Allosteric modulation of a neuronal K⁺ channel by 1-alkanols is linked to a key residue in the activation gate

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Harris, Thanawath, Andrew R. Graber, and Manuel Covarrubias. Allosteric modulation of a neuronal K⁺ channel by 1-alkanols is linked to a key residue in the activation gate. Am J Physiol Cell Physiol 285: C788–C796, 2003; 10.1152/ajpcell.00113.2003.—The selective inhibition of neuronal Shaw2 K⁺ channels by 1-alkanols is conferred by the internal S4-S5 loop, a region that also contributes to the gating of voltage-gated K⁺ channels. Here, we applied alanine scanning mutagenesis to examine the contribution of the S5 and S6 segments to the allosteric modulation of Shaw2 K⁺ channels by 1-alkanols. The internal section of S6 is the main activation gate of K⁺ channels. While several mutations in S5 and S6 modulated the inhibition of the channels by 1-butanol and others had no effect, a single mutation at a key site in S6 (P410A) converted this inhibition into a dramatic dose-dependent potentiation (~2-fold at 15 mM and ~6-fold at 50 mM). P410 is the second proline in the highly conserved PVP motif that may cause a significant α-helix kink. The P410A currents in the presence of 1-butanol also exhibited novel kinetics (faster activation and slow inactivation). Internal application of 15 mM 1-butanol to inside-out patches expressing P410A did not significantly affect the mean unitary currents (~2 pA at 0 mV) or the mean open time (5–6 ms) but clearly increased the opening frequency and open probability (~2- to 4-fold). All effects displayed a fast onset and were fully reversible upon washout. The results suggest that the allosteric modulation of the Shaw2 K⁺ channel by 1-alkanols depends on a critical link within the FVP motif and activation gating. This study establishes the Shaw2 K⁺ channel as a robust model to investigate the mechanisms of alcohol intoxication and general anesthesia.

Key Words: alcohol; anesthesia; gating; scanning mutagenesis; Shaw channels

Aliphatic alcohols (1-alkanols) and general anesthetics directly interact with critical sites in neuronal ion channels and, thereby, alter their function (7, 28, 29, 34, 36). Whereas in some instances ion channel function is inhibited, in others it is potentiated. Such effects could result from pore blockade or altered gating and are thought to constitute the physiological basis of acute alcohol intoxication and general anesthesia. However, little is known about the structural basis of these interactions and how they may change gating of ion channels. A current hypothesis proposes that 1-alkanols and general anesthetics bind to amphiphilic protein-protein interfaces involved in the conformational changes that underlie channel gating (2, 10, 14, 28). We have tested this hypothesis by examining the role of regions that contribute to the putative gating machinery of voltage-gated K⁺ channels (Kv channels). The neuronal Shaw2 K⁺ channel from Drosophila melanogaster is suitable for these studies because it is selectively inhibited by pharmacologically relevant concentrations of alcohol (3, 4). Shaw2 K⁺ channels are also special members of the superfamily of Kv channels because they exhibit very weak voltage dependence and a very low open probability over the range of physiological membrane potentials (33). Voltage-dependent gating of most Kv channels involves multiple conformational changes that move 10–14 charges across the membrane electric field (1). In contrast, gating of Shaw2 K⁺ channels appears to be a first-order process with an apparent charge movement of ~1 e₀ (23). This process may correspond to the last concerted conformational change that opens the pore of Kv channels (16, 30–32, 38, 40, 41). In most Kv channels, the opening of the pore is tightly coupled to the voltage-dependent movement of the voltage sensor (the S4 segment) and probably depends on propagated conformational changes involving the S4-S5 loop and the COOH-terminal section of the S6 segment in the pore-forming subunit (18–20, 25, 35, 39, 40).

Our earlier studies showed that the selective inhibition of Shaw2 K⁺ channels by 1-alkanols results from the apparent stabilization of the closed state (4); and from studies with chimeric channels, we found that this inhibition is determined by a 13 amino acid internal segment that constitutes the S4-S5 loop of the Shaw2 pore-forming subunit (14). Here, we applied alanine-scanning mutagenesis to investigate the contribution of the Shaw2 S5 and S6 segments. P410A in the COOH-terminal section of S6 caused the most remarkable change by converting the inhibition into a novel dramatic potentiation. P410 is the second proline in the highly conserved PVP motif that might help to control voltage-dependent activation of eukaryotic Shaker-related Kv channels (5, 6, 39). These results...
are discussed in terms of a working hypothesis that proposes allosteric interactions involving the S4-S5 loop and the COOH-terminal section of S6. Notably, the structural perturbation of the second proline of the S6 PVP motif can act as a switch that determines the overall response of the Shaw2 K⁺ channel to 1-alkanols.

MATERIALS AND METHODS

Molecular biology and site-directed mutagenesis. Wild-type cDNA encoding the Shaw2 pore-forming subunit was maintained as previously described (4). All mutations were created using QuickChange (Stratagene, La Jolla, CA), according to the manufacturer’s specifications as described before (14), and were confirmed by automated sequencing (Nucleic Acid Facility, Jefferson Cancer Institute). Capped cRNA for expression in *Xenopus* oocytes was produced by in vitro transcription using the Message Machine kit (Ambion, Austin, TX). Mutant names use standard single-letter amino acid abbreviations.

Oocyte injection and electrophysiology. *Xenopus laevis* oocytes were harvested according to a protocol approved by the IACUC of Thomas Jefferson University. Wild-type and mutant cRNAs were injected into defolliculated *Xenopus* oocytes (~5–50 ng/cell) using a Nanoject microinjector (Drummond, Broomall, PA). Currents were recorded 3–7 days postinjection. The two-microelectrode voltage-clamp technique (TEV-200; Dagan, Minneapolis, MN) was used to record whole oocyte currents. Microelectrodes were filled with 3 M KCl (tip resistance was <1 MΩ). The bath solution contained (in mM) 96 NaCl, 2 KCl, 1.6 CaCl₂, 1 MgCl₂, and 5 HEPES (pH 7.4, adjusted with NaOH). Current traces (generally, 450–900-ms depolarizations) were low-pass filtered at 1 kHz (~3 dB) and digitized at 500 μs point. The voltage offset recorded at the end of an experiment was generally small (~0.75 ± 2.2, n = 22) and was not subtracted from the command voltage. Correction was applied when offset appeared to be greater than one standard deviation. The leak current was subtracted offline, assuming Ohmic leak or using a P/4 procedure. In whole oocyte experiments, 1-alkanols were applied externally as previously described (4, 14). Whole oocyte currents were recorded at 23°C using a temperature-controlled microscope stage (PDMI-2, Medical Systems, Greenvale, NY).

Patch-clamp recording was conducted as described before (17) using an Axopatch 200B (Axon Instruments, Foster City, CA). Patch pipettes were constructed from Corning glass 7052 (Warner Instrument, Hamden, CT). Typically, for macropatch recording, the tip resistance of the recording pipettes in the bath solution (see below) was 0.5–1 MΩ. The pipette solution (external) was as described above. For inside-out patches, the bath solution (internal) contained (in mM) 98 KCl, 1 EGTA, 0.5 MgCl₂, and 10 HEPES (pH 7.2, adjusted with KOH). For single-channel recording, the tip resistance was 15–20 MΩ. Single-channel records were low-pass filtered at 2 kHz (~3 dB, 8-pole Bessel filter; Frequency Devices, Haverhill, MA) and digitized at 10 kHz. All patch-clamp experiments were recorded at room temperature (22 ± 1°C).

Data acquisition and analysis. Voltage-clamp protocols and data acquisition were controlled by a Pentium-class computer interfaced to a 12-bit analog-to-digital converter (Digidata 1200B using pCLAMP 8.0; Axon Instruments). Data analysis and curve fitting were conducted using Clampfit (pCLAMP 8.0; Axon Instruments), SigmaPlot (Jandel, San Rafael, CA), or Origin 7.0 (Origin Lab). Equilibrium dose-inhibition experiments were analyzed as described before (4). With the pCLAMP 9.0 analysis software (Axon Instruments), a standard halfway threshold method was used to idealize the single-channel records. The resulting event list was then used to construct amplitude and open time histograms. To estimate the mean unitary current, the amplitude histograms were described assuming a Gaussian distribution. A Simplex maximum likelihood method was applied to fit the logarithmically transformed distributions of open times (≥300 μs) with an exponential function to estimate the mean open time. Unless indicated otherwise, all pooled values are expressed as means ± SE.

RESULTS

Alanine scanning mutagenesis revealed a switch of alcohol action at the internal gate of a potassium channel. Structural and functional analyses of Kv channels have suggested that the internal sections of the S5 and S6 segments of the pore-forming subunit may interact with the S4-S5 loop (9, 19, 20, 25, 35). Together, these segments constitute the machinery that probably couples voltage sensing by the S4 region to the opening of an internal gate (11, 12, 16, 19–21, 25, 32, 39, 40). To investigate the interactions between 1-alkanols and the components of the activation gate in Shaw2 K⁺ channels, we applied alanine scanning mutagenesis to probe the internal sections of the S5 and S6 segments. This perturbation may alter critical contacts in protein-protein interfaces and thereby influence the allosteric interactions between 1-alkanol binding and channel gating. When the residues between K327 and V337 in Shaw2 S5 were individually mutated to alanine, we found modest changes in current kinetics or voltage-dependent current activation (~80 to +50 mV; Fig. 1, A and B; e.g., F335A, L336A). L331A, L332A, and F334A, however, eliminated functional expression. Families of currents from mutants that exhibited the most significant changes are shown in Fig. 1A. Several mutants had little or no effect on the inhibition induced by 15 mM 1-butanol (Fig. 1C; 47, 50, 51, and 45% for wild-type, K328A, L329A, and T330A, respectively); and others either suppressed the inhibition (35 and 38% for V333A and L336A, respectively) or enhanced it (56, 68, and 55% for K327A, F335A, and V337A, respectively). Relative to wild-type Shaw2, a complete dose-response analysis of F335A (which produced the largest change in the 1-butanol response) revealed a decrease in the Kₐ₅ for 1-butanol from 17 to 13 mM (Shahidullah and Covarrubias, unpublished observations). Thus alanine mutations in the internal section of S5 have a modest allosteric impact on Shaw2 gating and the interaction with 1-alkanols.

Alanine substitutions in the distal section of S6 in Shaw2 (from T404A to S414A) affected the kinetics of current activation in a few instances (Fig. 2A). Families of currents from mutants that exhibited the most significant changes are shown in Fig. 2A. Whereas activation of I405A is slower and clearly biphasic, V409A activation appeared very fast and unresolved. The current-voltage relationships were, however, similar for all alanine mutants (Fig. 2B). The most significant change was observed with I405A, which appeared...
to operate at more depolarized voltages. In contrast to the results from the alanine mutants in S5, S6 alanine mutants produced a very different and striking pattern of responses to 15 mM 1-butanol (Fig. 2C). Between T404A and V409A, there was a progressive reduction in the inhibition by 1-butanol from 47% (wild-type, WT) to 20% (L407A and P408A produced nonfunctional channels). P410A, however, exhibited a dramatic potentiation by 1-butanol (105%). A subsequent alanine mutation (V411A) reduced the inhibition (26%), and the last two mutations (V413A and S414A) enhanced it modestly (64 and 53%, respectively). This striking pattern suggests that a single mutation (P410A) acts as a switch that controls the overall response of the Shaw2 K⁺ channel to 1-butanol. No other Kv channels studied so far (wild-type or mutant) exhibited this remarkable response to 1-alkanols or general anesthetics (3, 4, 13). Ethanol also potentiated the P410A currents, but, relative to 1-butanol, it is a less potent agonist. At 180 mM, ethanol quickly and reversibly enhanced the P410A current by 94% (not shown). Given the distinct polarities of ethanol and 1-butanol, this finding is consistent with the presence of hydrophobic site of agonist action for 1-alkanols in the P410A mutant.

Dose dependence and electrophysiological properties of the potentiation of P410A by 1-butanol. The dramatic potentiation of P410A by 1-butanol is dose dependent.
and fully reversible (Fig. 3, A–C). Upon exposure of a whole oocyte to a relatively high concentration of 1-butanol (100 mM), the P410A current was quickly enhanced more than 10-fold and, upon washout, the current quickly returned to the original control level (Fig. 3B). A double logarithmic dose-response curve shows that the potentiation induced by 1-butanol was apparent at concentrations as low as 1 mM (~4% above the control) and that higher concentrations potently enhanced the current up to a level that was ~1,100% above the control at 100 mM (Fig. 3C), but there was no evidence of saturation. In the presence of high 1-butanol concentrations, the currents also exhibited a faster rising phase (see below) and a slow decline that inactivated ~90% of the peak current at the end of a 900-ms depolarization to +70 mV (Fig. 3D and Fig. 4C). These kinetic changes were not caused by improper voltage clamping of the oocytes (e.g., series resistance errors), because similar changes were observed from oocytes expressing different levels of current after 1-butanol application (between 12 and 23 μA; not shown). Also, no comparable inactivation was observed when studying other Shaw2 mutants (e.g., F335A; Fig. 1) that expressed currents of similar magnitude (10–15 μA).

The potentiation of P410A by 1-butanol was further characterized to gain insights into the mechanism of this response. First, we examined the current-voltage relation of P410A in the absence and presence of 1-butanol (50 mM; Fig. 4, A and B). Although there was an Fig. 4. Voltage-dependent activation of Shaw2-P410A in the absence and presence of 1-butanol. A: control whole oocyte currents evoked by step depolarizations from −100 mV to membrane potentials ranging between −100 and +70 mV in 10-mV intervals. Pulses were delivered at intervals of 5 s. B: whole oocyte currents evoked as explained above but in the presence of 50 mM 1-butanol (same oocyte). Note that the peak current is enhanced and the currents develop slow inactivation. C: peak current-voltage relations from the families of currents in B (○) and a scaled version of peak currents in A (crosses). D: voltage dependence of the activation time constant from currents recorded in the absence (○) and presence of 50 mM 1-butanol (●). Symbols represent means of 5 independent experiments (±SE). The activation time constants were obtained from the outward currents before and after 1-butanol application by assuming an exponential rising phase.
eightfold difference between the peak currents under these conditions, both current-voltage relations gradually increased with membrane depolarization in a similar fashion (Fig. 4C). Due to the characteristic low-voltage dependence of Shaw2 K\(^+\) channels, a comparison of the conductance-voltage relations could not be reliably obtained from whole oocyte currents recorded with the two-electrode voltage-clamp method. The limited current-voltage relations observed here only activate a very small fraction of the available channels, and depolarizations to voltages \(\geq +180\) mV are necessary to approach maximum conductance (33). Therefore, it is difficult to test whether a leftward shift of the activation curve accounts for the current potentiation by 1-butanol (see DISCUSSION). Nevertheless, a closer examination of the current kinetics revealed evidence of leftward-shifted voltage-dependent gating in the presence of 1-butanol. Clearly, the activation time constants were reduced \(-23\)-fold in the presence of 1-butanol, but their voltage dependence was not significantly affected (parallel relationships in Fig. 4D). Assuming that the activation time constants depend exponentially on membrane potential (solid lines, Fig. 4D), the derived apparent charges were 0.5 and 0.4 \(e_0\) in the absence and presence of 1-butanol, respectively. This parallel shift in the voltage dependence of the activation time constants is consistent with a negative shift in voltage-dependent activation. The small apparent charges are also consistent with the low voltage dependence of activation gating in Shaw2 channels (33).

1-Butanol increases the open probability of the Shaw2-P410A channel. We examined the effect of 1-butanol on the P410A channels in cell-free patches to test the possible dependence on soluble cellular components (e.g., second messenger systems). Clearly, in an inside-out patch expressing P410A (at 0 mV), 15 mM 1-butanol exerted a potent reversible potentiation that resulted from a fourfold increase in open probability (0.2, 0.8, and 0.3, before and after 1-butanol and after washout, respectively; Fig. 5). Thus 1-alkanols might induce the potentiation by acting directly on the pore-forming subunit or tightly associated regulatory components. Furthermore, the relatively rapid onset and reversal of the macroscopic response is not consistent with an effect mediated by a second messenger system (Fig. 3B).

Analysis of a separate sparse record of unitary P410A currents from an inside-out patch (Fig. 6; opening frequency 0.26 Hz at 0 mV) allowed the estimation of the mean unitary amplitude \((i)\) and mean open time \((\tau_0)\). Most openings in this record occurred in isolation (Fig. 6, A–C). The mean closed time was, however, not measured because the number of channels in the patch could not be determined. Under control conditions \((n = 122\) openings\), \(i\) was 2.6 pA and \(\tau_0 = 6.3\) ms \((-6\)-fold longer than the mean open time of wild-type Shaw2 K\(^+\) channel; Ref. 4). Assuming a reversal potential of \(-90\) mV under the ionic conditions of this experiment, the estimated single-channel conductance was \(-25\) pS, which is similar to that of the wild-type Shaw2 K\(^+\) channel (4). 1-Butanol (15 mM) slightly reduced \(i\) (Fig. 6D, top) and \(\tau_0\) (2.3 pA and 4.9 ms, respectively; \(n = 254\) openings), but the opening frequency was approximately doubled (0.54 Hz; Fig. 6D, center). Upon washout, the estimated single-channel parameters were close to control values \((i = 2.3\) pA; \(\tau_0 = 6.9\) ms; \(n = 148\) openings) and the opening frequency returned to basal levels (0.31 Hz, corresponding to an open probability of \(-0.001\)). The absence of a significant effect of 1-butanol on open durations was confirmed by comparing the cumulative distributions of open times (Fig. 6D, bottom). Thus mainly an increase in the opening frequency without significant changes in unitary conductance or the stability of the open state could account for the potentiation of the P410A activity by 1-butanol.

DISCUSSION

This study further probed the contribution of components of the activation machinery to the allosteric interactions between Shaw2 K\(^+\) channel and 1-al-
kanols. The main observation demonstrated that a single alanine mutation of the second proline in the highly conserved PVP motif at the COOH-terminal end of S6 (P410A) readily confers a dramatic reversible potentiation by 1-alkanols. This potentiation results from an increased open probability without significantly changing the mean open time or the unitary conductance. Inhibition and potentiation by 1-alkanols and general anesthetics are commonly observed responses among neuronal ion channels (7, 13, 22, 24, 28, 29, 34, 36).

The allosteric modulation of the Shaw2 channel by 1-alkanols may involve critical interdomain interactions in the activation gate. Crystallographic and biophysical analyses of bacterial K⁺ channels (MthK and KvAP) firmly suggest that the COOH-terminal segment of S6 is likely to be the activation gate of Kv channels (18–20, 40). Although the S6 PVP motif is not present at the equivalent positions in these bacterial channels, it is highly conserved among all eukaryotic Shaker-related Kv channels, and separate experimental evidence strongly suggests that the prolines in this motif favor a kinked α-helix structure at the COOH-terminal end of S6 (5, 6, 15). Thus it is hypothesized that four kinked S6 segments at the inner mouth of the pore constitute an internal activation gate in tetrameric Kv channels of eukaryotes (5, 6). Our data showed that a single proline to alanine mutation at the second position in the PVP motif of the Shaw2 K⁺ channel (P410A) acts as a switch that controls the overall response of the channel to 1-alkanols (Fig. 7A). Upon membrane depolarization, the positively charged voltage sensors move outward, which drags the S4-S5 loop to allow the expansion of the S5 cuff around the pore, and consequently, a concerted lateral displacement of the S6 COOH-terminal segments follows. This displacement causes the opening of the channel’s internal mouth. Importantly, the S4-S5 loop is a region that critically influences the modulation of Shaw2 and Kv3.4 channels by 1-alkanols (4, 14). It is therefore conceivable that the 1-alkanol response of the Shaw2 K⁺ channel depends on how 1-alkanol binding is coupled to a gating mechanism that, in turn, depends on the relationship between the S4-S5 loop and the COOH-terminal segment of S6. When the S6 segment is kinked at the PVP motif, there is inhibition of Shaw2 channels by 1-alkanols (Fig. 7A). In the closed state, the S4-S5 loop and S6 form a protein-protein interface that may constitute the binding cavity of 1-alkanols, which is rapidly occupied only from the internal side (14). The concerted rearrangements that open the channel can only take place when all S4-S5/S6 interfaces are free. Thus, if a 1-alkanol occupies one site [the Hill coefficient for the inhibition at equilibrium is ~1 (4, 14)], the channel remains locked in the closed conformation. To explain the potentiation, we assumed that the P410A mutation reduces or elimi-
opposing subunits of the tetrameric Kv channel are shown. For a simple visualization of the gating mechanism, segments S1-S3 are not shown and only 2 (S4, white with plus sign), and the main elements of the gating machinery (the S4-S5 loop, solid; and the internal segment of S6). For a simple visualization of the gating mechanism, segments S1-S3 are not shown and only 2 opposing subunits of the tetrameric KV channel are shown. A: inhibition by 1-alkanols. Here, we assumed that the internal segment (COOH-terminal) of S6 is kinked at the PVP motif. The right pathway represents normal gating in the absence of 1-alkanols and the left pathway illustrates the binding of 1-butanol to a hypothetical binding site in the interface between the S4-S5 loop and the COOH-terminal segment of S6. 1-Alkanol binding in this interface locks the channel in the closed conformation. B: potentiation by 1-alkanols. Here, we assumed that the P410A mutation eliminates the S6 COOH-terminal kink. Thus there is no S4-S5/S6 interface as depicted in A. The top pathway represents gating in the absence of 1-alkanols, and the bottom pathway illustrates the interaction of 1-butanol with elements of the voltage sensor at the protein-lipid interface. Note that repositioning of the voltage sensor by 1-butanol facilitates voltage-dependent gating in the bottom pathway (thicker arrow).

Fig. 7. Hypothetical models of K⁺ channel gating and alcohol action. These models are based on the crystal structure of a bacterial Kv channel (KvAP; DISCUSSION), which is simplified here to illustrate the α-helical regions that contribute to the permeation pathway (P-loop helix, hatched; S5, open; and S6, stippled), the voltage sensor (S4, white with plus sign), and the main elements of the gating machinery (the S4-S5 loop, solid; and the internal segment of S6). For a simple visualization of the gating mechanism, segments S1-S3 are not shown and only 2 opposing subunits of the tetrameric Kv channel are shown. A: inhibition by 1-alkanols. Here, we assumed that the internal segment (COOH-terminal) of S6 is kinked at the PVP motif. The right pathway represents normal gating in the absence of 1-alkanols and the left pathway illustrates the binding of 1-butanol to a hypothetical binding site in the interface between the S4-S5 loop and the COOH-terminal segment of S6. 1-Alkanol binding in this interface locks the channel in the closed conformation. B: potentiation by 1-alkanols. Here, we assumed that the P410A mutation eliminates the S6 COOH-terminal kink. Thus there is no S4-S5/S6 interface as depicted in A. The top pathway represents gating in the absence of 1-alkanols, and the bottom pathway illustrates the interaction of 1-butanol with elements of the voltage sensor at the protein-lipid interface. Note that repositioning of the voltage sensor by 1-butanol facilitates voltage-dependent gating in the bottom pathway (thicker arrow).

inates the S6 COOH-terminal kink (Fig. 7B). In this case, there is no S4-S5/S6 interface, but rearrangements similar to those explained above still mediate channel activation and pore opening. The crystal structure of KvAP suggests that S4 and the S4-S5 loop may be in contact with membrane phospholipids (19). Thus, through an interaction with the lipid-protein interface, 1-alkanols could reposition S4 and the S4-S5 loop to facilitate voltage-dependent activation. Accordingly, faster current activation at lower voltages results from the repositioning of the voltage sensors (Figs. 3D and 4D). Mechanical models based on the crystal structure of KvAP are, however, still speculative. To develop a physical model of the allosteric modulation of Shaw2 K⁺ channels by 1-alkanols, it is necessary to understand the interactions between the S4-S5 loop and the COOH-terminal tail of S6. Interestingly, mutating the equivalent proline (P469A) in Kv3.4 (a mammalian Shaw homologue) impaired voltage-dependent gating and also conferred activation by 15 mM 1-butanol (~50%; not shown). The latter suggested that the potentiation by 1-alkanols may only be weakly dependent on the distinct structures of the S4-S5 loops in Shaw2 and Kv3.4 (contrary to the inhibition, Ref. 14).

Biophysical basis of the allosteric modulation of the Shaw2-P410A mutant by 1-alkanols. The electrophysiological characterization of the P410A channel provided further information about the mechanism underlying the potentiation by 1-butanol (Figs. 3-6). The main effect of 1-butanol was a rapid dose-dependent increase in peak current that was not associated with significant changes in single-channel conductance or mean open time. This rapid reversible response, which is independent of soluble cellular components, suggested a direct interaction between 1-butanol and a channel subunit or a closely associated regulatory protein. Namely, 1-butanol increased the open probability of the P410A mutant by enhancing the opening frequency. A more detailed analysis of closed times was, however, not possible because the number of channels in the patch could not be estimated (see RESULTS). Nevertheless, the simplest general interpretation of these observations suggests that 1-butanol increases the open probability of the channel through destabilizing nonconducting states connected to the main open state of the channel. If the affected closed state is in the activation pathway, the voltage-dependent activation curve is expected to exhibit a leftward shift. The analysis of the current-voltage relations (Fig. 4), however, revealed no indication of such a shift. This result is not surprising because the examined voltage range (up to +70 mV) only appears to activate a very small fraction of the available channels at the foot of the activation curve (<0.01; Ref. 33). Indeed, by modeling activation of Shaw2 K⁺ channels as a first-order transition (C = O; with a midpoint potential of +200 mV and an apparent gating charge of 0.6 e0), we found that a shift of ~90 mV cannot be detected upon normalizing the current-voltage relations at the foot of the activation curve (~100 to +100 mV) where the open probability is
<0.05 (even though a drastic eightfold potentiation of the current is clearly apparent). Interestingly, 1-butanol also induced faster current activation of the P410 mutant, which was fully reversible and appeared to be concentration dependent and voltage dependent (Fig. 3D and Fig. 4D). The presence of faster current activation in the presence of 1-butanol is consistent with the presence of a leftward-shifted voltage-dependent activation curve. Further electrophysiological studies are necessary to investigate the properties of the apparent inactivation and the exact relation between the kinetic changes and the potentiation of the current.

The dose dependence of the current potentiation by 1-butanol (Fig. 6C) is consistent with an allosteric mechanism involving a low-affinity interaction that enhances activation gating. At the examined membrane potentials (up to +70 mV), P410A channels operate at a very low open probability (<0.01). Analogously, in the presence of submaximal agonist concentrations that activate <10% of the available channels, similar dose-response curves have been observed for the potentiation of GABA and glycine receptor channels by 1-alkanols or general anesthetics (8, 26, 27, 37). Characteristically, the dose-response curves for the potentiation of ligand-gated ion channels and G protein-coupled K channels by 1-alkanols do not exhibit evidence of saturation, which is also consistent with the presence of a low-affinity interaction (8, 22, 24, 26, 27, 37). Taken together, our results shed more light on the electrophysiological, kinetic, and structural basis of the allosteric modulation of activation gating in Shaw2 K channels by 1-alkanols. In particular, by demonstrating the dramatic effect of a single mutation, this study underscores the significance of investigating Shaw2 K channels as an archetypal system to learn more about the intricate interactions that constitute the protein-based theories of alcohol intoxication and general anesthesia.

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DISCLOSURES

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