Molecular Physiology of pH-Sensitive Background K$_{2P}$ Channels

Background K$_{2P}$ channels are tightly regulated by different stimuli including variations of external and internal pH. pH sensitivity relies on proton-sensing residues that influence channel gating and activity. Gene inactivation in the mouse is a revealing implication of K$_{2P}$ channels in many physiological functions ranging from hormone secretion to central respiratory adaptation. Surprisingly, only a few phenotypic traits of these mice have yet been directly related to the pH sensitivity of K$_{2P}$ channels.

Beside their role in K$^+$ transport and homeostasis, K$^+$ channels are also crucial for generation and regulation of the electrical membrane potential. Modulation of this potential is essential for neuronal coding of the information, sensory processing, muscle contraction, cardiac rhythm, and neurotransmitter and hormone secretion. K$_{2P}$ channels comprise subunits forming the K$^+$-selective pore, often associated with auxiliary subunits and regulatory proteins. More than 78 human genes encode pore-forming subunits that are classified into three structural classes (45, 50, 65). Representative members of subunits with one P-loop are voltage-gated K$^+$ channels (Kv) that have six transmembrane segments (TMS) and inward rectifiers (K$_i$) that have two TMS. The third class of pore-forming subunits displays two P-loops (K$_{2P}$) and four TMS (FIGURE 1A). K$_v$ and K$_i$ subunits are active as tetramers, and K$_{2P}$ as dimers.

K$_{2P}$ channels generate K$^+$ currents over the whole membrane potential range, unlike K$_v$ channels that are mainly opened at negative voltages and K$_i$ channels that activate upon membrane depolarization. The existence of such background K$^+$ channels has been postulated more than 50 years ago (55), but their "leak" behavior combined with a lack of specific pharmacology has hampered their study for many years. In 1996, our laboratory isolated two K$_{2P}$ channels, tandem of P-domain in a weak inwardly rectifying K$^+$ channel (TWIK1) and TWIK-related K$^+$ channel (TREK1) that were the prototypic members of a novel family (41, 70). 15 human genes encode K$_{2P}$ channel subunits (KCNK genes in the Human Gene Organization nomenclature, K$_{2P}$XX gene products for the International Union of Basic and Clinical Pharmacology) (45). Electrophysiology of cloned channels, then recording of corresponding native conductances, together with in situ hybridization and immunohistochemistry, have revealed an unexpected broad tissue distribution. Another major outcome has been the discovery that these currents were tightly regulated by a variety of chemical and physical stimuli, demonstrating that the corresponding native K$^+$ conductances are not passive but precisely tuned and adapted to cell-specific functions. A number of questions are still under scrutiny concerning their physiological roles and the relevance of their modes of regulation, and also regarding their predictable implication in pathologies.

Among K$_{2P}$ subunits, 12 of them produce currents upon heterologous expression, and 10 are sensitive to variations of the extracellular and/or intracellular pH value, often in the physiological range (FIGURE 1C). This review focuses on this particular aspect. In the first part, pH-sensitive K$_{2P}$ subunits will be briefly described, and the mode of action of pH will be examined. In the second part, the physiological roles of these channels will be discussed as well as the potential modulatory effects of pH.

pH-Sensitive K$_{2P}$ Channels

**TASK1/TASK3**

TWIK-related acid-sensitive K$^+$ channels TASK1 (K$_{2P}$3.1) (37, 61, 69), TASK3 (K$_{2P}$3.1) (101), and the silent subunit TASK5 (K$_{2P}$15.1) (4, 58, 62) share >50% of sequence identity (FIGURE 1B), and heterodimerization between TASK1 and TASK3 has been demonstrated (27). TASK channels are expressed in the central nervous system (CNS) but also in other tissues including heart (TASK1, TASK5) and adrenal gland (TASK1, TASK3).

TASK channels produce currents with the typical behavior predicted by the Goldman Hodgkin Katz constant field equation for a leak K$^+$-selective pore (37). In symmetrical K$^+$ conditions, their whole-cell current-to-voltage relationship is linear, whereas in physiological K$^+$ conditions, this relationship is outwardly rectifying. A major feature of these channels that is at the origin of their acronym is their exquisite
**FIGURE 1. pH-sensitive K$_{2P}$ channels**

A: structural organization of K$_{2P}$ channel subunits. Transmembrane helices (M1 to M4), coiled-coil self-interacting domain (SID), pore domain (P1 and P2), pore helices (PH), and selectivity filter sequences (in bold) are indicated.

B: dendrogram of human K$_{2P}$ subunits. pH-sensitive subunits are in bold. KCNKx symbols correspond to the gene nomenclature.

C: sensitivity of K$_{2P}$ channels to external pH (currents recorded at $V_{H} = 50$ mV). Physiological pH range is shaded.

D: alignment of partial K$_{2P}$ sequences. Residues involved in sensing external pH are shown.
sensitivity to variations of the external pH ($\text{pH}_o$) in a narrow physiological range (FIGURE 1C). As much as 90% of the maximal TASK1 current is recorded at $\text{pH}_o$ 7.7 and <10% at $\text{pH}_o$ 6.7 (37). pK value is 7.3. TASK3 has a similar sensitivity, albeit shifted toward more acidic values. TASK3 is almost fully active at pH 7.4 and is inhibited by acidification with a pK value of 6.7 (101). TASK1/TASK3 heterodimers are inhibited with a pK value of 7.3 (27). TASK1/3 channels are not sensitive to variations of the cystolic pH ($\text{pH}_i$).

As with other $K_{zp}$ currents, TASK currents are relatively resistant to the classical $K^+$ channel blockers including $\text{Ba}^{2+}$, $\text{Cs}^+$, TEA, and 4-AP. However, they are inhibited by the endocannabinoid anandamide and by local anesthetics including bupivacaine. TASK currents are stimulated by volatile general anesthetics with differential sensitivities: TASK1 is stimulated by halothane and xenon but not by chloroform, diethyl ether, and isoflurane (95), whereas TASK3 is sensitive to halothane, isoflurane, and chloroform but not xenon (48). TASK channel activity is downregulated by stimulation of Gq-coupled receptors (19).

**TASK2/TALK2/TALK1**

Soon after TASK1, another $K_{zp}$ subunit was cloned and termed TASK2 ($K_{zp}6.1$) because of its sensitivity to $\text{pH}_o$ (102). However, TASK2 is a member of a distinct subgroup of $K_{zp}$ subunits comprising TWIK-related alkaline pH-activated $K^+$ channels TALK1 ($K_{zp}6.1$) and TALK2 ($K_{zp}17.1$) (FIGURE 1B) (31, 44). TASK2 and TALK1/2 expression are very low in the CNS. These channels are more present in peripheral tissues including kidney, liver, pancreas, and placenta for TASK2, and mainly in the pancreas for TALK1 and 2.

These channels are stimulated by alkalinization of the external medium in the 7.5–10 pH range (FIGURE 1C). pK value for TASK2 activation is 8. pH sensitivity of TALK channels is even more shifted in the alkaline direction. Maximal activation of TALK1 and 2 occurs above $\text{pH}_o$ 10. TALK1 and TALK2 are strongly activated by nitric oxide species (NOS) and reactive oxygen species (ROS), suggesting that ROS and NOS could act as endogenous openers able to rise TALK activity at physiological pH (36). TASK2 and TALK2 are not only opened by extracellular alkalinization but also by intracellular alkalinization (88).

**TREK1/TREK2/TRAAK**

Together with the TWIK-related arachidonic acid-stimulated $K^+$ (TRAAK; $K_{zp}4.1$) channel (42, 71), TREK1 ($K_{zp}2.1$) (41) and TREK2 ($K_{zp}10.1$) (6, 72) form a functional subclass of $K_{zp}$ channels (FIGURE 1B) well expressed in the nervous system but also in small intestine (TREK1), kidney (TREK1, TREK2), pancreas (TREK2), and placenta (TRAAK).

These channels show complex regulatory properties. They are strongly stimulated by temperature rise, mechanical stretch of the plasma membrane, and application of free polyunsaturated fatty acids (PUFAs; including arachidonic acid, as indicated in the acronym TRAAK) and phospho- and lysophospho-lipids (63, 64, 72, 76–79, 96). TREK1 and TREK2 are also regulated by phosphorylation and G-protein-coupled receptors to hormones and neurotransmitters. They are inhibited by Gs-coupled receptors (19, 72, 96). This regulation is explained by stimulation of protein kinase PKA and phosphorylation of serine/threonine residues in the channel (77). Conversely, TREK channels are stimulated by Gi-coupled receptors (19) because a significant fraction of these channels is phosphorylated in basal conditions and gets dephosphorylated when PKA activity is downregulated (17). Finally, stimulation of Gq-coupled receptors inhibits TREK activity by a mechanism involving protein kinase PKC and channel phosphorylation (19, 57, 87). The cytosolic carboxy terminal (C-ter) domain immediately following the fourth TMS (M4) (104) plays a key structural role in the regulation of TREK channels by PUFAs and membrane stretch, but also by neurotransmitters (56, 63, 64). It contains serine residues that are phosphorylated by PKA and PKC. Binding of A-kinase anchoring protein AKAP150 to this regulatory domain has a major effect on TREK channel activity and regulation (106). Another protein partner is the microtubule-interaction protein Mtap2 (105). It favors the addressing of TREK channels to the plasma membrane.

Intracellular protons activate both TREK1 and TREK2 (56, 78). TRAAK is not modified by cytoplasmic acidosis but is stimulated by alkalinization from pH 7.3 (63). TREK1 and TRAAK are inhibited by external acidification (22, 104). TREK1 inhibition is strong with a pK of 7.4 (FIGURE 2C). The all-or-none effect of $\text{pH}_o$ variation is steep and is observed within one pH unit. TREK2 is not inhibited but is activated by acidification in the same range of $\text{pH}_o$ (FIGURE 2C) (104). The maximal inhibition by acidification (TRAAK) or by alkalinization (TREK2) is 40–60% of maximal current.

**TWIK1/TWIK2**

Originally cloned from adult kidney, TWIK1 ($K_{zp}1.1$) is expressed in many other tissues including brain, heart, liver, and pancreas (70). Its closest relative, TWIK2 ($K_{zp}6.1$) (18, 97), has a similarly wide distribution with highest expression levels found in heart, placenta, spleen, and colon. TWIK1 produces only modest currents upon heterologous expression. This lack of current is due to the rapid and constitutive endocytosis of TWIK1 from the plasma membrane and then storing in a recycling
FIGURE 2. TASK2 and the stabilization of bicarbonate transport

A: TASK2-expressing cells (in blue) in the adult mouse kidney. Expression is found in convoluted and straight proximal tubules and in papillary collecting ducts. A cortical area at higher magnification is shown.

B: A model of TASK function in proximal tubule cells. NaHCO3 reabsorption involves Na+/H+ exchange (by NHE3) across the apical membrane. Na+ and HCO3− transport by kNBC1 causes basolateral membrane depolarization. In the extracellular space, rise in HCO3− concentration causes a pH increase that activates TASK2. TASK2 activity recycles K+ accumulated by Na+-K+-ATPase and leads to repolarization of the membrane that is needed as a driving force for ongoing NaHCO3 export.

CA, carbonic anhydrase.
endosomal compartment (39, 40). Sumoylation has also been proposed as a TWIK1 silencing mechanism (100), but this issue remains controversial (39). TWIK1 binds to the guanine nucleotide exchange factor EFA6 (32). TWIK1 relocates to the plasma membrane in the presence of EFA6 and a dominant-negative mutant of the small G protein Arf6, suggesting that TWIK1 favors its own endocytosis through a transient Arf6/EFA6/TWIK1 association, where EFA6 would act both upstream of Arf6 as a guanine nucleotide exchange factor and downstream of Arf6 in cargo selection (32).

TWIK1 and TWIK2 produce currents with a rapidly inactivating component. Because of this inactivation, their steady-state current-voltage relationships are much more similar to that of weak inwardly rectifying K$_v$1.1 current than those of TASK K$_{2p}$ currents (97). Both TWIK1 and TWIK2 currents are substantially reduced by pharmacological treatments known to lower intracellular pH (70). TWIK1, but not TWIK2, is inhibited by external acidification. The inhibition has a pK of 6.7 (100).

**Regulation of K$_{2p}$ Channel Activity by pH: Modes of Action**

How does pH influence channel activity? K$^+$ flux through K$_{2p}$ channels is modulated by stimuli converging on a few gating mechanisms common to the different types of K$_{2p}$ channels. An inner transmembrane helix bundle forms the primary gate at the cytoplasmic side (M2 and M4 in K$_{2p}$ channels, depicted in **FIGURE 1A**). Opening and closing of this so-called inner or activation gate, under the influence of cytoplasmic agents (ATP, Ca$^{2+}$, Na$^+$, phosphorylation, etc.) or membrane depolarization or membrane stretch, control K$^+$ flow through the pore. A second gate is present at the outer surface that involves the selectivity filter and the pore domains (**FIGURE 1A**). The constriction of this upper gate inhibits K$^+$ flux even when the inner gate is open. This phenomenon has been originally identified in K$_v$ channels and coined C-type inactivation. C-type inactivation is sensitive to external K$^+$ concentration and to mutations at the external mouth of the pore. In K$_v$ channels, activation and inactivation gates are negatively coupled, such as the opening of the first one causes the other to close (26). In K$_{2p}$ channels, the two gates seem to be positively gated: the opening of the activation gate stimulates the opening of the upper gate, allowing K$_{2p}$ channels to fulfill their role of background channels (10). However, two very recent reports suggest that, in the TREK1 K2P channels, the gating is primarily, if not exclusively, at the selectivity filter itself (5, 99). For more comprehensive discussions on K$_{2p}$ channel gating, see Refs. 23 and 81.

External pH modifies K$_{2p}$ channel activity by acting on the upper gate as shown previously for K$_v$1.5 (107) and K$_v$2.3 (25) channels. **FIGURE 1D** depicts residues involved in pH$^o$ sensitivity of K$_{2p}$ channels. In TASK1, TASK3, and TWIK1, acid sensitivity is conferred primarily by protonation of a histidine residue located in P1 (GYGH, H98 in TASK1) (84, 101). Data suggest that protonation of H98 is associated with conformational changes at the selectivity filter. By rotating and creating an electropositive barrier for K$^+$ ions traversing the outer mouth of the channel, protonated H98 would promote lower K$^+$ pore occupancy and open probability. Although both TASK1 and TASK3 possess the same proton-sensing histidine in P1, TASK1 is more sensitive to acidification than TASK3. Swapping M1P1 loops between TASK1 and TASK3 was used to show that this domain influences the pH sensor at the selectivity filter, providing an explanation for the observed difference in their pH$^o$ sensitivity (21). In TREK1, TREK2, and TRAAK, pH$^o$ sensitivity also relies on protonation of a histidine residue. But this residue is located NH$_2$ terminal to P1 between the self-interacting domain and the pore helix in a position similar to K$_v$1.5 and K$_v$2.1 (**FIGURE 1D**) (104). How does protonation result in closure of TREK1 and TRAAK and opening of TREK2? By structural modeling and mutagenesis, charged residues adjacent to second pore domain in the P2M4 extracellular loop were shown to be important for pH$^o$ sensitivity of TREK1 and TREK2 (104). Replacement of neutral and positively charged residues in TREK2 by the negatively charged residues of TREK1 present at equivalent positions was sufficient to inverse TREK2 response to acidification and to convert it into a TREK1-like response. These results suggest that electrostatic interactions between the protonated histidine and P2M4-charged residues influence the upper gate. In TASK2 and TALK channels, there is no histidine residue involved in alkaline sensitivity. Several charged residues in the M1P1 loop had been implicated in pH$^o$ sensitivity of TASK2 (83), but this result has been debated later. A more convincing pH sensor is an asparagine residue located COOH terminal to P2 in the P2M4 loop (**FIGURE 1D**) (89). Transplanting this residue to a pH$^o$-resistant H98N mutant of TASK3 led to the recovery of pH$^o$ gating (113). Initially, it has been proposed that, in the protonated form, R224 might decrease occupancy of the selectivity filter by K$^+$, a blocked state relieved by neutralization of R224 at alkaline pH$^o$ (89). However, more recent analysis suggests that protonated R224 is associated with an increased height of the energetic barriers for K$^+$ translocation that accounts for an absence of channel conduction at acidic pH rather than a pore blockade (113).
How does intracellular pH influence channel activity? Cytoplasmic acidification converts low-activity TREK channels into robust leak channels that are insensitive to AA, stretch, and phosphorylation. Alanine scanning of the C-ter of TREK1 pointed out glutamine 306 as the proton sensor (56). Mutation of this glutamine to alanine converts TREK1 into a constitutively active K+ channel that becomes insensitive to AA, stretch, and phosphorylation. E306 is located in the major regulatory domain immediately adjacent to the M4. Recently, it has been shown that its binding to the plasma membrane controls TREK1 activity. It involves interactions between phosphatidylinositol-4,5-bisphosphate (PIP2), the most abundant phosphoinositol in the inner leaflet of the membrane, and a polybasic motif in the TREK1 C-ter (103). Protonation of E306 resulting from acidosis would strengthen these interactions by adding a positive charge to this domain and therefore enhances channel activity. Neutralization of a lysine residue has been shown to be linked to TASK2 opening by intracellular alkalinization (88). This lysine 245 is located at the C-ter end of M4, suggesting that, there again, pH influences channel gating.

**Physiological Roles of K_{2P} Channels**

The basic role of background K+ currents is to generate the resting negative membrane potential in many cell types and also to oppose depolarization in excitable cells. The characterization of K_{2P} channels has disclosed an unsuspected molecular diversity and wide distribution. Unsuspected as well was the very diverse modes of regulation of these supposed “leak” channels. These modulations are expected to strongly influence cell electrical properties. For example, inhibition of background K+ channels can favor membrane depolarization leading to a Ca^{2+} entry that promotes neurotransmitter and hormone secretion or muscle contraction. In neurons, their inhibition will result in more membrane resistance and enhanced responsiveness to other depolarizing inputs. Many hypotheses have been advanced regarding their specific physiological functions in different organs. In the absence of specific blockers, the main criterion to implicate K_{2P} channels in a native measured leak conductance has often been limited to their differential sensitivity to extracellular pH, membrane stretch, PUFAs, or volatile anesthetics. Generation of K_{2P} knockout (KO) mice has allowed this limitation to be overcome. Here, we highlight situations in which the pH sensitivity of a K_{2P} channel is regarded as an important property clearly implicated in channel function (CO_{2}/pH sensor in brain stem, HCO_{3}− sensor in kidney, pH sensor in aldosterone secretion, H^{+}-modulated synaptic function) as well as situations where this property is not considered as essential.

**TASK2 and the Renal Reabsorption of Bicarbonates**

Proximal renal tubular acidosis syndrome is characterized by an inability to reabsorb bicarbonate, causing hyperchloremic acidosis (3). The acid-base status of task2-/− mice is very similar to that observed in human patients suffering from this syndrome, with metabolic acidosis, reduced blood pH and HCO_{3}− concentration, and increased urinary HCO_{3}− (110). TASK2 is strongly expressed in proximal tubules and papillary collecting ducts (FIGURE 2A). Patch-clamp experiments on proximal tubular cells indicated that TASK2 was activated during HCO_{3}− transport as a consequence of alkalinization of the basolateral compartment (66). This K+ current increase allows efficient recycling of K+ that enters the cell via Na+/H+ co-transporters. In task2-/− mice, this K+ conductance is dramatically reduced. These results show that pHo sensitivity of TASK2 is directly linked to its role in bicarbonate reabsorption and blood pH control.

It has also been shown that TASK2 is necessary for cell-volume regulation in proximal tubule cells protecting from osmotic cell swelling that might accompany electrolyte reabsorption (8, 66). It is well documented that cell volume decrease also accompanies apoptosis, a phenomenon known as apoptotic volume decrease (AVD) (67). This volume change results from a loss of cytosolic K+ and Cl− and therefore of water in response to apoptosis inducers. In proximal tubule cells, TASK2 is activated during AVD induced by staurosporine or cyclosporine treatments. In task2-/− cells, apoptosis is strongly reduced, suggesting that activation of TASK2 could participate in the massive proximal cell death that occurs after renal ischemia/reperfusion episodes (67).

**TASK2 and the Respiratory Control**

Central chemoreception is the process by which pH decreases in the brain stem caused by increased levels of CO_{2} contribute to the adaptive changes in the depth and frequency of breathing necessary for CO_{2} elimination. In the central nervous system, TASK2 expression is restricted to very few brain stem nuclei including the retrotrapezoid nucleus (RTN) (43) (FIGURE 3, A AND B). The implication of a population of RTN neurons in CO_{2} and O_{2} sensing has been highlighted by an elegant
study showing that such neurons are lost in a mouse model bearing a phox2b mutation responsible for the congenital central hypoventilation syndrome (CCHS) in humans (FIGURE 4C) (34, 35). Patients have negligible sensitivity to hypercapnia and also to hypoxemia, leading to the failure of automatic control of breathing during sleep and wakefulness (20). Interestingly, it was found that all TASK2-expressing RTN neurons are lost in the CCHS mouse model (43) (FIGURE 3C). Task2−/− mice display disturbed chemosensory function with hypersensitivity to low CO2 concentrations (43). TASK2 is probably needed to stabilize the membrane potential of chemoreceptive cells. In addition, task2−/− mice lost the long-term respiratory depression induced by hypoxia (FIGURE 3D) but not the acute carotid body mediated increase. Together with the lack of anoxia-induced respiratory depression in the isolated brain stem spinal cord preparation, these results support an essential role of TASK2 channels in the central O2 chemosensitivity. A current hypothesis is that TASK2 activation by ROS generated during hypoxia may silence RTN neurons, thus leading to respiratory depression (36, 43, 67).

**FIGURE 3. TASK2 and the central respiratory chemoreception**
A: localization of TASK2-expressing cells (in blue) in the adult mouse brain stem. B: TASK2-positive cells in the dorsal raphe nucleus. C: loss of TASK2-positive cells in mouse embryo of Phox2bΔTalm/−, a mouse model for human congenital central hypoventilation syndrome. D: Task2−/− mice show depression of respiration during prolonged severe hypoxia that is absent in wild-type mice.

**TASK1 and TASK3 as Chemosensors**
Well before TASK2, TASK1, and TASK3 were the best candidates among K2P channels to mediate central as well as peripheral chemoreception. Their exquisite sensitivity to extracellular pH as well as their distribution in multiple clusters of respiratory-related chemosensitive neurons, including the medullary raphe, RTN, pre-Bötzinger and Bötzinger complexes, lateral reticular nucleus, hypoglossal motoneurons, and locus coeruleus, prompted the widely shared hypothesis that their inhibition by external acidification would lead to depolarization, and increased excitability and respiratory motoneuronal output (9, 111). However, a critical role of TASK1 and TASK3 channels in central CO2 sensitivity was later questioned because hypercapnic response persisted in double task1−/−task3−/− mice, although the chemosensitivity of raphe neurons, but not RTN neurons, was abolished (86).

TASK1 and TASK3 are also expressed in the glomus cells of the carotid body that respond to hypoxia and acidosis with depolarization that initiates electrical activity (13, 14, 60). Heterodimers made
of TASK1 and TASK3 subunits form the major part of the O2-sensitive background K⁺ conductance in these cells (60). Studies on the systemic carotid body-mediated ventilatory response to hypoxia and hypercapnia in KO mice have yielded conflicting results. A study reported that the hypoxic and acidic responses were partially blunted in task1⁻/⁻ task3⁻/⁻ mice (109), whereas another one found no significant changes of the hypercapnic response compared with wild-type mice in plethysmography experiments (86). Glomus cells lacking TASK1 and TASK3 have significantly higher membrane resistance and less hyperpolarized resting potential than wild-type cells (93). However, responsiveness to hypoxia of task1⁻/⁻ task3⁻/⁻ cells is maintained. This ability to maintain a powerful response to hypoxia does not definitively rule out a role of TASK channels in O2 and CO2 sensing than wild-type cells (93). However, responsiveness to hypoxia does not definitively rule out a role of TASK channels in O2 and CO2 sensing experiments (86). Glomus cells lacking TASK1 and TASK3 have significantly higher membrane resistance and less hyperpolarized resting potential compared with wild-type mice in plethysmography (80). Glomus cells lacking TASK1 and TASK3 in ZG cells prompted the hypothesis that TASK channels were important for ZG cell depolarization, thus stimulating Ca²⁺ entry via voltage-gated Ca²⁺ channels and aldosterone synthesis (FIGURE 4C). An additional regulation is provided by plasma pH as part of a feedback loop that maintains acid-base balance by enhancing renal proton excretion. The fact that angiotensin II and acidosis promote inhibition of both cloned TASK and native background K⁺ channels in ZG cells resulted in a marked depolarization of ZG cells, resulting in moderate hyperaldosteronism poorly sensitive to salt intake, hypertension, and hypokalemia. In these double-KO mice, the angiotensin II-independent component of the aldosterone secretory response to metabolic acidosis was preserved and even augmented (49). Thus pH sensing by glomerulosa cells does not require the presence of TASK1 or TASK3 channels.

**TASK1 and TASK3 as Nutrient Sensors**

TASK1 and TASK3 have not only been proposed to sense O₂ and CO₂ but also glucose (15). Hypothalamic orexin neurons are able to sense extracellular glucose concentration to trigger adaptive responses in response to low body energy level, including wakefulness and food-seeking behavior. Firing activity of these neurons is stimulated by a decrease of glucose and also by acidosis and CO₂ (16). Glucose and acid inhibit a background K⁺ current promoting depolarization and enhanced excitability. However, pH and glucose responses are preserved in task1⁻/⁻ task3⁻/⁻ orexin neurons, ruling out TASK channels as exclusive sensors in these cells (46, 51).

**TASK1 and TASK3 in Adrenal Gland Development and Aldosterone Secretion**

Adrenal gland is a major site of TASK1 and TASK3 expression in both rodents and humans. The situation may be different in bovine gland where TREK1-like currents are recorded instead of TASK-like currents (38). Nevertheless, in mouse and human adrenals, TASK1 is expressed in the whole cortex, whereas TASK3 is restricted to the outer zona glomerulosa (ZG) layer that produces aldosterone (FIGURE 4A). This mineralocorticoid hormone stimulates K⁺ excretion and Na⁺ reabsorption in the kidney, contributing to fluid volume and blood pressure control. Aldosterone production is controlled by angiotensin II and plasmatic K⁺ concentration and to a lesser extent adrenocorticotropin (ACTH) (FIGURE 4B). A low-Na⁺ diet causes an increase of the angiotensin II circulating level that drives the membrane potential of ZG cells to depolarized values, thus stimulating Ca²⁺ entry via voltage-gated Ca²⁺ channels and aldosterone synthesis (FIGURE 4C). An additional regulation is provided by plasma pH as part of a feedback loop that maintains acid-base balance by enhancing renal proton excretion. The fact that angiotensin II and acidosis promote inhibition of both cloned TASK and native background K⁺ channels in ZG cells prompted the hypothesis that TASK channels were important for ZG cell potential and aldosterone secretion (28, 29, 75). Task1⁻/⁻ mice show severe hyperaldosteronism independent of salt intake, hypokalemia, and hypertension (52). This primary hyperaldosteronism is caused by a defect of the adrenocortical zonation. Aldosterone synthase is absent in the outer cortex normally corresponding to the ZG, but abundant in the reticulo-fasciculata layer (FIGURE 4B). Hyperaldosteronism and zonation are restricted to females in adults. Zonation is corrected by testosterone in adult female task1⁻/⁻ mice that show restored salt controlled aldosterone synthesis and blood pressure. The molecular link between the missing TASK1 function and the altered adrenocortical zonation is not yet understood. Patch-clamp recordings showed that adrenal cells from task1⁻/⁻ mice were slightly depolarized compared with wild-type cells (~68 vs. ~75 mV, respectively). Additional depolarization by extracellular acidic pH indicated the presence of other pH-sensitive K⁺ currents, most probably TASK3 (52). In task1⁻/⁻ task3⁻/⁻ male mice, zonation of the adrenal cortex is preserved (30). However, inactivation of both TASK1 and TASK3 resulted in a marked depolarization of ZG cells, resulting in moderate hyperaldosteronism poorly sensitive to salt intake, hypertension, and hypokalemia. In these double-KO mice, the angiotensin II-independent component of the aldosterone secretory response to metabolic acidosis was preserved and even augmented (49). Thus pH sensing by glomerulosa cells does not require the presence of TASK1 or TASK3 channels.
**TASK Channels and Cell Proliferation/Cell Death**

Reducing K⁺ efflux trough TASK3 by extracellular acidosis or genetic inactivation prevents apoptosis of cultured cerebellar granule cells. Conversely, genetic transfer of TASK1 or TASK3 in hippocampal neurons induces cell death (68). These results suggest an important role for these TASK subunits in cell survival. This is also supported by the fact that TASK3 expression is upregulated in breast cancers (85). Overexpression of TASK3 in cell lines is associated with tumor formation and resistance to hypoxia and serum deprivation (85), whereas genetic inactivation of TASK3 in tumor cells abrogates its oncogenic functions (98), confirming that the dysregulation of TASK3 expression plays a role in breast cancer.

**K₂P Channels and Neuronal Excitability**

In the brain, pH changes occur in both physiological and pathophysiological conditions. For example, neurotransmitter release produces transient acidifications in the extracellular space, particularly in synapses, whereas metabolic activity linked to neuronal activity produce intracellular acidification. Also, modifications in blood flow, seizure, spreading depression, ischemia, and inflammation are all associated with pH shifts that can persist significantly. By affecting ion channel activity, these pH variations adversely affect neuronal activity.

The general importance of background K₂P channels for neuronal excitability is confirmed by the fact that K₂P KO animals show a substantial decrease in sensitivity for volatile anesthetics. In trek1⁻/⁻, task1⁻/⁻, and task3⁻/⁻ mice, immobilization and analgesia require significantly higher doses of halothane (53, 73, 74). TASK1 and TASK3 KO mice also exhibit compromised motor performance consistent with their high expression in the cerebellum. Inactivation of TASK1 has no significant effect on the electrophysiological properties of cerebellar granule cells because TASK3 homodimers can substitute to TASK1/TASK3 heterodimers (1). In task3⁻/⁻ mice, granule neurons are depolarized, and smaller current injections are necessary to produce action potentials. Sustained activity is affected, showing that TASK3 plays a role in repolarization. In its absence, voltage-gated conductances could not completely recover from inactivation, interfering with sustained repetitive firing (12). More recently, it has also been shown that task3⁻/⁻ mice exhibit pronounced sleep disturbances and a marked decrease in the sensitivity to the hypnotic effects of halothane in a loss-of-righting reflex assay. This has been linked to the disruption of intrinsic neural rhythms, i.e., the type II theta oscillations that accompany anesthetic-induced hypnosis and natural sleep (94). In humans, a missense mutation in TASK3 fully abolishes TASK currents both when functioning as a TASK3 homodimer or as a heterodimer with TASK1. This mutation is associated with mental retardation, hypotonia, and unique dysmorphism with elongated face (7).

In several assays designed to evaluate depression, trek1⁻/⁻ mice displayed a resistance to helplessness and despair (54). In addition, these mice were insensitive to antidepressant drugs including serotonin-specific reuptake inhibitors (SSRIs). This depression-resistant phenotype was attributed to an increased activity of serotonin systems because firing activity of dorsal raphe neurons that express TREK1 is increased in KO mice and because serotonin tone is increased in the hippocampus of these animals. Background currents have been identified in serotonin-synthesizing raphe neurons and initially assigned to TASK family on the basis of their sensitivity to pH and halothane (112). A pH-sensitive current is indeed abolished in dorsal raphe neurons from task⁻/⁻ mice (82). More recently, it has been shown that, like trek1⁻/⁻, task3⁻/⁻ mice are resistant to depression and insensitive to Fluoxetine (47). Several SSRIs directly inhibit TREK1, raising the possibility that TREK1 inhibition could account for some of the effect of these antidepressant drugs (59, 108). Together, these results support both the TREK1 and TASK3 channels as therapeutic targets for antidepressant action. TREK1 and TRAAK were also shown to contribute to pain detection. KO mice are more sensitive not only to painful heat and cold but also to low-threshold mechanical stimuli (2, 91). Inhibition of these channels by acidosis that rises during injury, inflammation, and cancer may therefore contribute to the associated pain (24).

**FIGURE 4. TASK channels in adrenal gland function**

A: distribution of TASK1 and TASK3 mRNAs in adrenal gland from adult mouse. Both TASK1 and TASK3 are expressed in the zona glomerulosa (ZG); TASK1 is also present in the zona reticulo-fasciculata zona (ZR). B: mislocation of the aldosterone synthase (in green) in adult female task1⁻/⁻ mouse. C: regulation of aldosterone secretion by glomerulosa cells. The different steps are 1) depolarization of the membrane induced by K⁺ channel inhibition by angiotensin II of by an hyperkalemia that shifts the Nernst potential, 2) a Ca²⁺ influx through voltage-gated Ca²⁺ channels activated by the depolarization and also by angiotensin receptor activation that leads to generation of IP3 and store release of Ca²⁺, 3) Ca²⁺ binding to calmodulin, 4) Ca²⁺-calmodulin activation of CaM-kinases, 5) activation of transcription factors, and 6) increased transcription of the aldosterone synthase that ultimately causes aldosterone production and release.
larger infract volumes following transient middle cerebral artery occlusion (82). The protection from ischemia afforded by linolenic acid (LIN) or lysophosphatidylcholine was absent in trek1−/− mice, suggesting that activation of these channels following PUFAs release and intracellular acidosis, both occurring during ischemia, limit neuronal depolarization and damage. An alternative mechanism of protection involves cerebral blood flow modulation by TREK1. In trek1−/− mice, the vasodilator acetylcholine is unable to relax basilar arteries, and LIN is unable to induce artery vasodilatation and enhance cerebral blood flow, suggesting that TREK1 may have a protective effect by favoring an increased blood flow during ischemia (11). If TREK1 activation by PUFAs and intracellular acidification is protective, then how do we explain that TASK1 currents are also protective despite their inhibition by acidification? Brain neurons have developed a broad array of immediate and long-term homeostatic mechanisms to suppress hyperexcitability during ischemic episodes. TASK1 channels may act before TREK1 channels and provide a protective polarization in the early phase of ischemia as long as they remain active during moderate acidification. This hypothesis is corroborated by the findings that TASK1 protection is more efficient during shorter episodes of ischemia or in earlier evaluations of stroke volume after artery occlusion (82).

Conclusions and Perspectives

Sensitivity to pH variations has been reported as a remarkable property of many types of K+ channels. Among K2P s, TASK and TALK channels were originally thought to be the only background channels sensitive to external pH in the physiological range. Actually, this property appears to be shared by many more K2P channels. Nevertheless, it is a lesson from the study of K2P KO mice that it is not obvious to make the relation between pH sensitivity and the effective role of a particular channel, nor even to demonstrate that proton sensing is relevant for channel functions. Works from our laboratory have shown that the functions assigned to TASK2 in bicarbonate transport in the kidney, or in the relation between cerebrospinal fluid pH and RTN neuron potential, are clearly related to their ability to sense and react to external pH changes. On the other hand, we and others failed to notice a major influence of the loss of TASK channels on breathing adaptation to CO2 and acidification. In adrenal glands, TASK1 inactivation has major consequences on aldosterone secretion but no clear relationship to their pH sensitivity. Also, gene inactivation of K2P channels has multiple consequences on neuronal excitability, yet again not clearly related to pH sensitivity. In the brain, pH changes occur in both physiological and pathophysiological conditions. By affecting ion channel activity, these pH variations adversely affect neuronal activity. Acid-sensing ion channels (ASICs) form another important class of pH-sensitive ion channels expressed in the nervous system (33, 90). Both activation of ASIC cationic channels and inhibition of K2P K+ channels by acidosis favor depolarization, suggesting that activity of ASIC channels could mask, at least partially, the functional importance of K2P channel pH sensitivity in KO mice. Probing the physiological relevance of K2P channel pH sensitivity clearly requires the development of new tools. Identification of residues involved in pH sensing in K2P channels makes it now possible to generate knock-in mice expressing pH-resistant channels to specifically address this issue.

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