decreased the expression of glial fibrillar acid protein, a specific marker of glial cells; reduced the abundance of Kir4.1 channel protein; and activated the complement cascade in the cerebellum.

**A. Are Kir Channels Involved in Central Chemoreception?**

Kir4.1 and Kir5.1, highly prominently expressed in several brain stem nuclei involved in cardiorespiratory control (418), associated as heterodimers are influenced by hypercapnia-induced intracellular acidification (421). Jiang and co-workers (67, 421, 427) have studied the pH sensitivity of Kir4.1/Kir5.1 channels using CO\(_2\)/HCO\(_3\)- mixtures and were the first to suggest that owing to their high sensitivity to pH\(_i\) around the physiological level, these channels are poised to play an important role in central CO\(_2\) chemoreception. The LC is a noradrenergic neuronal cluster connected with most parts of the forebrain and involved in several functions such as attention, cognitive behaviors, arousal states, cardiovascular function, and control of breathing (107). It is also documented that most neurons in this region are sensitive to changes in extracellular pH (71, 107). Kir4.1 and Kir5.1 proteins are present in the mouse LC, and their messengers have been detected in the rat (418, 441); however, immunohistochemistry has failed to discern Kir4.1 and Kir5.1 coexpression in rat medulla oblongata (424). D’Adamo et al. (71) demonstrated a reduced response of LC neurons to cytoplasmic alkalization and acidification in a Kir5.1 null mouse, concluding that the channel is essential in the chemosensitivity process.

Rett syndrome, a genetic disease due to mutations in the X-linked MeCP2 gene, is characterized by dramatic defects in the development of the brain and respiratory abnormalities. It has been hypothesized that LC noradrenergic neurons are involved in these disturbances of MeCP2-null mouse. Similar to humans affected by Rett syndrome, the null mice breathing atmospheric air display irregular respiratory patterns, gasping, and apnea. Affected mice also had a selective loss of respiratory response to 1–3% CO\(_2\) but exhibited regular breathing in response to 6–9% CO\(_2\) (441). Interestingly, LC neurons obtained from null mice lacked sensitivity to mild hypercapnia (1–3% CO\(_2\)), along with an increase in Kir4.1 protein expression without changes in Kir5.1. Electrophysiological experiments with isolated neurons evidenced a reduced pH\(_i\) sensitivity in the Kir currents of MeCP2-null neurons. Similar type of experiments in HEK-293 cells cotransfected with Kir4.1 and Kir5.1 in various ratios led to the conclusion that the decrease of pH\(_i\) sensitivity observed in MeCP2-null cells was due to an overexpression of Kir4.1 (441). Mice KO for Kir4.1 present 100% mortality at P24 associated with marked motor impairment secondary to dysmyelination and axonal degeneration; nevertheless, there were no reports of respiratory abnormalities (67, 197). Neither peripheral nor central chemosensitivity is impaired by ablation of Kir5.1 (392). Kir5.1 inactivation nevertheless results in a well-defined respiratory phenotype with reduced ventilatory responses to hypoxia or hypercapnia that is probably the consequence of an altered signal transmission from carotid body to CNS. This could be due to a compensatory modulation that develops in response to the profound metabolic acidosis shown by Kir5.1 KO mice (308, 392).

The hypothesis that Kir4.1 might influence chemoreception by modulating astrocyte activity, rather than having a direct influence on chemosensitive neurons, has also been examined. Kir4.1 is abundantly expressed in the astrocytes of the ventral respiratory group, mostly in the processes surrounding capillaries and also in close proximity of the neurons. But although Kir4.1 makes a major contribution to K\(^+\) conductance, it has no effect on the rhythmic bursting activity of the pre-Bötzinger complex (270). There is also...
convincing functional evidence for the presence of the Kir4.1/Kir5.1 complex in astrocytes of the RTN. It has been hypothesized that acidification-induced inhibition of Kir4.1/Kir5.1 would induce ATP secretion, which in turn would influence neuronal activity (410). Indeed, pH-dependent secretion of ATP by astrocytes in chemosensitive regions of the brain stem has been proposed as a possible modulator of chemosensitive neurons (124, 125, 187). Nevertheless, membrane depolarization of astrocytes is not required for ATP secretion according to Trapp et al. (392).

The possible role of Kir5.1/Kir4.1 channels in the respiratory response to hypercapnia has been explored through genetic inactivation of Kir5.1 in mice. Chemosensitive neurons of the LC from Kir5.1 KO animals become unresponsive to changes in cytoplasmic pH, hinting that Kir5.1 may be involved in the response to hypercapnic acidosis (71). However, experiments in the same animals show that Kir5.1 channels are not essential for functional central and peripheral respiratory chemosensitivity (392).

### B. Role of the K2p Channels in Chemoreception

TASK subfamily K2p channels TASK-1 and TASK-3 as well as TALK channel TASK-2 have been considered as part of the intrinsic chemosensitivity of neurons owing to their high responsiveness to extracellular pH changes. TASK-1 and TASK-3 channels are sensitive to pHo in the physiological range and are expressed in a number of different neuron types in the respiratory network. Simultaneous deletion of TASK-1 and TASK-3 in the mouse abolishes the acid-sensitive background K+ current of raphe neurons, but leaves central ventilatory chemosensory response unaffected (264).

The involvement of TASK-1 or TASK-3 in the control of ventilation by peripheral chemoreceptors is a matter of controversy. Oxygen-regulated K+ channels have been postulated to contribute importantly to chemosensory transduction in the carotid body (CB). Indeed, it is generally accepted that depolarization of glomus cells via inhibition of K+ channels leading to Ca2+ influx and transmitter release is at the heart of chemosensing in the CB (106, 232). More recently, the attention has been focused on K+ channels of the K2p family as candidates to underlie the chemosensitive K+ conductance of the CB. Experiments using CB type 1 cells demonstrated a close similarity in biophysical and pharmacological properties between O2-sensitive currents and those generated in heterologous systems by K2p-channel expression. TASK-1 and TASK-3 were both postulated as O2-sensing CB channels (44, 413) with the TASK-1/TASK-3 heteromer better emulating the characteristics of native O2-sensitive channels (192). The possible role of TASK-1 and TASK-3 in peripheral chemoreception was addressed directly by examining the ventilatory response to hypoxia and moderate normoxic hypercapnia in mice deficient in TASK-1, TASK-3, or both channels simultaneously (391). The results of these experiments indicated the participation of TASK-1, but not TASK-3, in the peripheral control of ventilation. More recent experiments have used the same animals to explore the function of these channels in CB cells in vitro. TASK-1 was dispensable for O2/CO2 sensing in mouse CB cells, whilst TASK-3 channels, or its heteromer with TASK-1, could be involved in the depolarization of glomus cells under hypoxia. Catecholamine release in response to O2/CO2 changes was, however, normal in glomus cells from either TASK-1 or TASK-1/TASK-3 null animals (297). Similar results were obtained more recently in which Ca2+ responses to hypoxia and metabolic inhibition were unaltered in the absence of TASK-1 or TASK-3, or the combined absence of TASK-1 and TASK-3 (43). There might be other O2-sensitive K+ currents in the CB that take over the role of the genetically removed TASK channels in the KO animals. This possible redundancy of sensor and effector mechanisms in the CB has been mentioned as an adaptation to the important function the CB cells plays in the physiology of the animal (297).

TASK-2 expression in the brain is circumscribed to certain RTN neurons and based on work with a KO mouse has been proposed to be important in central CO2 and O2 chemosensitivity (110, 400). It is interesting that in addition to its extracellular pH sensitivity, TASK-2 is regulated by pHi, thus offering a possible way to couple channel activity to CO2 levels (281). Intriguingly, TASK-2 has also been found to be directly inhibited by CO2 without any need for changes in pHi. The molecular determinants of this modulation have not been unveiled (313). Gestreau et al. (110) reported that TASK-2 is expressed in a scattered manner in brain stem nuclei that are involved in central chemoreception, including the dorsal raphe, the RTN, and the parafacial respiratory group. Transgenic expression in mice of a mutated Phox2b gene, coding for the paired-like homeobox 2b transcription factor, led to disappearance of certain parafacial neurons, unresponsiveness to an increase in CO2 and premature death from central apnea (89). All expression of TASK-2 in the RTN is also lost under the effect of this transgene. The respiratory effects of TASK-2 inactivation were studied by whole body plethysmography. The acute response to hypoxia was normal, suggesting appropriate O2 sensitivity of the carotid body chemoreception. Exposure to a 20-h hypoxia did not elicit the normal ventilatory depression followed by acclimatization in the KO mice. This indicated that TASK-2 channels could be involved in the changes in the CO2 set-point in RTN neurons. The use of an en bloc preparation of neonatal brain stem-spinal cord allowed to determine that a 5-min anoxia provoked a reduction of the respiratory frequency in the WT preparation but not in that derived from KO mice. The authors concluded that TASK-2 channels are involved in the stabilization of membrane potential of chemosensitive neurons of brain stem nuclei, including the RTN region. TASK-2 might not be the sole chemosensory
ion channel since KO animals had a definite ventilatory response to hypercapnia (110).

The role of TASK-2 in central chemosensing was initially put in doubt because its ablation, in contrast to what is seen after deletion of RTN neurons, does not produce the expected alteration in pH sensitivity of the neonate breathing network in vitro. Additionally, the metabolic acidosis presented by the TASK-2 null mice secondary to the impaired bicarbonate renal reabsorption by itself could have altered the central response to CO₂ (53, 138). A direct electrophysiological recording of RTN neurons was therefore needed to test whether TASK-2 deficit leads to a modification of their intrinsic pH sensitivity.

The answer to this criticism (400) came through the use of two different genetically modified mice to study the contribution of TASK-2 to the intrinsic pH sensitivity of the RTN chemoreceptor neurons: the β-galactosidase expressing TASK-2 KO mouse (110) and a Phox2b-eGFP BAC transgenic line (213). Patch-clamp recordings were made in brain stem slices from RTN neurons identified by Phox2b promoter-driven expression of GFP or β-galactosidase. Nearly all RTN cells from WT animals were pH sensitive, and only about one half of GFP-expressing RTN neurons from TASK-2 KO mice responded to this description. No TASK-2 KO RTN neuron, identified by the ability to express β-galactosidase and that made a subpopulation of GFP-expressing neurons, showed pH sensitivity. RTN neurons from TASK-2 KO mice that had some pH sensitivity, but not those that were pH insensitive, had some kind of background K⁺ current of unknown molecular identity activated by alkalinization of reduced magnitude. The use of a working heart-brain stem preparation disclosed that a respiratory alkalosis caused a blunted decrease in phrenic burst amplitude in the KO preparation. The pH at which the phrenic activity stops, the apneic threshold, was significantly higher in the KO preparations (400). These results undoubtedly demonstrate the contribution of TASK-2 channel to the intrinsic pH sensitivity of the major part of Phox2b expressing RTN neurons. In those cells where the expression of TASK-2 is higher, this conductance accounts fully for the pH-sensitive background K⁺ current and pH-dependent effects on neuronal firing. However, there is a subset of RTN neurons where a redundant or compensatory pH-sensitive background K⁺ conductance of unknown molecular identity becomes evident.

### VIII. ROLE OF POTASSIUM CHANNELS IN ADRENAL BIOSYNTHESIS OF ALDOSTERONE

#### A. Modulation of K⁺ Channels in the Production of Aldosterone

Cells of the adrenal zona glomerulosa (ZG) are central in water and electrolyte homeostasis and consequently in the regulation of blood pressure via aldosterone secretion. The most relevant physiological stimuli for aldosterone secretion are the plasma concentrations of K⁺ and angiotensin II (ANG II), either acting independently or synergistically (368). Aldosterone is not accumulated in secretory granules, but its rate of synthesis and secretion critically depends on Ca²⁺ signaling at several points of the steroidogenesis pathway. An example is Ca²⁺/calmodulin kinase I-dependent transcription of CYP11B2, the aldosterone synthase that catalyzes the conversion of 11-deoxycorticosterone to aldosterone (20, 65). The activation of the ANG II type I receptor (AT1) elicits a transient intracellular store release of Ca²⁺ followed by influx of the ion mainly through T-type Ca²⁺ channels that sustain the aldosterone secretion (368). This last phase requires depolarization of the plasma membrane which is achieved through inhibition of K⁺ channels responsible for the resting membrane potential of ZG cells. In fact, both increases in extracellular K⁺ and ANG II depolarize the ZG cells (234). Patch-clamp experiments have identified inwardly rectifying, outwardly delayed rectifying, Ca²⁺-activated, and background K⁺ current in different species (121, 135). The resting membrane potential of rat ZG cells is close to the equilibrium potential for K⁺ at which only Kir and K₂P channels may give rise to conductances; however, in that situation only background K⁺ currents have been recorded (68, 135). In addition, ANG II and acidosis lead to inhibition of cloned TASK-1 and TASK-3 and native background K⁺ channels in ZG cells, suggesting that these channels in homo- or heterodimeric association give rise to the background hyperpolarizing conductance of ZG cells (68, 70).

An alternative model to explain sustained production and secretion of aldosterone has been raised by Barrett and co-workers (167). They found that ZG cells behave as intrinsic oscillators which depend on Cav3.2 currents (167). Under stimulation with ANG II or K⁺, the elicited depolarization is small (4–10 mV), insufficient to elicit a significant Ca²⁺ influx. The voltage oscillations in the plasma membrane will amplify small changes in the potential, allowing repeated cycles of Ca²⁺ entry into the ZG cell through Cav3.2 channels. In this scenario, TASK channels would play their role principally during the depolarization phase of the oscillatory cycle by affecting the rate of depolarization between spikes. Delayed rectifiers and Ca²⁺-activated K⁺ channels will enter into action when the membrane is depolarized by controlling the rate of repolarization (167). Modulation of K⁺ channel activity therefore would be a major way of controlling the production of aldosterone.

Studies in patients with autonomous secretion of aldosterone (primary aldosteronism) and KO mouse models have further highlighted the importance of K⁺ channels in glomerulosa cell physiology and pathophysiology.
B. KCNJ5 Gene Mutations and Aldosterone-Producing Adenomas

Primary aldosteronism is a main cause of secondary hypertension. Mutations in the human KCNJ5 gene encoding the G protein-activated inward rectifier K⁺ channel 4 (GIRK4) have been found in familial hyperaldosteronism type III patients and ~40% of sporadic aldosterone-producing adenomas (APA). Choi et al. (57) identified somatic and germline tumor-causing mutations in the human KCNJ5 gene which are heterozygous and located at or near the selectivity filter, G151R and L168R, respectively. Whole cell patch-clamp studies of the mutant channels revealed loss of channel selectivity, with increased sodium conductance leading to membrane depolarization and activation of voltage-gated calcium channels. In the same study, the T158A mutation was found in familial hyperaldosteronism type III, a Mendelian autosomal-dominant form of primary aldosteronism. T158 lies in the loop between the selectivity filter and the second transmembrane domain and also led to loss of K⁺ selectivity. After these seminal discoveries, families carrying G151R and G151E mutations and somatic KCNJ5 mutations surrounding the selectivity filter in APA have been reported (121, 262). The authors postulate that KCNJ5 mutations involved in inherited and acquired aldosteronism with cell autonomous proliferation is the result of increased calcium influx (57, 352). The role of GIRK4 channels in glomerulosa cells in normal physiology is unknown.

C. Aldosterone Production Studies Using TASK-1 and TASK-3 KO Mice

TASK-1 is expressed in the whole cortex, and TASK-3 is restricted to the ZG in rodents and human adrenal glands. Their genetic ablation results in a range of phenotypes with abnormal aldosterone production.

The main pathophysiological feature in TASK-1 null mice is mislocalization of aldosterone synthase observed before puberty and in adult females but not in males (146). The enzyme was absent from the zona glomerulosa, but abundant in the reticulofasciculata zone. The observed phenotype was severe hyperaldosteronism that is reversed by ACTH inhibition and is independent of salt intake, hypokalemia, and low renin hypertension. The pathological change in adrenocortical zonation and the phenotype could be reproduced in KO males by castration. Moreover, administrations of testosterone to KO females provoked a redirection of the aldosterone synthase to the zona glomerulosa and suppression of the phenotype. These results suggest an androgen compensatory mechanism, such as an increased expression of TASK-3 channels. Electrophysiological studies demonstrate that TASK-1 KO adrenal cells were depolarized compared with WT cells (~68 vs. ~75 mV, respectively). A significant depolarization by extracellular acidic pH in TASK-1 KO cells indicated the presence of other pH-sensitive K⁺ currents that again could correspond to homomeric TASK-3 channels (14, 146).

Double TASK-1 and TASK-3 KO adrenal glands had normal cellular organization (74). The complete loss of TASK-like current in glomerulosa cells provoked an ~20-mV depolarization of the cells resulting in autonomous aldosterone overproduction. Consequently, the double KO mice show elevated plasma levels of aldosterone with low levels of renin, hypertension, and hypokalemia. Plasma aldosterone remains increased under a low-salt diet, and it is not suppressed by high-salt diet. Finally, the aldosterone levels were resistant to AT1 receptor blockage. All these features are reminiscent of patients with idiopathic primary hyperaldosteronism (74). Guagliardo et al. (134) working with this double KO mouse found that physiological inhibition of TASK channels by H⁺ is not required to evoke an increase in aldosterone production and concluded that pH-sensing by glomerulosa cells does not require TASK-1 or TASK-3 channels.

The effect of deleting only TASK-3 on the control of aldosterone production and blood pressure has also been examined (136). The KO mice displayed mild hyperaldosteronism associated with low renin levels, increased aldosterone-renin ratio, and hypersensitivity to angiotensin II. Interestingly, this phenotype recalls the human low-renin essential hypertension, and the loss of the TASK-3 channel activity might be part of the pathophysiological mechanism of this hypertensive syndrome. A similar phenotype using another TASK-3 KO mouse was described by Penton et al. (314) who measured Ca²⁺ transients and aldosterone secretion in ex vivo adrenal glands, finding that a significant part of the aldosterone secretion is autonomous and not due to angiotensin II hypersensitivity.

Bandulik et al. (16) investigated the age dependence of the adrenal phenotype of TASK-3 KO mice. The newborn KO mice displayed a severe adrenal phenotype with strongly increased plasma levels of aldosterone, corticosterone, and progesterone. They found an increase of the activity of the local intra-adrenal renin-angiotensin system and hypothesized that abnormal local renin expression could also be relevant for the development of primary hyperaldosteronism in a subset of human patients.

The KO models discussed are very suggestive that abnormalities in TASK-1 and/or TASK-3 in humans might be involved in the pathogenesis of primary aldosteronism. Nevertheless, sequencing of these genes in 22 patients with APA by Choi et al. (57) did not identify any causative mutations. However, a study was designed to explore genetic variation in KCNK3 and KCNK9 genes coding for TASK-1 and TASK-3, respectively, in relation...
to blood pressure and aldosterone production in healthy young humans (182). The main finding was that multiple single nucleotide polymorphisms in KCNK9 were associated with systolic blood pressure in African Americans, whereas associations were primarily with aldosterone production in European American individuals. These interesting preliminary results should be replicated in other populations fully to establish a role for KCNK9 in causing blood pressure variability.

IX. UNEXPLORED AND SURPRISING RECENT DEVELOPMENTS IN K2P AND Kir CHANNEL PHYSIOLOGY AND PATHOPHYSIOLOGY

A. Kir7.1 Follows the Na+/K+ Pump in Subcellular Distribution

Kir7.1 is prominently expressed in epithelial tissues where it follows the subcellular distribution of the Na+/K+ pump: Kir7.1 is present at the apical membrane of retinal pigmented epithelium (RPE) and in the choroid plexus that also express the pump apically (207, 267, 361, 425), whilst it is located at the basolateral membrane of intestinal epithelial cells, thyroid follicular cells, and epithelial cells of proximal and distal convoluted tubule, all of which present the pump at the more usual basolateral membrane location (81, 267, 294). Inwardly rectifying K+ currents probably corresponding to the activity of native Kir7.1 are present in RPE (306) and small intestinal villus enterocytes (358). The remarkably faithful subcellular colocalization of Kir7.1 and the Na+/K+ pump has prompted the hypothesis that this channel might be responsible for K+ recycling, allowing continuous pump function in epithelia to sustain high rates of ion transport (153).

A mutation in KCNJ13, the gene encoding the Kir7.1, leading to replacement of methionine-162 by tryptophan, has been associated with the retinal disease known as snowflake vitreoretinal degeneration (SVD) (150). The mutated channel reaches the plasma membrane where it is inactive (440), or mediates only weak nonselective cation current (307). In both cases, coexpression of mutated channels reduced the activity of the WT protein. Recessive mutations in KCNJ13 are also a cause of Leber congenital amaurosis, another retinal degenerative disease (359). These mutations, R166X and L241P, lead respectively to loss of COOH terminus and probable failure of membrane surface trafficking, and perhaps loss of function by a COOH-terminal folding defect. No formal functional evaluation of these mutants has yet been done.

B. Kir4.2 Channels

Little is known about the physiological function of Kir4.2 despite its broad tissue distribution in kidneys, brain, lungs, liver, and pancreas (35, 315, 362). The KCNJ15 gene coding for Kir4.2 is located close to the locus of the Down syndrome chromosome region 1, a fact that initially suggested a possible link with this disease (123). Okamoto et al. (291) recently identified KCNJ15 as a susceptibility gene for lean type 2 diabetes, which is highly prevalent in Japan. A synonymous SNP in exon 4 resulted in increased Kir4.2 protein abundance. Kir4.2 is present in β-cells of the endocrine pancreas, where its expression is stimulated by glucose. It is thought that increased expression of Kir4.2 stabilizes the resting membrane potential of β-cells, preventing membrane depolarization, which is necessary for insulin secretion (291), as previously indicated by gain-of-function mutations in the KCNJ11 gene encoding Kir6.2 channel (252). No similar association was found in the European population (291). Most interestingly, knock-down of KCNJ15 increased insulin secretion in vivo and in vitro (290).

C. K2P Channels in CNS Function

Transient changes in pHo in the brain occur in the extracellular space near neurons and glia during neurotransmitter or hormone action or after changes in metabolic activity and ion transport that might also change pHi (54). Local change in pHo at endfeet of astrocytes might link neuronal activity and blood flow (277). In the retina, pHo in the extracellular space of photoreceptors responds to light stimulus and to a circadian rhythm (84, 85). It is therefore conceivable that K2p channels expressed in the CNS might respond to these changes and regulate excitability. Evidence that TASK-1, TASK-3, and TREK-1 are important for neuronal excitability has come from the study of the respective KO mice and has been reviewed in detail recently (93, 218, 342). KO mice for TASK-1, TASK-3, and TREK-1 show increased resistance to halothane whilst TASK-1 and TASK-3 KO animals have altered motor performance. Cerebellar granule cells express both TASK-1 and TASK-3 and are not too sensitive to inactivation of either individually. TASK-3 KO mice have altered sleep patterns, probably due to the alteration of intrinsic neural rhythms. Studies with TREK-1 KO mice support a participation of this channel in neuroprotection, depression, and pain perception (162, 342). In addition, TREK-1 KO mice have a defective acetylcholine- and bradykinin-dependent vasodilation in mesenteric arteries, an effect that is dependent on the endothelium (108). TREK-1 might be important in the endothelial cells forming the mammalian blood-brain barrier (BBB), and recent studies show altered expression of TREK-1 in human BBB upon inflammation and BBB defects in TREK-1 KO mice (31; reviewed in Ref. 30).

D. K2p Channels and Human Disease

Inhibition of TASK-1 mediates the depolarizing effects of hypoxia and endothelin-1 in human pulmonary artery
smooth muscle cells (383), although this does not occur in the mouse (250). This looks like a genuine species difference, and indeed, mutations affecting human TASK-1 have been found in familial and idiopathic pulmonary arterial hypertension (242). The effect of the pathological mutations assayed in a heterologous expression system resulted in nonfunctional channels, but some activity could be recovered using a phospholipase inhibitor. Unfortunately, for a possible therapeutic intervention, the increase in current occurred at highly depolarized voltages well outside the physiological range of a background conductance.

A missense mutation in the maternal copy of KCNK9, the TASK-3 gene, has been associated with Birk-Barel syndrome, a maternally transmitted genomic-imprinting disorder leading to mental retardation, hypotonia, and elongated face dysmorphism (18). The mutated TASK-3-G236R channel was described as nonfunctional either in homo- or in heterodimeric form with nonmutated TASK-3. A more recent detailed study by Veale et al. (399) shows that TASK3-G236R channels generate decreased but significant currents when compared with WT channels. The currents are mildly inwardly rectified and differentially sensitive to extracellular acidification and muscarinic activation. Importantly, a partial recovery of the current magnitude could be achieved through the use of flufenamic acid, opening a possible avenue for the development of drugs effective in treating the Birk-Barel syndrome.

To our knowledge, the only other genetic human disease involving K2p channels refers to a dominant-negative mutation affecting pH-independent TRESK channel linked to familial migraine with aura (211). The mutation, a F139WfsX24 frameshift, abolishes function and exerts a dominant negative effect. Surprisingly, however, several other TRESK channel missense variants in unrelated patients did not have functional effect, and one point mutation, C110R, leading to complete loss of TRESK function when expressed in Xenopus oocytes was present in affected and control individuals (3). Intriguingly, expression of the C110R variant in small-diameter trigeminal ganglion neurons, unlike migraine-associated frameshift TRESK mutant, does not lead to hyperexcitability, indicating that it is incapable of exerting a dominant negative in a physiological context (137). As is often the case with human inherited diseases, migraine with aura belongs to the group of genetically complex disorders, and future work will have to elucidate the role played in it by TRESK malfunction.

Although as yet unrelated to human disease, it has been reported that TWIK-2 null mice present elevated systolic blood pressure associated with an increased peripheral vascular resistance and normal cardiac function. Isolated aortic smooth muscle cells are depolarized, and isolated segments of aorta presented greater contractile responses (226). Unexpectedly, endothelium-dependent relaxation was enhanced in the aortae of KO mice, which could represent a compensatory mechanism to the increased vascular tone (227).

E. Fickle Filters in K2p Channels: Possible Physiological or Pathophysiological Roles

Examples of changes in ion selectivity of K2p channels of possible physiological importance have multiplied recently. Some of these findings will have to await confirmation, but their accretion speaks of some sort of functional plasticity in the SF of these channels.

A paradoxical depolarization in hypokalemia is observed in some cardiomyocytes and has recently been linked to the expression of TWIK-1 channels (243). Using the channel activity-boosted TWIK-1-K274E mutant, Ma et al. (243) reported that this phenomenon is probably due to the remarkable acquisition of high relative Na+/K+ permeability of TWIK-1 channels in response to low extracellular K+. TWIK-1 has an unusual SF in which the TIGYG motif normally encountered in K+ channels is replaced by TTGYG in P1 domain and TIGLG in P2. This variation in SF is partly to blame as introducing the TTGYG motif into TASK-3 made these normally solidly K+-selective channels also Na+ permeable in low extracellular K+. Observing that many K+ channels exposed to low-K+/high-Na+ media experience an SF collapse which often passes through a phase of Na+ coordination, Goldstein (117) postulated that this normally brief intermediate state is stabilized in TWIK-1.

The loss of the first 56 NH2-terminal residues in TREK-1 channels through alternative translation initiation (ATI) may lead to the permanent acquisition of Na+ permeability. The full-length TREK-1 is highly K+ selective, whereas the shorter channel allows Na+ permeation and mediates depolarization (388, 426). Both the long and short versions of TREK-1 are present in the rat CNS (388), but there are as yet no functional reports of a Na+-permeable form in native cells. A recent paper by Veale et al. (398) reports an enhancement of TREK-1 by fenamates that affects both the full-length form and the ATI short form of the channel. In addition to establishing fenamate derivatives as potential pharmacological agents to modulate TREK-1, the investigation failed to observe the proposed change in selectivity of the shorter version of the channel. Both pieces of work use macroscopic currents to measure selectivity, the first after expression in Xenopus oocytes (388) and the second after expression in mammalian cells (398). Single-channel selectivity studies might help to resolve this discrepancy.

Intriguingly, similar ATI in TREK-2 deleting 54 or 66 amino acids of the NH2 terminus alters single-channel conductance without any change in ion selectivity (363). High-and low-conductance channel activity that probably corresponds to the expression of different forms of TREK-2 is
detected in cerebellar granule neurons (185). High- and low-conductance forms of TREK-1 have been seen in cardiac myocytes and in heterologous expression systems, but it is not clear if they correspond to species with alternative sequences (419).

The anomalous SF sequence of TWIK-1 plus other crucial sequence variations are thought to be responsible of the remarkable change in selectivity experienced by TWIK-1 upon extracellular acidification (49). TWIK-1, despite sharing the same extracellular H⁺ sensor with TASK-1 and TASK-3, responds quite differently to acidification, becoming highly Na⁺ permeable rather than simply closing at low pH₆ (49, 244). Independently of the mechanism mediating it, what might be the physiological impact of such a change of selectivity if any? Chatelain et al. (49) have attempted to respond to this query. According to their own previous results, TWIK1 is detected mainly in the recycling endosomes of native cells or those in which it has been heterologously expressed (76), and this is dependent on a COOH-terminal di-isoleucine motif (96). TWIK-1 outward currents first increase and then diminish as TWIK1 becomes permeable to Na⁺ and mediates its influx. This switch in selectivity develops slowly, requiring several minutes for completion and tens of minutes for recovery (49, 244). Considering that the pH of intracellular compartments is quite acidic, around 6 in the lumen of the trans-Golgi network and the endoplasmic reticulum (47), any TWIK-1 briefly visiting the plasma membrane would elicit a depolarizing effect before being recycled by endocytosis that leads to complete internalization in <10 min (76). Such a scenario helps to explain the observation that TWIK1 gene inactivation in mice causes a paradoxical hyperpolarization of kidney principal cells, pancreatic β-cells, and hippocampal astrocytes (49, 257, 401). According to this reasoning, TWIK-1 would be mainly a monovalent cation-permeable channel of intracellular compartments. Its more rare visits to the plasma membrane could be important in the physiology of β-cells, but otherwise remains enigmatic.

A rather more extreme case of SF eccentricity in K₂P channels is provided by Woo et al. (416) who report a direct effect of G protein βγ-subunits on TREK-1. This effect observed in astrocytes remarkably appeared to change TREK-1 selectivity so that the channel became anion selective, supporting astrocytic glutamate release to affect neighboring neurons. According to Hwang et al. (175), TWIK-1 becomes functional in cortical astrocytes only when forming dimers with TREK-1 in the plasma membrane of cortical astrocytes. This TWIK-1/TREK-1 dimer is present at the plasma membrane of astrocytes, is regulated by endocytosis but not by sumoylation, and is stabilized by disulfide bond cross-linking of the subunits at the extracellular cap or SID. Upon activation of cannabinoid receptor 1, and release of Gβγ subunits, it switches from being a K⁺-selective channel into an anion-selective conductance capable of mediating the release of glutamate (175). These extraordinary and exciting claims clamor for further probing, and it will be interesting to follow up future work in this front. A possible note of caution is that work on hippocampal astrocytes shows that although they also express TWIK-1 and TREK-1, TWIK-1 is present in intracellular compartments only (401).

X. CONCLUSIONS

Recent years have seen remarkable progress in our understanding of the molecular aspects of gating of pH-regulated K₂P and Kir K⁺-transport channels and in the physiological roles they play in various important cellular processes. Kir K⁺-transport channels are essential to renal function, regulation of K⁺ homeostasis in the brain, and acid secretion in the stomach. pH-dependent K₂P channels are critical in central chemoreception, regulation of aldosterone secretion in the adrenal gland, and bicarbonate conservation. Although their pH dependence is significant in some of these functions, its importance remains to be ascertained in many others. Mouse models attempting to understand the functions of these channels are increasingly becoming available, and it is predicted that judicious use of the advances in structure-function knowledge will increase their sophistication to interrogate specific channel function in a very direct way. Important diseases are associated with mutations in the genes encoding Kir K⁺-transport channels. The discovery of human inherited defects affecting K₂P channels is starting for this most recently discovered group of K⁺ channels.

Very important advances into how form relates to function has been made recently thanks to the discovery of new Kir structures that give clues about the mechanisms of gating of these channels. The reports of the first atomic structures of K₂P channels, on the other hand, reveal unique features that are already spurring fresh experimental work to improve our knowledge of the very special gating characteristics of these channels. These new insights come at a time when concepts about rather unorthodox gating behavior of K₂P channels has come to light.

Our examination of the recent literature on pH-regulated K₂P and Kir K⁺-transport channels also reveals some quite unexplored areas that are ready to be investigated: KO animals that have never reportedly been attempted and strange gating or ion handling behaviors awaiting confirmation and mechanistic explanation.

The evidence accumulated these years shows clearly that in addition to being important in setting the resting potential, Kir transport channels are crucial in the control of cell excitability, K⁺ homeostasis, and epithelial transport. The same can be said about the K₂P channels examined here: from being the molecular substratum of the long sought,
rather inert leak K⁺ conductance present in all cells, they have revealed themselves as a group of channels with a wide range of functions and exquisite regulation. The many questions still open about these channels augur a future of intense and satisfying research on these proteins in years to come.

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Address for reprint requests and other correspondence: F. V. Sepúlveda, Centro de Estudios Científicos (CECs), Avenida Arturo Prat 514, Valdivia 5110466, Chile (e-mail: fsepuveda@cecs.cl).

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