A Novel Mechanism for Human K_{2p}2.1 Channel Gating

FACILITATION OF C-TYPE GATING BY PROTONATION OF EXTRACELLULAR HISTIDINE RESIDUES

Asi Cohen, Yuval Ben-Abu, Shelly Hen, and Noam Zilberberg

From the Department of Life Sciences and the Zlotowski Center for Neuroscience, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel

The mammalian K_{2p}2.1 potassium channel (TREK-1, KCNK2) is highly expressed in excitable tissues, where it plays a key role in the cellular mechanisms of neuroprotection, anesthesia, pain perception, and depression. Here, we report that external acidification, within the physiological range, strongly inhibits the human K_{2p}2.1 channel by inducing “C-type” closure. We have identified two histidine residues (i.e. His-87 and His-141), located in the first external loop of the channel, that govern the response of the channel to external pH. We demonstrate that these residues are within physical proximity to glutamate 84, homologous to Shaker Glu-418, KcsA Glu-51, and KCNK0 Glu-28 residues, all previously argued to stabilize the outer pore gate in the open conformation by forming hydrogen bonds with pore-adjacent residues. We thus propose a novel mechanism for pH sensing in which protonation of His-141 and His-87 generates a local positive charge that serves to draw Glu-84 away from its natural interactions, facilitating the collapse of the selectivity filter region. In accordance with this proposed mechanism, low pH modified K_{2p}2.1 selectivity toward potassium. Moreover, the proton-mediated effect was inhibited by external potassium ions and was enhanced by a mutation (S164Y) known to accelerate C-type gating. Furthermore, proton-induced current inhibition was more pronounced at negative potentials. Thus, voltage-dependent C-type gating acceleration by protons represents a novel mechanism for K_{2p}2.1 outward rectification.

Potassium leak channels comprise the newest branch of the potassium channel superfamily serving to carry leak or “background” currents that are mostly time- and voltage-independent. They are structurally unique in that each subunit possesses four transmembrane segments and two pore-forming domains and hence are often referred to as two-pore domain K+ channels or K_{2p} channels. By influencing the cell membrane resting potential, leak currents shape the duration, frequency, and amplitude of action potentials and, therefore, modulate cell responsiveness and excitability (1).

The best studied member of the K_{2p} family is the mammalian mechanosensitive K_{2p}2.1 (KCNK2, TREK-1) channel, expressed at high levels in excitable tissues such as the nervous system (2), heart (3), and smooth muscle (4). K_{2p}2.1 channels have attracted increasing interest in recent years as their activity and biophysical properties are strongly regulated by various physical and chemical signals (5). In addition to activation by mechanical stretch, K_{2p}2.1 opens in response to high temperatures (6), lysophospholipids (7), internal acidosis (8), arachidonic acid (9), volatile general anesthetics (10), and other agents. K_{2p}2.1 activity is down-regulated upon phosphorylation by protein kinases A and C (2) and inhibited by various compounds such as fluoxetine (Prozac) (12), caffeine, and theophylline (13). It has been suggested that K_{2p}2.1 is an important target for volatile anesthetics and participates in protection against epilepsy and neuroprotection during brain and spinal cord ischemia (14). Recently, it has been shown that K_{2p}2.1 channels expressed in small dorsal root ganglion neurons (6) can act as one of the molecular sensors involved in pain perception (15).

Although the regulation and contribution of the carboxyl-terminus domain to the modulation of K_{2p}2.1 activity have been studied in detail, the conformational changes and gating events that take place at the ion-conducting pore during channel regulation remained unknown. It had been previously shown that gating of another member of the K_{2p} family, i.e. the Drosophila KCNK0, entails protein rearrangement of the externally oriented portion of the pore and shares many characteristics with the slow inactivation (C-type inactivation) of voltage-gated potassium channels (16). Now, we provide evidence that K_{2p}2.1 channels rely on this mechanism for gating as well.

We report that external acidification, within the physiological range, strongly inhibits the human K_{2p}2.1 channel. Mutations in two outer pore surface-exposed histidine residues (i.e. His-87 and His-141), located in the first turret loop of the channel, dramatically decreased channel responsiveness to external acidification. Our results also indicate that external protons inhibit the channel by inducing closure of the outer pore gate, similar to the C-type inactivation of voltage-gated potassium channels. Moreover, we propose a mechanistic model for proton-induced pore destabilization of potassium leak channels. Finally, as the magnitude of the pH effect was voltage-dependent, we present a novel mechanism for K_{2p}2.1 outward rectification.

* This work was supported by grants from the Israel Science Foundation (431/03) and the Zlotowski Center for Neuroscience (to N.Z.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom correspondence should be addressed. Tel.: 972-8-6472640; Fax: 972-8-6479208; E-mail: noamz@bgu.ac.il.
Inhibition of Human K$_{2p}.2.1$ Channel by External Protons

**EXPERIMENTAL PROCEDURES**

*Molecular Biology—*The human K$_{2p}.2.1$ channel (NM_001017425) was cloned into the pRAT plasmid as described previously (17). The murine K$_{2p}.2.1$ channel, cloned into the pcDNA3.1 plasmid, was generously provided by Prof. K. Sanders from the University of Nevada. Mutants were generated using the QuikChange site-directed mutagenesis technique (Stratagene, La Jolla, CA). The mutations were confirmed by DNA sequencing. cRNA was transcribed in vitro using T7 polymerase and the AmpliCap High Yield Message Maker (EPICENTRE Biotechnologies) kit.

*Electrophysiology—*Xenopus laevis oocytes were isolated and injected with 23 nl containing 0.2–8 ng of cRNA. Whole-cell currents were measured 1–3 days after injection by the two-electrode voltage clamp technique, using a GeneClamp 500B amplifier (Axon Instruments, Union City, CA). Data were sampled at 2 kHz and filtered at 0.5 kHz with Clampex 9.0 software.

For two-electrode voltage clamp experiments, the pipette contained 3 M KCl and the bath solution contained, unless otherwise noted (in mM): 4 KCl, 96 NaCl, 1 MgCl$_2$, 0.3 CaCl$_2$, 5 HEPES, pH 7.4, with NaOH (standard solution). HEPES buffer was replaced by Tris in alkali solutions (pH 8.5, 9.0) and MES$_2$ in acidic solutions (pH 5.5–6.5). When required, bath solution sodium ions were isotonically replaced by potassium ions. To avoid possible effects of pH on channel regulation (18), the sodium ions were isotonically replaced by potassium ions. To lower the external pH from 9.0 to 5.5 decreased current levels by >70% (Fig. 1). For steady state currents recorded at +40 mV, inhibition by acidic pH values was characterized by an apparent $pK$ of 7.47 ± 0.2 (Fig. 1C). A calculated Hill coefficient of 0.51 ± 0.07 implies the existence of multiple proton-binding sites in the K$_{2p}.2.1$ channels, acting with negative cooperative interactions. Single channel analysis shows that at low pH$_{2o}$ values, channel open probability, but not single channel conductance, is decreased (Fig. 1D), suggesting an effect on channel gating.

**Identification of the pH Sensor of K$_{2p}.2.1$ Channel—**To date, three mechanisms have been proposed as being responsible for the pH$_{2o}$ sensitivity of K$_{2p}$ channels. In members of the K$_{2p}.3.1$ (TASK1) group, current decreases are due to protonation of a histidine residue, located next to the first pore domain selectivity filter signature (i.e., GYGH) (23, 24). In K$_{2p}.5.1$ (TASK2) channels, several extracellular charged residues have been implicated as carrying the channel response to alkalization (25). This was later disputed and an alternative mechanism in which a single arginine residue, located near the second pore domain (Arg-224), was proposed as being responsible for the pH-dependent activity of K$_{2p}.5.1$ as well as TALK (K$_{2p}.16.1$ and K$_{2p}.17.1$) channels (26). None of these explanations seemingly applies to K$_{2p}.2.1$. K$_{2p}.2.1$ channels neither possess a histidine residue at the selectivity filter, like K$_{2p}.3.1$, nor do they sense pH$_{2o}$ by Lys-286 (homologous to Arg-224 in K$_{2p}.5.1$), as mutating Lys-286 to alanine failed to abolish K$_{2p}.2.1$ inhibition by protons (data not shown).

In seeking the pH sensor of human K$_{2p}.2.1$, we studied the mouse variant of the channel, not considered to be highly pH$_{2o}$-sensitive (27). In our hands, the murine K$_{2p}.2.1$ channel indeed showed low sensitivity to external pH, with only 35% inhibition noted at pH 5.5 (Fig. 2B). A comparison of the two sequences revealed the presence of a histidine residue (His-87) in the human sequence not found in its murine counterpart (Fig. 2A). Indeed, mutating the human His-87 to glutamine, as found at position 87 of the murine K$_{2p}.2.1$ sequence, resulted in a pH sensitivity profile almost identical to that of the murine variant (Fig. 2B). In light of this finding, we evaluated the roles of the three other human K$_{2p}.2.1$ histidine residues predicted to reside in extracellular loops of the channel by individually mutating them to alanine (i.e., Fig. 2A, H106A, H141A, and H247A). The H106A and H247A mutants did not significantly affect the pH$_{2o}$ sensitivity of human K$_{2p}.2.1$ (Fig. 2B). By contrast, His-141 was found to participate in the channel response to pH$_{2o}$, as mutating this residue dramatically decreased channel responsiveness to external acidification, shifting the sensitivity curve away from the physiological range (Fig. 2B). The H87Q,H141A double mutant, however, showed a pH sensitivity similar to the

---

**RESULTS**

**Human K$_{2p}.2.1$ Channels Sense External pH—**Recent work has suggested the existence of unspecified, pH-sensitive, leak-like K$^+$-selective conductance in different neuronal tissues, such as dorsal root ganglion neurons and the retrotrapezoid nucleus (21, 22). We thus examined the sensitivity of K$_{2p}.2.1$ leak channels to external pH (pH$_{2o}$), given the wide distribution of this channel in the central nervous system. We found human K$_{2p}.2.1$ channels to be highly sensitive to pH$_{2o}$ (at values within the physiological range) when expressed and studied in X. laevis oocytes. Under physiological potassium concentrations (4 mM), lowering the external pH from 9.0 to 5.5 decreased current levels by >70% (Fig. 1). For steady state currents recorded at +40 mV, inhibition by acidic pH values was characterized by an apparent $pK$ of 7.47 ± 0.2 (Fig. 1C). A calculated Hill coefficient of 0.51 ± 0.07 implies the existence of multiple proton-binding sites in the K$_{2p}.2.1$ channels, acting with negative cooperative interactions. Single channel analysis shows that at low pH$_{2o}$ values, channel open probability, but not single channel conductance, is decreased (Fig. 1D), suggesting an effect on channel gating.

---

2 The abbreviations used are: MES, 4-morpholineethanesulfonic acid; pH$_{2o}$, external pH; WT, wild type.
Inhibition of Human K$_{2p2.1}$ Channel by External Protons

Voltage-dependent Proton-mediated Inhibition Increases Outward Rectification—We found that the proton-mediated inhibition is voltage-dependent, being more pronounced at negative potentials, with $V_{1/2}$ of -79 mV ± 1.7 (Fig. 3, A and B). This differential inhibition causes the K$_{2p2.1}$ currents to be significantly more outwardly rectifying at acidic than at basic pH values (Fig. 3A). Accordingly, in the absence of proton-mediated inhibition (H141A mutant) or Mg$^{2+}$ blockade, the channel behaves as an open rectifier channel (Fig. 3, C and D). These results suggest a new mechanism contributing to the exceptional capability of K$_{2p2.1}$ to function in a voltage-gated mode. We believe that the voltage dependence of the channel reflects, in part, release from voltage-dependent proton-mediated inhibition.

External Acidification Facilitates C-type Closure of K$_{2p2.1}$—What is the mechanism by which external protons inhibit K$_{2p2.1}$ currents? Neither His-87 nor His-141 is predicted, according to the known K$^+$ channel structures (28), to lie along the ion conduction pathway. This, therefore, rules out the possibility that protons act through pore blockade. Hence, to elucidate the manner by which these residues sense pH$_O$ levels, we studied the effect of pH inhibition on K$_{2p2.1}$ gating. In a homologous channel, i.e. the Drosophila KCNK0 channel, closing of the channel involves conformational changes in the external pore (16) through a mechanism resembling the C-type inactivation of voltage-gated potassium channels (29–31). Might K$_{2p2.1}$ pH sensors, i.e. His-87 and His-141, located at the first turret loop, induce closure of the external gate by a mechanism similar to C-type inactivation?

C-type inactivation is known to be slowed by permeable external ions (29). As such, high external potassium concentrations might reduce the extent of K$_{2p2.1}$ inhibition observed at acidic pH values. Indeed, elevating external potassium concentrations from 4 to either 20 or 100 mM dramatically interfered with pH inhibition of channel activity (Fig. 4A). Although lowering the pH from 9.0 to 7.0 produced a 42% inhibition in K$_{2p2.1}$ currents attained in the presence of 4 mM external potassium, only 18% inhibition was measured in 20 mM potassium solution and a mere 11% inhibition was observed in the presence of 100 mM external potassium (Fig. 4B, right panel). In light of these results, we measured the ionic selectivity of K$_{2p2.1}$ at different pH$_O$ values as conformational modification of the pore area...
Inhibition of Human K_{2P}2.1 Channel by External Protons

alters the selectivity filter arrangement, thereby increasing sodium permeability (32–34). Lowering the pH of a 100 mM sodium solution from 9.0 to 7.0 caused a significant right shift of 20 mV (−134.4 ± 0.6 to −114.3 ± 0.9, mean ± S.E., n = 17) in the measured reversal potential of oocytes expressing wild type (WT) K_{2P}2.1 channels (Fig. 4B, left panel). To exclude any nonspecific influences of low pH in these studies, the same experiment was repeated using the pH-insensitive mutant K_{2P}2.1-H141A. In cells expressing the mutant, pH changes failed to alter the reversal potential (Fig. 4B, right panel). Accordingly, the K^+/Na^+ permeability ratio in WT channels was considerably decreased (close to a 3-fold change) as the external pH was reduced (Fig. 4C). By contrast, the H141A mutant presented only a mild decrease (<20%) in the K^+/Na^+ permeability ratio, with the change only being noted at pH 6.5, when a slight inhibition by protons was noted as well (Fig. 4C).

The changes in the K^+/Na^+ permeability ratio most likely result from an increase in Na^+ permeability rather than a decrease in K^+ permeability, as the single channel current amplitude, measured in symmetrical K^+ concentrations (140 mM K^+ and no Na^+ in the pipette solution), was not affected by external pH changes (Fig. 1D). Rb^+ and Cs^+ selectivities were not changed in either the WT or H141A channels under acidic conditions (data not shown).

We next considered the effects on K_{2P}2.1 pH sensitivity of two mutations, homologous to those shown previously to accelerate C-type gating in other potassium channels. In Shaker channels, mutation of an external threonine residue (i.e. Thr-449) located next to the selectivity filter alters C-type gating kinetics (31). Mutating the homologous position in the KCNK0 first pore domain (i.e. S112Y) enhanced C-type gating (16). A similar enhancement resulted from mutation of an extracellular glutamate, present in most potassium channels in the amino-terminal of the pore turret (i.e. Glu-28 in KCNK channels and Glu-418 in Shaker channels) (35, 36). Ser-164, the K_{2P}2.1 homolog of Shaker Thr-449 and KCNK0 Ser-112, was mutated to tyrosine (S164Y) and Glu-84, homologous to Shaker Glu-418 and KCNK0 Glu-28, was mutated to alanine (E84A). The K_{2P}2.1-S164Y mutant demonstrated increased pH sensitivity, with a pK of 8.1 and >90% inhibition at low pH values (Fig. 4D).

Surprisingly, the K_{2P}2.1 E84A mutation, expected to affect C-type gating in the same manner, presented currents that were significantly less sensitive to external pH, with a maximal inhibition of only 40% (Fig. 4D).

The Molecular Mechanism of pH Sensing—The insensitivity of the E84A mutant to changes in pH_{o} implies that Glu-84 takes part in the pH-sensing mechanism itself. It was previously suggested that Shaker Glu-418, which lies adjacent to the pore, stabilizes the open conformation of the slow inactivation gate by forming hydrogen bonds with residues in the P-S6 loop (36). Breaking this bond during conformational changes enables the rotation of the P-S6 loop and the subsequent collapse of the slow inactivation gate. Accordingly, we hypothesized that protonation of the external histidines, His-87 and His-141, would add positive charges that could interact with the negative charge of Glu-84 to draw this residue away from its natural interactions, thus causing a collapse of the selectivity filter region.

In Fig. 5A, we present one subunit of the bacterial KcsA potassium channel, based on its published structure (28). The predicted interactions of K_{2P}2.1 at the homologous sites in KcsA are indicated as follows: at the primary sequence level, KcsA residues Glu-51, Val-84, and Thr-85 align with K_{2P}2.1 residues Glu-84, Arg-166, and Thr-167, respectively. The side chain of KcsA Glu-51 is predicted to form hydrogen bonds (orange lines) with the backbone amides of Arg-166 and Thr-167 and the side chain hydroxyl group of Thr-167. KcsA Leu-59 and Ala-54 are replaced in this presentation by histidine resi-
Inhibition of Human $K_{2p2.1}$ Channel by External Protons

FIGURE 3. Voltage-dependent proton-mediated blockage as a mechanism for outward rectification in $K_{2p2.1}$. A, normalized currents of oocytes expressing $K_{2p2.1}$ channels. Oocytes were held at $-80$ mV and pulsed from $-155$ to $+40$ mV at $15$-mV intervals in “symmetrical” (140 mM) potassium solution at external pH values of 9.0 and 6.5, as indicated (mean ± S.E., $n = 7$). B, voltage dependence of proton-mediated inhibition, calculated as the current measured at pH 6.5 divided by the current measured at pH 9.0, for each voltage step measured in A (mean ± S.E., $n = 7$). The apparent electrical distance (δ) traversed by protons, as modeled by a simplification of the Woodhull approach (19), was 0.22. C, normalized currents of oocytes expressing WT or H141A mutant channels. Oocytes were held at $-80$ mV and pulsed from $-155$ to $+40$ mV at 15-mV intervals in symmetrical potassium solution (pH 7.4), with or without 3 mM Mg$^{2+}$, as indicated. The dashed line represents the predicted I-V relationship of an open rectifier channel, based on the Goldman-Hodgkin-Katz (GHK) current equation. D, outward rectification quantification, calculated as the current measured at $-155$ mV divided by the current measured at $+40$ mV (mean ± S.E., $n = 7$). The dashed line represents the predicted value for an open rectifier channel, based on the GHK current equation.

FIGURE 4. Proton-mediated inhibition facilitates C-type inactivation. A, external proton reduces pH inhibition of $K_{2p2.1}$ currents. Left, human WT channel studied at different pH values in 4 mM (solid circles), 20 mM (solid triangles), and 100 mM (solid squares) potassium solutions. Right, inhibition percentage obtained by lowering the external solution pH from 9.0 to 7.0 in different external potassium concentrations, as indicated (mean ± S.E., $n = 7–12$). B, pH affects the ion selectivity of $K_{2p2.1}$ channels. Whole-cell macroscopic permeability ratios were approximated (for each pH value separately) according to: $P_{K}/P_{Na} = \exp(-FE_{rev}/RT)$, where $P_{K}$ and $P_{Na}$ represent the permeabilities of potassium and sodium, respectively. Results are shown as means ± S.E. ($n = 10–29$). C, extracellular pH dependence curves of mutant E84A (triangles) and S164Y (squares) channels as compared with human WT channels (circles). Currents were studied and analyzed as in Fig. 1. Results are shown as means ± S.E. ($n = 7$). The solid lines represent a fit of the data to the Hill equation.

dues, as present at the homologous positions in $K_{2p2.1}$ (i.e. His-141 and His-87, respectively). Potential hydrogen bonds between the Glu-84 hydroxyl groups and the histidine amide groups are drawn as orange lines. For the proposed mechanism of $K_{2p2.1}$ pH$_{p}$ sensitivity to be valid, the three residues, namely Glu-84, His-87, and His-141, must be close enough to one another to interact. To test whether this is indeed the case, we first produced paired cysteine mutants, i.e. E84C and H87C, and E84C and H141C. Formation of either of the disulfide bonds (i.e. Cys-84-Cys-87 or Cys-84-Cys-141) would be expected to modify $K_{2p2.1}$ currents whereas reduction would be expected to reverse this effect. Although channels containing a single cysteine mutation (i.e. E84C, H87C, or H141C) were active, channels including double mutations failed to produce any measurable currents (data not shown). Adding the reducing agent dithiothreitol (10 mM) to the bath solution did not affect this situation (data not shown), possibly because the introduced disulfide bonds locking the channel into the closed state were not accessible to the reducing agent. However, we cannot exclude other possibilities at this point, such as improper assembly or massive conformational changes of the channel caused by introducing two mutations in this vital domain of the channel.

Seeking an alternative manner to demonstrate the physical proximities of Glu-84 and His-141/His-87, we exploited the ability of three or more histidine and cysteine residues to coordinate the binding of Cd$^{2+}$ ions. If Glu-84 is indeed sufficiently
adjacent to both His-141 and His-87, replacing this glutamate with a cysteine residue (E84C) should create a new Cd\(^{2+}\)-binding site. As proposed, K\(_{2p2.1}\).E84C was >10-fold more sensitive to Cd\(^{2+}\) ions than was the WT channel (Fig. 5B). Furthermore, damaging the newly created Cd\(^{2+}\)-binding site by mutating either of the histidine residues in the K\(_{2p2.1}\).E84C mutant background (i.e. E84C,H141A; E84C,H87Q; or E84C,H87Q,H141A) reduced Cd\(^{2+}\) binding back to WT levels (Fig. 5B).

**DISCUSSION**

In the present report, we have shown that extracellular protons within the physiological range strongly inhibit the human K\(_{2p2.1}\) channel by affecting the channel open probability (Fig. 1). Transient pH variations occur in all three compartments of nervous tissue, i.e. in neurons, glial cells, and extracellular spaces, in response to neuronal stimulation to neurotransmitters and hormones as well as in a secondary manner to metabolic activity and ion transport (37). Shifts in blood flow, synchronous activation of nerve cells, seizure, or spreading depression have been associated with interstitial alkaline shift or acidosis that could persist for minutes in different segments of the central nervous system (reviewed in Ref. 38). Such changes are known to dramatically modulate ion channel activity and hence affect neuronal tissue excitability. As decreased tissue pH is a common feature of the ischemic brain that can lead to neuronal injury, Xiong et al. (39) had recently suggested acid-sensing ion channels as novel therapeutic targets for ischemic brain injury. Being modulated by intra- and extracellular acidification, the K\(_{2p2.1}\) channel is an important sensor for pH changes in the central nervous system.

We have identified two histidine residues located in the first turret loop (i.e. His-87 and His-141), predicted to lie in the outer vestibule of the conduction pathway, as being responsible for the K\(_{2p2.1}\) response to external pH changes (Fig. 2). The location of the two histidine residues implies a new pH-sensing mechanism, different from those previously suggested for K\(_{2p}\) channels (23–26). In this context, the His-87 residue is especially interesting, as it is present in the human K\(_{2p2.1}\) variant (as well as in all other known non-rodent, yet unstudied, mammalian homologs) but is replaced by glutamine in the mouse K\(_{2p2.1}\) channel, which is only mildly affected by pHO (Fig. 2). It is possible that the two variants thus serve distinct physiological roles as their general expression patterns, as well as their central nervous system distribution, diverge (2, 40). His-87 is also absent from the human K\(_{2p10.1}\) (TREK-2) channel, closely related to K\(_{2p2.1}\) (78% homology) and presenting significant similarity to K\(_{2p2.1}\) in terms of biophysical properties, as well as with respect to channel regulation traits (41, 42). By placing a phenylalanine residue instead of histidine at position 102 (homologues to K\(_{2p2.1}\) His-87), the K\(_{2p10.1}\) channel was found to modestly react to extracellular pH changes (42). Hence, the addition of a single histidine residue in the turret loop may offer an efficient means to expand the physiological diversity of K\(_{2p}\) channels. This may represent a more general phenomenon to govern functional variance of potassium channels. For example, the pH sensitivity of the rKV1.5 channel is mediated by a histi-
dine residue in the turret loop (His-452), absent in the closely related, pH-insensitive, Kv1.2 channel (43).

Our results indicate that the outward rectification of K_{2p}.2.1 is more pronounced at low external pH values and almost completely lost in the pH-insensitive mutant H141A at physiological pH values (Fig. 3). Because neither of the two studied histidine residues is predicted to lie within the membrane electrical field (Fig. 5A), we presuppose a non-direct effect of the membrane potential. Thus, the identity of the actual membrane-embedded voltage sensor has yet to be revealed. Two mechanisms were previously suggested to explain the limitation of K_{2p}.2.1 currents at negative potentials, namely voltage-dependent external Mg^{2+} blockade or an intrinsic voltage-sensing mechanism, argued to depend on an essential carboxyl-terminal protein kinase A phosphorylation site in human K_{2p}.2.1, Ser-348 (V_{1/2} = 10 ± 7 mV) (17), or on the carboxyl-terminal domain of the mouse K_{2p}.2.1 variant (44). In addition, it was shown that agonist-induced phosphatidylinositol-4,5-biphosphate hydrolysis shifts the voltage sensitivity of K_{2p}.2.1 channels toward depolarized potentials (45). Here, we present a novel mechanism for outward rectification of K_{2p}.2.1, depending on extracellular pH sensors, presenting a pH-insensitive mutant H141A at physiological pH changes in the central nervous system, with external pH changes modifying both their currents and membrane potential dependence. Moreover, we offer new insight into the molecular mechanism and structural rearrangements that occur during the K_{2p}.2.1 response to pH_{1/2}, involving an external pH sensor, identified here for the first time.

Taken together, our results highlight the physiological importance of human K_{2p}.2.1 channels as important sensors of pH changes in the central nervous system, with external pH changes modifying both their currents and membrane potential dependence. Moreover, we offer new insight into the molecular mechanism and structural rearrangements that occur during the K_{2p}.2.1 response to pH_{1/2}, involving an external pH sensor, identified here for the first time.

Acknowledgments—We thank Ofer Yifrach, Shai Silberberg, and Jerry Eichler for advice on the manuscript.

REFERENCES

1. Goldstein, S. A., Bockenhauer, D., O’Kelly, I., and Zilberberg, N. (2001) Nat. Rev. Neurosci. 2, 175–184
2. Fink, M., Duprat, F., Lesage, F., Reyes, R., Romey, G., Heurteaux, C., and Lazdunski, M. (1996) EMBO J. 15, 6685–6682
3. Aimond, F., Rauzier, J. M., Bony, C., and Vassort, G. (2000) J. Biol. Chem. 275, 39110–39116
4. Koh, S. D., Monaghan, K., Sergeant, G. P., Ro, S., Walker, R. L., Sanders, K. M., and Horowitz, B. (2001) J. Biol. Chem. 276, 44338–44436
5. Honore, E. (2007) Nat. Rev. Neurosci. 8, 251–261
6. Maingret, F., Lauritzen, I., Patel, A. I., Heurteaux, C., Reyes, R., Lesage, F., Lazdunski, M., and Honore, E. (2000) EMBO J. 19, 2483–2491
7. Maingret, F., Patel, A. I., Lesage, F., Lazdunski, M., and Honore, E. (2000) J. Biol. Chem. 275, 10128–10133
8. Maingret, F., Patel, A. I., Lesage, F., Lazdunski, M., and Honore, E. (1999) J. Biol. Chem. 274, 26691–26696
9. Lauritzen, I., Blondeau, N., Heurteaux, C., Widmann, C., Romey, G., and Lazdunski, M. (2000) EMBO J. 19, 1784–1793
10. Patel, A. J., Honore, E., Lesage, F., Fink, M., Romey, G., and Lazdunski, M. (1999) Nat. Neurosci. 2, 422–426
11. Clydon, T. W., Makary, S. Y., Dibb, K. M., and Boyett, M. R. (2004) Biophys. J. 87, 2407–2418
12. Kennard, L. E., Chumbley, J. R., Ranatunga, K. M., Armstrong, S. J., Veale, E. L., and Mathie, A. (2005) Br. J. Pharmacol. 144, 821–829
13. Harinath, S., and Sildar, S. K. (2005) Epilepsy Res. 64, 127–135
14. Heurteaux, C., Guy, N., Laigle, C., Blondeau, N., Duprat, F., Mazzauc, M., Lang-Lazdunski, L., Widmann, C., Zanzorri, M., Romey, G., and Lazdunski, M. (2004) EMBO J. 23, 2684–2695
15. Alloui, A., Zimmermann, K., Mamet, J., Duprat, F., Noel, J., Chemin, I., Guy, N., Blondeau, N., Voilley, N., Rubat-Coudert, C., Borsotto, M., Romey, G., Heurteaux, C., Reep, P., Eschaller, A., and Lazdunski, M. (2006) EMBO J. 25, 2368–2376
16. Zilberberg, N., Iban, N., and Goldstein, S. A. (2001) Neuron 32, 635–648
17. Bockenhauer, D., Zilberberg, N., and Goldstein, S. A. (2001) Nat. Neurosci. 4, 486–491
18. Cohen, A., and Zilberberg, N. (2006) J. Neurosci. Methods 153, 62–70
19. Woodhull, A. (1973) J. Gen. Physiol. 61, 687–708
20. Lopes, C. M., Gallagher, P. G., Buck, M. E., Butler, M. H., and Goldstein,
S. A. (2000) *J. Biol. Chem.* 275, 16969–16978

21. Mulkey, D. K., Talley, E. M., Stornetta, R. L., Siegel, A. R., West, G. H., Chen, X., Sen, N., Mistry, A. M., Guyenet, P. G., and Bayliss, D. A. (2007) *J. Neurosci.* 27, 14049–14058

22. La, J. H., Kang, D., Park, J. Y., Hong, S. G., and Han, J. (2006) *Neurosci. Lett.* 406, 244–249

23. Kim, Y., Bang, H., and Kim, D. (2000) *J. Biol. Chem.* 275, 9340–9347

24. Lopes, C. M., Zilberberg, N., and Goldstein, S. A. (2001) *J. Biol. Chem.* 276, 24449–24452

25. Morton, M. J., Abohamed, A., Sivaprasadarao, A., and Hunter, M. (2005) *Proc. Natl. Acad. Sci. U. S. A.* 102, 16102–16106

26. Niemeyer, M., González-Nilo, F., Zuñiga, L., González, W., Cid, L., and Sepúlveda, F. (2007) *Proc. Natl. Acad. Sci. U. S. A.* 104, 666–671

27. Patel, A. J., and Honore, E. (2001) *Trends Neurosci.* 24, 339–346

28. Doyle, D. A., Morais Cabral, J., Pfuetzner, R. A., Kuo, A., Gulbis, J. M., Cohen, S. L., Chait, B. T., and MacKinnon, R. (1998) *Science* 280, 69–77

29. Ogielska, E. M., and Aldrich, R. W. (1999) *J. Gen. Physiol.* 113, 347–358

30. Liu, Y., Holmgren, M., Jurman, M. E., and Yellen, G. (1997) *Neuron* 19, 175–184

31. Lopez-Barneo, J., Hoshi, T., Heinemann, S. H., and Aldrich, R. W. (1993) *Receptors Channels* 1, 61–71

32. Yuill, K. H., Stansfeld, P. J., Ashmole, I., Sutcliffe, M. J., and Stanfield, P. R. (2007) *Pfluegers Arch. Eur. J. Physiol.* 455, 333–348

33. Starkus, J. G., Kuschel, L., Rayner, M. D., and Heinemann, S. H. (1997) *J. Gen. Physiol.* 110, 539–550

34. Starkus, J. G., Kuschel, L., Rayner, M. D., and Heinemann, S. H. (1998) *J. Gen. Physiol.* 112, 85–93

35. Ortega-Saenz, P., Pardal, R., Castellano, A., and Lopez-Barneo, J. (2000) *J. Gen. Physiol.* 116, 181–190

36. Larsson, H. P., and Elinder, F. (2000) *Neuron* 27, 573–583

37. Deitmer, J. W., and Rose, C. R. (1996) *Prog. Neurobiol. (Oxf.* 48, 73–103

38. Chesler, M. (2003) *Physiol. Rev.* 83, 1183–1221

39. Xiong, Z. G., Chu, X. P., and Simon, R. P. (2007) *Front. Biosci.* 12, 1376–1386

40. Meadows, H. J., Benham, C. D., Cairns, W., Gloger, I., Jennings, C., Medhurst, A. D., Murdoch, P., and Chapman, C. G. (2000) *Pfluegers Arch. Eur. J. Physiol.* 439, 714–722e

41. Kang, D., Choe, C., and Kim, D. (2005) *J. Physiol.* 564, Pt. 1, 103–116

42. Lesage, F., Terrenoire, C., Romey, G., and Lazdunski, M. (2000) *J. Biol. Chem.* 275, 28398–28405

43. Steidl, J. V., and Yool, A. J. (1999) *Mol. Pharmacol.* 55, 812–820

44. Maingret, F., Honore, E., Lazdunski, M., and Patel, A. J. (2002) *Biochem. Biophys. Res. Commun.* 292, 339–346

45. Lopes, C. M., Rohacs, T., Czirjak, G., Balla, T., Enyedi, P., and Logothetis, D. E. (2005) *J. Physiol.* 564, Pt. 1, 117–129

46. Starkus, J. G., Varga, Z., Schonherr, R., and Heinemann, S. H. (2003) *Pfluegers Arch. Eur. J. Physiol.* 447, 44–54

**Inhibition of Human K_{2P}2.1 Channel by External Protons**

JULY 11, 2008 • VOLUME 283 • NUMBER 28 • JOURNAL OF BIOLOGICAL CHEMISTRY 19455