Identification of a Critical Motif Responsible for Gating of Kir2.3 Channel by Intracellular Protons*

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The modulation of membrane excitability is a significant cellular property, which not only provides cells with an important strategy for responding to their external stimuli but also enables them to monitor their internal environment and intermediary metabolism (1). Inward rectifier K⁺ channels play a part in this process in which several intracellular molecules such as nucleotides and protons are involved (2, 3). Whereas the concentration of protons is normally controlled by several buffering and feedback-controlling systems, pH can fall beyond its normal levels under certain pathophysiological conditions. For instance, CO₂ retention, or hypercapnia, can cause a reduction in intra- and extracellular pH leading to respiratory acidosis (4).

It is known that CO₂ sensing, which plays an important role in the feedback regulation of CO₂ and pH homeostasis in mammalian systems, is carried out by chemoreceptors, especially the central chemoreceptors (5–8). Through these chemoreceptors, a high PCO₂ level stimulates respiratory neuronal networks in the brain stem and enhances respiratory motor output. During this process, the information of PCO₂ levels received by these chemoreceptor cells may first be conveyed to the change in their membrane excitability and then passed to the respiratory neuronal networks through chemical or electrical synaptic transmissions (9, 10). Hence, the change in membrane excitability constitutes an important step in the CO₂ sensing.

The alteration in membrane excitability with hypercapnia is known to be mediated by specific ion channels. CO₂ has been shown to enhance the firing activity of locus ceruleus neurons by suppressing a proton- and polyamine-sensitive inward rectifying K⁺ current (11). Our previous studies have shown that CO₂ induces a depolarization in Lymnaea snail neurons by inhibiting a K⁺ channel with modest inward rectification (12). Recently, we have further demonstrated that CO₂ inhibits specific inward rectifier K⁺ channels including Kir2.3 (13). Because the Kir2.3 is expressed in the central nervous system and contributes to the maintenance of membrane excitability (14), detailed studies of the modulation of Kir2.3 during hypercapnia may bring about an understanding of the molecular mechanisms for CO₂ sensing.

Hypercapnia can modulate channel activity directly by acting on channel protein and indirectly by recruiting second messengers and other intermediate molecules. Our previous studies showed that the Kir2.3 inhibition during hypercapnia is largely mediated by decreases in the intra- and extracellular pH (pHi and pHo) and demonstrated that this channel has two pH sensors on either side of the plasma membranes (13). Whereas the pHo sensor has been well studied (15), the pHi sensor, which contributes to more than 80% of channel inhibition, works at near physiological pH levels, and seemingly plays a more important role during hypercapnia, is still not understood. Therefore, we designed these experiments using a molecular genetic approach combined with patch clamp experiments to have an intervention of the structural-functional relationship of the Kir2.3 in CO₂/pH sensing.

MATERIALS AND METHODS

Frogs (Xenopus laevis) were anesthetized by bathing in 0.3% 3-aminobenzoic acid ethyl ester. A few lobes of the ovaries were removed after a small abdominal incision (~5 mm). Xenopus oocytes were treated with 2 mg/ml collagenase (Type I, Sigma) in OR2 solution (82 mM NaCl, 2 mM KCl, 1 mM MgCl₂, and 5 mM HEPES) for 90 min at room temperature. After washing, the oocytes were then incubated at 18 °C in ND-96 solution containing (in mM) 96 NaCl, 2 KCl, 1 MgCl₂, 1.8 CaCl₂, 5 HEPES, and 2.5 sodium pyruvate with 100 mg/liter Geneticin added. A vector for eukaryotic expression (pcDNA3.1, Invitrogen, Carlsbad, CA) was used to express Kir2.1 and Kir2.3 channels. Chimerical constructs between Kir2.1 and Kir2.3 were prepared by an overlap extension at the junction of the interested domains using polymerase chain reaction (Pfu or Taq DNA polymerases, Stratagene, La Jolla, CA). The resulting polymerase chain reaction products were subcloned into pcDNA 3.1. Site-specific mutations were made using a site-directed mutagenesis kit (Quickchange, Stratagene). Correct mutations in both the overlapping polymerase chain reaction and site-directed mutagen-
Whole cell currents were recorded from oocytes 2–5 days after cDNA injection using a two-electrode voltage clamp. Currents were recorded before, during, and after 15% CO2 exposure. A, Kir2.3 currents were reversibly inhibited by CO2. The currents decreased by 80% of their base-line values during a 5-min CO2 exposure and returned to the control level 5 min after the exposure ended. B, a similar exposure however, had no effect on Kir2.1 currents.

**RESULTS**

**CO2 and Low pH Inhibit Kir2.3 Currents**—Kir2.3 currents were studied by expressing these K+ channels in *Xenopus* oocytes. In the voltage clamp mode, whole cell Kir2.3 currents showed a strong inward rectification and were highly sensitive to extracellular Ba2+ (IC50 = 11 ± 2 μM, n = 4). Exposure of the oocytes to 15% CO2 produced a marked inhibition of the whole cell Kir2.3 currents by 50 ± 2% (n = 7) (Fig. 1A). This effect was reversible and dependent on concentrations of CO2 (5, 10, and 15%, data not shown). Selective intracellular acidification without changing the extracellular pH also inhibited Kir2.3 to an extent comparable with that of CO2 when changes in pH levels were considered. The measured intracellular pH level during 15% CO2 exposure was 6.58 ± 0.13 (n = 6) using ion-selective microelectrodes (20). Selective intracellular acidification to this pH level inhibited Kir2.3 by 74 ± 4% (n = 5).

The inhibition of Kir2.3 by CO2 or intracellular acidification can be a direct effect of protons on the channel protein or an indirect effect through changes in concentrations of intracellular second messengers, protein kinases, phosphatases, and other cytosol-soluble factors. To delineate the mechanisms underlying the channel modulation, we performed experiments using cell-free excised patches. In the inside-out patch configuration, Kir2.3 currents were strongly inhibited when the cytosolic surface of the plasma membrane was exposed to low pH solutions. This inhibition was fast, reversible, and concentration-dependent (Fig. 2). Another member of the Kir2 family, Kir2.1, however, did not respond to either high CO2 (Fig. 1B) or pH 6.6 (Fig. 3), suggesting that this effect was rather specific. Because cytosol-soluble factors were vastly diluted or washed out under

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The abbreviations and trivial names used are: PIPES, piperazine-N,N'-bis-(2-ethanesulfonic acid); H7, 1-[5-isouquinolinesulfonyl]-2-methylpiperazine dihydrochloride.
our experimental condition, the inhibition of Kir2.3 channels is unlikely to be mediated by second messengers and other cytosol-soluble factors. Moreover, because our intracellular solutions contained chemicals that were unfavorable to protein dephosphorylation (see “Materials and Methods”), the modulation of Kir2.3 channel activity by pH levels may not be related to the fast turn-over of protein phosphorylation and dephosphorylation.

The CO₂/pH-sensitive Structure Is Located in the N-terminal Region of Kir2.3—Our results in excised patches support an idea that the proton-sensitive mechanism for channel gating is located on the Kir2.3 channel protein. To further test this idea, we chose to use molecular genetic approaches to determine the molecular basis of Kir2.3 channel modulation by intracellular protons. According to the generally accepted membrane-spanning topology of inward rectifier K⁺ channels, the Kir2.3 channel should have its N and C termini in the cytosol. In addition, part of the pore (P or H5) sequence should be accessible from the intracellular side. Based on these characteristics of Kir channels, we constructed chimeras in which each of these three sequences was replaced by its counterpart in Kir2.1. CO₂ and pH sensitivities of these recombinant HIR-IRK channels were then studied in whole cell voltage clamp and excised patches. Substitution of the N terminus in Kir2.3 with that in Kir2.1 (IN-HIR) completely eliminated the channel response to low pHᵢ (Figs. 3A and 5). A chimerical Kir2.1 channel carrying an N-terminal sequence of Kir2.3 (HN-IRK) became pH-sensitive, although the degree of the pH sensitivity was smaller than that of wild-type Kir2.3 (Figs. 3A and 5). Switches of the C terminus (IRK-HC, Fig. 5) or the H5 region from Kir2.3 to Kir2.1 (IN-HIR, Figs. 3A and 5), however, failed to produce any CO₂/pHᵢ sensitivity in these mutant channels. Thus, these results indicate that the CO₂/pHᵢ-sensitive mechanism is located on the N terminus rather than the H5 and C terminus of Kir2.3.

Titratable Amino Acid Residues in the N Terminus Are Not Involved—It is known that a large number of protein molecules can be modulated by pH levels. Binding of protons to certain titratable amino acids can cause changes in the electrical charges of these residues and thus alter the protein conformation and channel activity. If this is the case in Kir2.3, there should be amino acid residues on the channel protein acting as receptive sites for protons. To test this hypothesis, we examined titratable amino acids in the N terminus of Kir2.3, especially histidines in which the side chain has a pKᵣ value of 6.04. Using site-directed mutagenesis, these histidines were changed to aspartates in which positive charges at these positions were converted to negative charges during hypercapnia. A simultaneous replacement of all three histidines (H2D, H4D, H11D) had no effect on channel expression and sensitivity to CO₂ and pHᵢ (Fig. 4, A and B). Neither did replacements of individual ones, suggesting that these histidines do not constitute the pH sensor in Kir2.3. To further strengthen this idea, we deleted all of these histidines plus the other nine residues at the beginning of the N terminus and found that the CO₂/pHᵢ sensitivity was unaffected (see below). In addition to these histidines, several residues that exist only in Kir2.3 and are
Proton Sensing in Kir2.3

The crucial role of the N-terminal region in pH sensing. A, because Kir2.3 (HIR) responds to CO2 and low pH, but Kir2.1 (IRK) does not, chimeras were constructed between these two channels to identify the intracellular pH-sensing domain. In IN-HIR, the Kir2.3 N-terminal region (from the N terminus to the beginning of M1) was substituted with the counterpart of Kir2.1. This IN-HIR recombinant lost its sensitivity to intracellular acidification. When the Kir2.1 N-terminal domain was replaced with the corresponding sequence in Kir2.3, the mutant channel became pH-sensitive. Note that pH sensitivity of these chimeras was studied by two-electrode clamp experiments in different oocytes. B, concentration-dependent inhibition of K+ currents in inside-out patches. Whereas Kir2.3 currents showed strong pH sensitivity with pH 6.77, Kir2.1 and IN-HIR had almost no response to a pH change from 7.4 to 5.8. Although its pH sensitivity decreased (pH 6.45), IN-IRK responded to pH changes in a manner more like Kir2.3 than Kir2.1. Data are presented as means ± S.E.

CO2/pH Sensing Is Related to a Short Sequence Near the M1 Domain—To understand the pH-dependent mechanisms for Kir2.3 gating, we divided the N-terminal region into three segments and reconstructed several N-terminal chimeras using Kir2.3 and Kir2.1 sequences. Most of these chimeras were expressed in oocytes as functional channels, although the current amplitude in some of them was reduced. To eliminate the extracellular pH sensor, the sequence from M1 through M2 in Kir2.3 was replaced with a corresponding one in Kir2.1. This mutant channel carrying N and C termini from Kir2.3 (HIH) showed CO2 and pH sensitivities almost identical to the wild-type Kir2.3 (Fig. 6B). Thus, several chimeras were constructed based on the HIH.

The first 12 amino acids at the N terminus of Kir2.3 (N1–12IH) were not involved in the CO2 sensitivity. A truncation of these residues did not have any significant effect on the channel expression, base-line currents, and sensitivity to CO2 (70 ± 3%, n = 4, p > 0.05) (Fig. 5). Although a deletion of the next 12 residues did not produce functional channels, chimera with the first 19 amino acids replaced by those in Kir2.1 (N1–19IH) showed a CO2 sensitivity very close to that of the wild-type Kir2.3 (61 ± 4%, n = 6). The middle part of the N-terminal region includes 20 residues with 9 of them different between Kir2.3 and Kir2.1. Substitution of this sequence in Kir2.3 (N25–44IH) moderately reduced the CO2 sensitivity (54 ± 6%, n = 4). Unlike the two N-terminal regions mentioned above, an amino acid sequence near the M1 membrane-spanning domain containing about 10 residues was critical for CO2 sensitivity. Replacement of this short sequence alone (N51–60IH) was sufficient to eliminate 80% of the CO2 sensitivity of the mutant Kir2.3 (13 ± 2%, n = 4). When this short sequence was constructed to Kir2.1 (N77–86IRK), we found that the mutant channel gained a substantial sensitivity to CO2 (18 ± 5%, n = 4) and pH (Fig. 5 and 7). Extension of this sequence to 41 residues prior to the M1 domain of Kir2.3 (N46–86IRK) gave the mutant Kir2.1 a CO2 sensitivity (36 ± 3%, n = 4) nearly the same as the HN-IRK (40 ± 4%, n = 6, p > 0.05) (Fig. 5). Therefore, this 10-residue motif is critical for the sensing of CO2/pH in Kir2.3, although another 30 amino acids (total 41 residues) around this motif are also needed to achieve the full effect of the entire N terminus.

CO2/pH Sensing in Kir2.3 Depends on a Few Critical Residues—There is a high sequence homology in these 10 residues between Kir2.3 and Kir2.1, in which Kir2.3 differs from Kir2.1 in only three residues (Thr23, Tyr27, and Met60). Mutation of all of them into residues in Kir2.1 (T53I, Y57W, and M60V) greatly reduced the pH sensitivity by 0.6 pH units and generally disabled this mutant channel in terms of CO2 sensing (Fig. 6). Replacements of two of these residues (T53I, Y57W) had the same effect (Fig. 6B). Even with a single mutation (T53I), the CO2 and pH sensitivities were severely diminished. Mutation

![Image](image-url)
Fig. 5. Localization of a CO₂-sensing motif in the N terminus of Kir2.3.

Even though the wild-type Kir2.3 (HIR) was strongly inhibited, Kir2.1 (IRK) was not sensitive to CO₂. When its N terminus was replaced with that in IRK, the mutant Kir2.3, IN-HIR, lost its pH sensitivity (note that the abbreviated names of chimeras are explained in the lower panel). Transferring the N terminus from HIR to IRK made the HN-IRK CO₂ sensitive, whereas inducing the HIR C terminus to IRK (IRK-HC) was ineffective. To further understand the CO₂-sensing motif, the N-terminal regions of Kir2.3 and Kir2.1 were divided into three segments at two conserved areas. A deletion of the first 12 residues in the N terminus did not significantly affect the CO₂ sensitivity of the N1–12IH. Substitution of amino acids 1–19 in Kir2.3 with those in IRK (N1–19IH) had only a modest effect on its CO₂ sensitivity. When the middle part of the N-terminal region (amino acids 25–44) was replaced, chimera N25–44IH was still fairly CO₂-sensitive, although its sensitivity was slightly reduced. In contrast to these three chimeras, a substitution of 10 amino acids near the M1 membrane-spanning sequence of Kir2.3 (N51–60IH) drastically reduced its CO₂ sensitivity. Construction of residues 20–60 of Kir2.3 to Kir2.1 gave this mutant IRK (N46–86IRK) a CO₂ sensitivity as great as that of the HN-IRK. This was seen even with the introduction of 10 residues to IRK (N77–86 IRK). Data are presented as means ± S.E.

of either tyrosine or methionine alone (Y57W or M60V) also caused a reduction in the CO₂ and pH sensitivities, though their effects were much less than that of the T53I mutation. When these three residues were constructed to Kir2.1 (N77–86IRK), the effect was dramatic. The mutant Kir2.1 started to be inhibited at pH 6.6 with a pK value of 6.0 and h (Hill coefficient) of 2.3 (n = 5). Its pH sensitivity became more like that of HN-IRK, a chimera carrying the full N terminus from Kir2.3, than of the wild-type Kir2.1 (Fig. 7). Mutation of isoleucine 79 alone (I79T-IRK) had an effect on converting the pH sensitivity of Kir2.1 as effectively as the N77–86IRK mutation (pK 5.93, h 2.3, n = 5) (Fig. 7), demonstrating the critical role of the threonine 53 of Kir2.3 in CO₂ and pH sensing.

Because threonine 53 in Kir2.3 is a protein kinase C phosphorylation site (21), channel sensitivity to CO₂ was studied in the presence of high concentration H7 (1-[5-isouquinolinesulfonyl]-2-methylpiperezine dihydrochloride, a blocker of protein kinases C and A; Sigma). After bathing the oocytes with 30 μM H7 for 20 min, exposure of the oocytes to 15% CO₂ produced the same degree of inhibition of Kir2.3 currents (74 ± 3% control versus 72 ± 1% with H7, p > 0.05, paired Student’s t test, n = 4), a result consistent with our studies of wild-type Kir2.3 using excised patches (see above).

DISCUSSION

In our current studies, we have demonstrated that a molecular motif that is located on the N terminus consisting of about 10 amino acids is responsible for gating the Kir2.3 channel by high CO₂ and low intracellular pH.

Direct Effect of Protons on Kir2.3 Currents—Like Ca²⁺, nucleotides, and other second messengers, proton is an important regulator of cellular functions. A number of ion channels have been known to be modulated by protons; these include large conductance Ca²⁺-activated K⁺ channels, h currents, gap junctions, and γ-aminobutyric acid and N-monomethyl arginine receptor channels (22–27). Changes in the activity of these ion channels can take place under several physiological and pathophysiological conditions when intra- and extracellular pH levels are low. One of these conditions is high CO₂ or hypercapnia. CO₂ sensing by the central chemoreceptors is critical in feedback mechanisms controlling CO₂ levels in mammals (5, 7, 8, 28), which may involve the modulation of K⁺ channel activity. Inhibition of inward rectifier K⁺ channels can produce depolarization and an increase in membrane excitability (2, 3). Although there is a number of ways to inhibit these K⁺ channels during hypercapnia, our current studies indicate that the mechanisms for the inhibition of Kir2.3 are related to the specific structure of the channel protein. We have found that fast and reversible inhibition of Kir2.3 activity by high CO₂ or low pH can be seen in cell-free excised patches, indicating that cystolic soluble factors such as second messengers, Mg²⁺, and polyanimes are not players. Also, CO₂/pH sensing is seen in Kir2.3 but not in Kir2.1, which has a nearly 80% homology to Kir2.3 in amino acid sequence, suggesting that a specific sequence in Kir2.3 protein underlies this sensitivity. Moreover, our data do not support the idea that the inhibition of Kir2.3 during hypercapnia or intracellular acidification is mediated by phosphorylation of channel proteins. There are chemical compounds in our intracellular solution to prevent channel rundown that are inhibitors of phosphatases and phosphodiesterases. This observation plus the lack of ATP and Mg²⁺ in our intracellular solution strongly suggests that the turnover of protein phosphorylation and dephosphorylation in gating...
Kir2.3 may not occur under such an experimental condition. Also, our results have shown that the inhibition of Kir2.3 activity by CO₂ is not affected by the blockade of protein kinases C and A. Therefore, specific structures on Kir2.3 channel protein seem to be responsible for CO₂ and pH sensing.

Mechanisms for Gating Kir2.3 Activity by Protons—If protons have a direct effect on channel functions, they may bind to the channel protein through certain titratable amino acid residues and alter conformation and activity of the channel. Also, protons can affect channel activity if they are involved in the binding of the channel protein with another protein. In any case, the channel protein should have specific sequences for these interactions. To locate these sites, we have studied the pH sensitivity of Kir2.3 channel by selectively replacing amino acid residues and peptide sequences in this channel with those in Kir2.1, which is not sensitive to a pH change in physiological ranges. These molecular genetic interventions have led us to look closely at the structure-functional relationship in the Kir2.3 channel in CO₂/pH sensing.

Consistent with the idea that the sensing mechanism is related to the channel protein, we have found that CO₂/pH sensitivity requires the presence of the N-terminal region of Kir2.3. To find proton-binding sites in the N terminus, we have examined all of the histidine residues by substituting them with aspartate, a negatively charged amino acid, or by deleting all of them with another nine residues at the very end of the N terminus. However, none of these mutants has shown a change in its sensitivity to CO₂ and pH, indicating that these histidines are not the pH sensor. Interestingly, the extracellular pH sensor does not involve titratable histidine residues either. Although an extracellular domain including a histidine is crucial in pH₄ sensing, it does not seem to have a proton-binding site. Indeed, selective substitution of the histidine residue in this cluster has no effect on the pHo sensitivity of Kir2.3 (15).

Thus, Coulter et al. (15) believe that an unidentified cysteine residue may play a role.

Using these chimerical and site-directed mutant Kir channels, we have identified a consensus sequence in the N-terminal region of Kir2.3 that is crucial for Kir2.3 gating by CO₂ and pH₄. This motif is located in a conserved region near the M1 membrane-spanning domain. Although there are only three residues that are different between Kir2.3 and Kir2.1, this motif may involve some neighboring residues. Mutation of any of these residues causes a marked reduction in CO₂ sensitivity. Of these three residues, the Thr⁵³ is the key. Mutation of this residue leads to an almost complete loss of channel sensitivity to pH₄ and CO₂, whereas creation of this residue in Kir2.1 makes the mutant channel pH-sensitive. Interestingly, threonine is not a titratable amino acid, and thereby pH change should not affect either its charges or its polarity. Thus, protons may not act directly on this residue to change channel conformation and activity. Then how can this residue be involved in CO₂/pH sensing and the modulation of channel activity? We believe that there are at least three potential ways by which this threonine residue can participate in the modulation of channel activity during hypercapnia. 1) This residue may be engaged in the maintenance of the pK value of another amino acid residue(s) titratable at high or low pH, as suggested previously in Kir1.1 (20, 29). 2) This residue may be involved in a motif that is required for protein-protein interactions in...
CO₂/pH sensing. 3) Phosphorylation at this residue may occur during CO₂/pH exposure.

Is it possible that protons bind to ionizable amino acids other than histidine residues in the N terminus? To answer this question, we have changed nonconserved lysine and arginine residues in the Kir2.3 N terminus into corresponding ones in the Kir2.1 (R66G, R14T, K16Q, and R17Q). Our data reveal that mutations of these residues do not affect CO₂/pH sensitivity. Also, we have systematically divided the N termini of Kir2.3 and Kir2.1 into several segments and rejoined them alternatively to construct chimerical N termini. These extensive interventions would allow us to interrupt the pH sensitivity of Kir2.3 if there were such a proton-binding site, whether positively or negatively charged residues. Although some of these chimeras, such as the N51–60IH, are shown to be more critical than others in CO₂/pH sensing, residues potentially titratable in physiological pH levels do not exist in the sequence of Kir2.3. Interestingly, there are three residues (Asp52, Arg54, and Arg55) that are titratable at extremely high or low pH in the short motif. We have never found any mutation of these into neutral and polar residues (asparagine) in Kir1.1 channel. Because they are highly conserved in all Kir channels, these mutations did not produce any functional channels (30). Therefore, whether some of them are proton sensors with pK levels that can be affected by the Thr53 is still unknown.

Because mutations of Thr53 and Tyr57 into nonpolar isoleucine and tryptophan dramatically reduced the CO₂ sensitivity of Kir2.3, the polarity of positions 53 and 57 seems important in pH sensing. The periodicity of the three crucial residues (Thr53, Tyr57, and Met60) may suggest a helical structure in the region, but our search using the Chou-Fasman and Robson-Granier analyses did not show any helical structure in this short motif.

Our results indicate amino acid sequences other than this motif are also needed to fulfill a complete CO₂ and pH sensitivity. In addition to these 10 residues, we have observed that a sequence composed of a total 41 amino acids can produce an effect of CO₂/pH as effectively as the entire N terminus. Also, the presence of the C terminus enhances the CO₂/pH sensitivity of the mutant Kir channels, although it has no effect on CO₂/pH sensing by itself. A simple explanation of these data would be that the C terminus interacts with the N terminus in gating the channel, as suggested recently in Kir1.1 by Schulte et al. (31). Our data, however, do not support the idea that CO₂/pH sensing in Kir2.3 is a result of protein phosphorylation, as has been described above. Therefore, it is possible that CO₂/pH sensing in Kir2.3 is mediated by an interaction of amino acid residues on the Kir2.3, which is affected by protons and controls the channel activity. Clearly, further understanding of the molecular mechanisms for Kir2.3 modulation by protons may provide information of channel gating under a variety of pathophysiologic conditions, and the demonstration of these critical residues in CO₂/pH sensing in our current studies constitutes an important step toward this goal.

**Physiological Significance of Kir2.3 Sensitivity to Intracellular Acidification**—We believe that the molecular basis of pH sensing in Kir2.3 may underlie the CO₂ chemoreception in the central nervous system. Studies on central chemoreceptors have shown that a large number of these cells depolarize and their membrane excitability increases during hypcapnia. This change in membrane excitability is not mediated by a change in synaptic transmission, because the depolarization persists with the removal of extracellular Ca²⁺ or exposure to tetrodotoxin (32). Although several ionic mechanisms may be involved in the regulation of membrane excitability during hypcapnia, K⁺ channels, especially the inward rectifier K⁺ channels, seem to be the major player (11, 12, 33). The inhibition of these inward rectifier K⁺ channels during hypcapnia produces depolarization and increases membrane excitability (13). With two pH sensors on Kir2.3, cells can sense pH changes on either side of the plasma membranes, in their internal and external environment. Because the primary sensor is a K⁺ channel, the changes in intra- and extracellular pH can be readily coupled to membrane excitability in the nerve cells that are endowed with this pH sensing mechanism and in the neuronal networks to which these cells project. Because Kir2.3 is expressed in the central nervous system (14, 34), the determination of molecular mechanisms in proton sensing may be applicable to the search for unidentified homologues of the Kir2.3 channel in brain stem neurons, which may eventually result in the identification of the CO₂ central chemoreceptors.

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