Effects of Almitrine Bismesylate on the Ionic Currents of Chemoreceptor Cells from the Carotid Body

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ABSTRACT

Almitrine is a drug used in the treatment of hypoxemic chronic lung diseases such as bronchitis and emphysema because it is a potent stimulant of the carotid bodies in human and different animal species that produces a long-lasting enhancement of alveolar ventilation, ameliorating arterial blood gases. However, the mechanism of action of almitrine remains unknown. We investigated the effect of almitrine on ionic currents of chemoreceptor cells isolated from the carotid body of rat and rabbits by using the whole-cell and inside-out configurations of the patch-clamp technique. Almitrine at concentrations up to 10 μM did not affect whole-cell voltage-dependent K⁺, Ca²⁺, or Na⁺ currents in rat or rabbit cells. However, this concentration of almitrine significantly inhibited the Ca²⁺-dependent component of K⁺ currents in rat chemoreceptor cells. This effect of almitrine on the Ca²⁺-dependent component of K⁺ currents was investigated further at the single-channel level in excised patches in the inside-out configuration. In this preparation, almitrine inhibited the activity of a high-conductance (152 ± 13 pS), Ca²⁺-dependent K⁺ channel by decreasing its open probability. The IC₅₀ value of the effect was 0.22 μM. The inhibitory effect of almitrine on Ca²⁺-dependent K⁺ channels also was observed in GH3 cells. We conclude that almitrine inhibits selectively the Ca²⁺-dependent K⁺ channel and that in rat chemoreceptor cells, this inhibition could represent an important mechanism of action underlying the therapeutic actions of the drug.

The CB is an arterial chemoreceptor origin of ventilatory reflexes directed to maintain blood levels of O₂, CO₂, and H⁺ under physiological limits. Chemoreceptor cells are the CB elements that sense blood PO₂ and PCO₂/[H⁺], being activated when PO₂ decreases and PCO₂/[H⁺] increases. Activated chemoreceptor cells release neurotransmitters in amounts that are proportional to the decrease in PO₂ and to the increase in PCO₂/[H⁺]; parallel increases in the action potential frequency of the sensory nerve of the CB and in ventilation follow (Gonzalez et al., 1994).

The coupling of the decrease in PO₂ to the exocytotic machinery responsible for the release of neurotransmitters in chemoreceptor cells (i.e., the chemotransduction process) is incompletely understood, but it is well documented that plasma membrane mechanisms are involved. The presence in rabbit chemoreceptor cells of O₂-sensitive K⁺ channels (López-Barneo et al., 1988; López-López et al., 1989), whose open probability decreases as a function of PO₂ (Ganfornina and López-Barneo, 1991), led to the proposal that hypoxia could control the excitability of the cells, causing cell depolarization, activation of Na⁺ and Ca²⁺ channels, an increase in [Ca²⁺]ᵢ, and release of neurotransmitters (Gonzalez et al., 1992; Gonzalez et al., 1994). O₂-sensitive K⁺ channels also have been found in neonatal (Peers, 1990; Buckler, 1997) and adult (Hatton et al., 1997; López-López et al., 1997) rat chemoreceptor cells, and a similar transduction sequence has been proposed. The detailed characterization of the different CB chemoreceptor cell preparations showed some discrepancies that have been reported to be mainly species related [see López-López and Peers (1997) for a review]. In particular, O₂-sensitive K⁺ channels seem to be different between rabbit and rat chemoreceptor cells. Although hypoxia inhibits a transient voltage-dependent Iₖ in rabbit cells (López-Barneo et al., 1988), the O₂-sensitive currents in rats are both a ChTX-sensitive Ca²⁺-dependent Iₖ (Peers, 1990; Wyatt and Peers, 1995; López-López et al., 1997) and a leak Iₖ (Buckler, 1997).

In patients with chronic respiratory failure, acute exacerbations brought about by respiratory infections may further impair their blood gas levels. Treatment with central respi-
ratory stimulants provides limited clinical success and many side effects; long term oxygen therapy, being more successful, is both expensive and hard for patients. A third possibility to improve oxygenation to the blood is to stimulate the CBs with a drug such as almitrine bismesylate, which improves ventilation without central nervous system disturbances. The effect of almitrine enhancing alveolar ventilation through stimulation of the CB has been reported in several studies (Laubie and Schmitt, 1980; Bisgard, 1981; McQueen et al., 1989; Lahiri et al., 1989), but its mechanism of action remains unknown. It was reported recently that almitrine produces a long-lasting increase in the release of catecholamines from chemoreceptor cells in resting normoxic conditions and potentiates low Po2-induced catecholamine release (Almaraz et al., 1992). It also has been shown that almitrine inhibits I_K from rabbit or rat cells. However, almitrine inhibited the Ca2+-dependent component of the I_K recorded from CB chemoreceptor cells (López-López and Peers, 1997), the effects of almitrine on I_Na, I_K, and I_Ca were studied in freshly or acutely cultured cells isolated from adult rabbits or rats using the whole-cell configuration of the patch-clamp technique. Almitrine did not modify voltage-dependent I_Na, I_K, or I_Ca from rabbit or rat cells. However, almitrine inhibited the Ca2+-dependent component of the I_K recorded from rat cells in the whole-cell configuration. This selective effect was characterized further at the single-channel level in membrane patches excised from rat chemoreceptor cells.

**Materials and Methods**

**Cell isolation and culture.** Experiments were performed on cultured rat and rabbit CB chemoreceptor cells. Adult Wistar rats (3–4 months old) or adult New Zealand White rabbits (1.5–2 kg) were anesthetized with pentobarbital sodium (100 mg/kg administered intraperitoneally to the rats or 40 mg/kg administered through the lateral vein of the ear to the rabbits). After tracheotomy, the carotid artery bifurcations were removed, and the animals were killed by an intracardiac bolus injection of pentobarbital sodium. The CBs were cleaned of surrounding connective tissue and enzymatically dispersed as described previously (Pérez-García et al., 1992; López-López et al., 1997). Dispersed cells were plated onto small poly-L-lysine-coated coverslips and maintained in culture for up to 36 hr.

**Electrophysiological recording.** Ionic currents were recorded at room temperature (20–25°C) using the whole-cell and inside-out modes of the patch-clamp techniques (Hamill et al., 1981). Whole-cell current recordings and data acquisition were made as described previously (López-López et al., 1997). Patch pipettes used for single-channel recordings were made from borosilicate glass (0.8 mm; World Precision Instruments, New Haven, CT) and double-pulled (Narishige PP-83) and heat-polished (Narishige MF-83) to resistances of 12–20 MΩ when filled with the internal solution.

Recordings were made with an Axopatch-200A patch-clamp amplifier and a Digidata 1200 A/D interface, driven by pCLAMP version 6.02 software (Axon Instruments, Burlingame, CA) with a Pentium computer. Single-channel records were filtered at 1 kHz and digitized at 10 kHz.

**Analysis.** Analysis of the data was performed with the CLAMPFIT and FETCHAN subroutines of the pCLAMP software. Single-channel amplitudes and open probabilities were measured from amplitude histograms generated with FETCHAN. The amplitude histograms consisted of 256 bins with each bin containing the number of sample points falling within the bin width. The amplitude of the single-channel currents was taken as the difference between the peaks for opened and closed currents levels. Because most of the patches had multiple channels, open probabilities were expressed as NPo, where N represents the number of single channels present in the patch, and Po represents the open probability of a single channel. NPo was calculated using the following expression (Kajioka et al., 1991):

\[
NPo = \frac{(A_1 + 2A_2 + 3A_3 + \ldots + nA_n)/(A_0 + A_1 + A_2 + \ldots + A_n)}
\]

where A0 is the area under the curve of the amplitude histogram corresponding to current in the closed state, and A1, . . . , An represents the histogram areas reflecting the different open-state current levels for 1 to n channels present in the patch. Histogram parameters were obtained from multiple least-squares gaussian fits of the data using ORIGIN 4.0 software (MicroCal, Northampton, MA).

When pooled data are shown, they are expressed as mean ± standard error. Statistical comparisons were performed with the two-tailed t test for paired or unpaired data as appropriate, and values of p < 0.05 were considered statistically different.

**Solutions.** The compositions of the bathing and pipette solutions for all recording conditions are given in Table 1. Gigaseals were formed in the standard extracellular solution (standard). Whole-cell I_K were recorded in this same extracellular solution with 125K in the pipette. When we studied I_Na or I_Ca, the 0K solution was used in the pipette; for I_Ca, the external solution was

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switched to 10 Ca (or 10 Ba), and in some experiments TTX was added to block IKCa.

For the solutions used in inside-out patch experiments, careful attention was paid to hold the Ca2+ concentration facing the internal side of the channel at fixed levels. Ca2+ chemical activity was fixed to the values indicated by buffering with EGTA according to the software CHELATOR (Schoenmakers et al., 1992). Titrated stock solutions of CaCl2 and EGTA were used to minimize errors due to impurities of EGTA and hydration of CaCl2.

Chemicals and drugs. All chemicals used in pipette and bath solutions were obtained from Sigma Chemical (St. Louis, MO). ChTX (Alomone Labs, Jerusalem, Israel) was used as described previously (López-López et al., 1997). Almitrine bismesylate [1-(4’,-6’-diallylamin-o-2’-triazinyl)-4’-(bis-4’, 4’-fluorobenzoydryl)piperazine bis-methane sulfonate; Vectorion, Servier International, Paris, France] was prepared in a 4.5 mM stock, with a solvent of a solution of 45 mM malic acid. At the highest concentration used (0.1 mM), malic acid alone had no effect on whole-cell ionic currents from chemoreceptor cells (data not shown).

Results

Effects of almitrine on ionic currents from rat chemoreceptor cells. The effect of almitrine (10 μM) on whole-cell IK from rat CB chemoreceptor cells is shown in Fig. 1. After establishment of the whole-cell configuration, I-V relationships for IK were obtained every minute, with the application of groups of 200-msec depolarizing steps from −60 to +100 mV in 10-mV steps. The holding potential was −60 mV. After several minutes under control conditions, almitrine (10 μM) was applied for 5 min, and the recording continued for 6 min after removal of the drug. Peak current values obtained at +20 and +90 mV in one representative cell are plotted against time in Fig. 1a. The slope of the usual decay of IK amplitude at +20 mV (△), due to washout of IKCa (see López-López et al., 1997), sharply increased after the application of almitrine 10 μM (Fig. 1, hatched rectangle). The effect of almitrine on the current elicited at +90 mV was much less pronounced. In the 6-min period after the removal of the drug from the bathing solution, there was no recovery from inhibition. Fig. 1b shows sample records and the whole I-V relationships obtained before (time 2 min) and after (time 12 min) application of the drug. It is evident that the prominent hump in the I-V curve, which is due to the activation of Ca2+-dependent K+ channels (Peers, 1990; López-López et al., 1997), disappeared in the presence of almitrine. Results obtained in 11 cells with the same protocol were averaged and presented in Fig. 1c as percentage of inhibition produced by the application of 10 μM almitrine. In seven cells (●), the inhibition was clearly voltage dependent, being maximal between the range of potentials of activation of Ca2+ channels (0–20 mV; p < 0.001 at 0 mV). In four cells (○), there was almost no effect of almitrine in the entire range of tested voltages; two of these four cells did not exhibit a clear hump in their I-V relationships, but we do not have any explanation for the lack of effect of almitrine in the other two cells with a clear Ca2+-activated component. The voltage dependence of the inhibition strongly suggested that almitrine effectively inhibited IKCa in rat cells; indeed, the effect of the abolishment by almitrine of the hump of the I-V relationship is comparable to that observed with the IKCa blocker ChTX (Peers, 1990; López-López et al., 1997). To confirm the specificity of almitrine on IKCa, we studied the effect of the drug on IK recorded in the presence of 20 nM ChTX. Fig. 2 shows the Ipeak at +20 mV elicited by voltage ramps (0.023 mV/ms) from −60 to +100 mV applied every 30 sec. This ramp protocol was used instead of step depolarizations to enhance the Ca2+-dependent component of IK (López-López et al., 1997). The application of ChTX produces a marked decrease of the current amplitude that is maximal at voltages between +10 and +20 mV (Fig. 2, inset). In the presence of ChTX, almitrine did not modify IK, suggesting a common target (IKCa) for the inhibitory effect of the two drugs. The same lack of effect of almitrine on ChTX treatment was observed in an additional three cells. However, because IKCa decayed slowly along the experiments (Fig. 1a, initial 4 min and △; see also López-López et al., 1997) and because the washout of almitrine seemed to be very slow, it was very difficult to quantify the inhibition due to the drug; the two effects (current decay and current inhibition) combine in an apparently irreversible fashion. Moreover, the effect of almitrine can be due to a direct inhibition of Ca2+-activated K+ channels, an
inhibition of the entry of Ca\(^{2+}\) through the Ca\(^{2+}\) channels, or both.

This latter possibility was tested by studying the effect of almitrine on whole-cell I\(_{Ca}\) in rat chemoreceptor cells is shown in Fig. 3. Families of I\(_{Ca}\) were obtained through the application every 2 min of a group of 7-msec depolarizing pulses from a holding potential of \(-80\) mV to \(+60\) mV in 10-mV steps. I\(_K\) were blocked with Cs\(^+\) in the pipette (solution 0K\(_i\)), and I\(_{Ca}\) were maximized with 10 mM Ba\(^{2+}\) in the bath (solution 10 Ba\(_e\); see Table 1). Fig. 3a shows a typical experiment in which current amplitudes were measured immediately before the end of pulses to three different potentials and plotted against time. Almitrine (10 \(\mu M\)) was applied for 5 min (hatched bar). Actual records at the indicated times illustrating the progressive run-down of the currents also are shown; again, almitrine did not modify the time course of this rundown. Mean I-V relationships obtained in five cells during the application of almitrine were normalized to the averaged control and recovery I-V curves and are represented in the figure. Although the currents were completely blocked by 100 \(\mu M\) Cd\(^{2+}\) excludes the possibility of any contaminating I\(_{Na}\) in our records, confirming previous reports that indicate that Na\(^+\) channels are either absent in rat CB chemoreceptor cells or present in only a low percentage (López-López et al., 1997).

**Effects of almitrine on rat Ca\(^{2+}\)-activated K\(^+\) channels.** The inhibition of the Ca\(^{2+}\)-dependent component of IK and the lack of effect of almitrine on I\(_{Ca}\) strongly suggested that Ca\(^{2+}\)-activated K\(^+\) channels were in fact the targets for the action of the drug. This possibility was tested by studying the effect of almitrine on the activity of single Ca\(^{2+}\)-activated K\(^+\) channels present in excised chemoreceptor cell membrane patches and recorded in the inside-out configuration of the patch-clamp technique. It is well known that Ca\(^{2+}\)-activated K\(^+\) channels (Peers and Buckler, 1995; Wyatt and Peers, 1995; López-López et al., 1997). BK channels in the isolated patches were identified in this study on the basis of their voltage dependence, large conductance, and Ca\(^{2+}\) sensitivity (Fig. 4). In asymmetrical solutions (4.7 mM K\(^+\) at internal membrane face, 125 mM K\(^+\) at external membrane face), the I-V relationship of the isolated channels showed some inward rectification (Fig. 4a, △), which is well described by the Goldman-Hodgkin-Katz current equation. The extrapolated reversal potential was close to the K\(^+\) equilibrium potential, which was \(+84\) mV in these experiments. When the bath solution was changed and single-channel currents were recorded under symmetrical conditions (125 mM K\(^+\) at both sides of the membrane), the rectification disappeared and the reversal potential shifted to 0 mV, which is as expected for a K\(^+\)-selective channel (Fig. 4a, □). The averaged I-V relationships obtained with symmetrical high K\(^+\) conditions from different patches (○). The average slope conductance under these conditions was 152 ± 13 pS (five cells).

The other requirement used to classify these high-conductance K\(^+\) channels as BK channels was their dependence on bath (“intracellular”) Ca\(^{2+}\); as shown in Fig. 4b. Channel activity was recorded under symmetrical K\(^+\) conditions (125 mM) at \(+60\) mV with 0, 1, or 10 \(\mu M\) Ca\(^{2+}\) in the bathing solution. NPo in each situation (see Materials and Methods) was calculated from all-points histograms generated in all cases from \(\approx0\)-min recording of channel activity. Mean NPo values of 4–12 patches at the three Ca\(^{2+}\) concentrations are represented in the figure. Although the Ca\(^{2+}\) dependence of the channels has not been characterized thoroughly, it is evident that channels were almost silent in 0 Ca\(^{2+}\) and that on increasing Ca\(^{2+}\) concentration, channel activity increased markedly.

When 10 \(\mu M\) almitrine was added to the bathing solution, BK activity recorded under symmetrical K\(^+\) conditions at \(+60\) mV clearly was inhibited, in both 1 \(\mu M\) and 10 \(\mu M\) Ca\(^{2+}\) (Fig. 5). Lower doses of the drug (0.01 \(\mu M\)) or malic acid (0.1 mM) did not affect the activity of the channels (Fig. 5). This malic acid concentration corresponds to that in the bathing solution with 10 \(\mu M\) almitrine.

The dose dependence of the effect of almitrine on BK
channel activity was explored further in several patches. The holding potential was +60 mV, and the Ca\(^{2+}\) concentration in the bath was kept at 1 \(\mu\)M. All-point histograms obtained during 4-min periods at different concentrations of almitrine in a single patch are shown in Fig. 6a. The concentration of almitrine was increased progressively from 0.1 to 10 \(\mu\)M in this particular case. At 8–12 min after removal of the drug, the channel activity recovered almost completely. Due to this slow recovery, washout of the drug could not be detected in the whole-cell experiments; rundown of IK\(_{Ca}\) usually is faster. Also evident in Fig. 6a is the fact that almitrine inhibited channel activity decreasing the open probability without affecting the channel conductance. The relationship between the normalized channel activity (NPo in the presence of almitrine divided by NPo under control conditions) and the concentration of almitrine obtained in several different patches are shown in Fig. 6b. The continuous curve was drawn by fitting all data to the following equation:

\[
\frac{N_{Po}}{N_{Po\_control}} = \frac{1}{1 + \left(\frac{IC_{50}}{[Almitrine]}\right)^n}
\]

where IC\(_{50}\) and \(n\) were 0.22 \(\mu\)M and 0.68, respectively. Importantly, the mean blood levels attained with therapeutic doses of almitrine in patients have been estimated to be \(-0.3\) \(\mu\)M, which is very close to the IC\(_{50}\) value obtained in the current study (Campbell et al., 1983).

We further characterize the inhibition of BK channels by almitrine by considering its dependence of the intracellular Ca\(^{2+}\) concentrations. Fig. 7 shows the percentage of decrease of NPo in the presence of 10 \(\mu\)M almitrine at two intracellular Ca\(^{2+}\) concentrations: 1 and 10 \(\mu\)M. Although this concentration of almitrine almost completely blocks BK channels recorded in 1 \(\mu\)M Ca\(^{2+}\), there is only a 39% inhibition when the
Ca$^{2+}$ concentration is raised to 10 $\mu$M. Also illustrated in the figure is our observation that the effect of almitrine on BK channels in rat chemoreceptor cells is not tissue specific; BK channels recorded from GH3 cells also are inhibited by almitrine to a very similar extent. Furthermore, this effect of almitrine on BK channel activity in GH3 cells shows the same Ca$^{2+}$ dependence.

Effects of almitrine on ionic currents from rabbit chemoreceptor cells. The effect of almitrine (10 $\mu$M) on whole-cell voltage-dependent currents of rabbit CB chemoreceptor cells is shown in Fig. 8. Voltage-dependent $I_K$ were studied in seven cells. A protocol similar to the one described above for $I_K$ in rat cells was used, but current families were obtained every 2 min. For each cell, the I-V relationship was obtained by determining $I_{\text{peak}}$ and the amplitude just before the end of the 200-msec pulses ($I_{\text{ss}}$). I-V relationships obtained before, during, and after the application of 10 $\mu$M almitrine were normalized to the peak current at 180 mV in control conditions (i.e., before the application of the drug), and the results obtained with the seven cells (mean ± standard error) are represented in Fig. 8a. In the presence of almitrine, there is a small reduction in the current amplitude at very depolarized values (> +40 mV). However, this reduction is due to the rundown of the currents along the experiment, and its time course is the same in almitrine-treated and untreated cells. Traces in Fig. 8a show the $I_K$ elicited by depolarizing pulses to +40 mV before, during, and after the application of almitrine in one of the studied cells.
The effect of almitrine on IC\text{a} in rabbit chemoreceptor cells was investigated as well (Fig. 8b). The recording protocol is the same used for IC\text{a} in rat chemoreceptor cells. Internal solution was solution 0K\text{a} and, because of the higher density of Ca\text{2+} channels in rabbit compared with rat chemoreceptor cells (López-López and Peers, 1997), the bath solution contained Ca\text{2+} as the charge carrier instead of Ba\text{2+} (solution 10 Ca\text{a}, see Table 1). The bath solution also contained 300 nM TTX, a concentration known to completely block INa in rabbit chemoreceptor cells (López-López and Gonzalez, 1992). Almitrine (10 \text{mM}) was applied for 4 min. Traces obtained in a typical experiment with the pulses to 10 mV are shown. Current amplitudes decayed along the experiment, due to the well-documented progressive washing-out of the Ca\text{2+} channels in chemoreceptor cells (Duchen et al. 1988; Ureña et al. 1989), and almitrine did not modify the time course of the washing-out. I-V relationships obtained in several cells (six) during the application of almitrine were normalized to the average between the maximal currents elicited in control and recovery conditions to correct for washing-out.

Finally, the effect of almitrine on INa was tested in rabbit chemoreceptor cells (Fig. 8c). A protocol similar to that described for IC\text{a} was used, but the cells were bathed with the solution standard, containing 100 \text{\mu M} Cd\text{2+}, and I-V relationships were obtained every 30 sec. Almitrine (10 \text{mM}) was added to the bathing solution for >5 min. The I-V relationships obtained in four cells before, during, and after the application of the drug were normalized to the maximal current obtained in control conditions (i.e., before drug application); averaged; and represented in Fig. 8c. The normalized I-V relationships obtained in the absence and presence of the drug are not statistically different. The current traces obtained in one cell with the depolarizing pulses to +10 mV also are shown in Fig. 8c.

Discussion

We examined the effect of almitrine on ionic currents from chemoreceptor cells from rat and rabbit carotid bodies. In whole-cell recordings, the only significant effect observed has been an inhibition of IKCa in rat cells. Almitrine does not affect IKCa in rabbit cells (Fig. 8), or in rat cells, as suggested for the voltage dependence of the drug effect (Fig. 1b) and for the lack of effect in the presence of ChTX (Fig. 2). Furthermore, the effect on IKCa is not due to an inhibition of IC\text{a} (Figs. 3 and 8) but to a direct action of the drug on high-conductance Ca\text{2+}-activated K\text{+} channels (Figs. 5–7). The effect of almitrine on BK channels is fully reversible, but the
There is clear evidence for the contribution of BK to the active cells increases action potential frequency. In addition, thereby selective inhibition of this channel in spontaneously studied because the Ca\(^{2+}\) present in a very variable amount and IK is mainly a voltage-dependent current (Uren˜a et al., 1989). In fact, the lack of a marked hump in the I-V relation show several differences with a previous work in chemoreceptor cells, almitrine, at concentrations up to 10 \(\mu\)M, inhibits selectively BK channels.

The single-channel properties of BK currents in our preparation show several differences with a previous work in neonatal rat CB cells (Wyatt and Peers, 1995), including a smaller unitary conductance (152 versus 190 pS) and a higher open probability for a given intracellular Ca\(^{2+}\) concentration (\(\Delta P_0 = 0.3\) at 1 \(\mu\)M Ca\(^{2+}\) versus 0.04). These discrepancies could reflect differences in the developmental stage between both preparations, because the experimental conditions and recording solutions are quite similar.

The selective effect of almitrine on BK channels in chemoreceptor cells provides a molecular target that can contribute to understanding of the reported chemostimulant effects of the drug in several preparations. We know that the main function of BK in excitable cells is to contribute to action potential repolarization (Garcia et al., 1995; Sah, 1996), and thereby selective inhibition of this channel in spontaneously active cells increases action potential frequency. In addition, there is clear evidence for the contribution of BK to the washout of the drug is very slow. The apparent lack of reversibility in whole-cell recordings certainly is due to the overlapping of the slow recovery and the washout of IK\(_{Ca}\).

The percent inhibition was calculated as 100 \(\times (P_0_{control} - P_0_{almitrine}) / P_0_{control}\), with \(P_0_{control}\) being the average open probability before and after almitrine application. In all cases, almitrine was present in the bath for 4–6 min. Each bar, mean ± standard error of three to six patches.

The results of electrophysiological studies have shown that most rat CB chemoreceptor cells possess whole-cell IK with a ChTX-sensitive Ca\(^{2+}\)-dependent (BK) component that represents a major percentage of the entire IK at membrane voltages between −10 and +40 mV (Peers and Buckler, 1995; López-López et al., 1997; see Fig. 1). It is a well-established fact that BK currents in rat chemoreceptor cells are reversibly inhibited by low \(P_{O_2}\) and that this inhibition may play an important role in the modulation of the response of the cells.

**Fig. 7.** Ca\(^{2+}\) dependence of the effect of 10 \(\mu\)M almitrine on BK channel activity recorded in excised membrane patches from rat CB chemoreceptor cells or GH3 cells at two different Ca\(^{2+}\) concentrations: 1 and 10 \(\mu\)M. The percent inhibition was calculated as 100 \(\times (P_0_{control} - P_0_{almitrine}) / P_0_{control}\), with \(P_0_{control}\) being the average open probability before and after almitrine application. In all cases, almitrine was present in the bath for 4–6 min. Each bar, mean ± standard error of three to six patches. Solutions: 0 Ca (pipette)/1 \(\mu\)M Ca or 10 \(\mu\)M Ca (bath).

**Fig. 8.** Effect of 10 \(\mu\)M almitrine on whole-cell currents from rabbit chemoreceptor cells. a, I-V relationships of \(I_K\) measured at the peak \(\left(I_{peak}\right)\) or at 200 msec \(\left(I_{200}\right)\) and obtained before, during, and after the application of 10 \(\mu\)M almitrine. Current amplitudes were normalized in respect to \(I_{peak}\) at +80 mV under control conditions. Values are mean ± standard error for seven cells. Solutions: standard/125K. Traces obtained in a single cell during a 200-msec step from −60 to +40 mV are shown. b, I-V relationships of \(I_{Na}\) measured during the application of almitrine were normalized against the average of those obtained under control and recovery conditions to correct for rundown. Values are mean ± standard error for six cells. Solutions: standard, 100 \(\mu\)M CdCl\(_2\)/0K. Traces obtained in one cell in steps to +10 mV before, during, and after the application of 10 \(\mu\)M almitrine are shown. c, I-V relationships of \(I_{Na}\) measured under control conditions, during the application of almitrine, and after washing of the drug were normalized against the maximal current under control conditions. Values are mean ± standard error for four cells. Solutions: standard, 100 \(\mu\)M CdCl\(_2\)/0K. Traces, obtained in one cell with voltage steps to +10 mV are shown.
to hypoxia (Peers and Buckler, 1995; Wyatt and Peers, 1995; López-López et al., 1997). However, because rat CB chemoreceptor cells in normoxic conditions do not present spontaneous activity (López-López and Peers, 1997), the functional significance of this inhibition is in dispute. Although some workers have shown that BK contributes significantly to the genesis and maintenance of resting membrane potential (Wyatt and Peers, 1995), others found that ChTX does not affect membrane potential (Buckler, 1997), implying that BK inhibition produced by low PO2 cannot represent the trigger for the chemotransduction process. The chemostimulant action of almitrine in normoxic rats (Behm et al., 1993; Lagneaux, 1994), in light of the findings reported in the current work, could be accounted for if BK contributes to the genesis of membrane potential, but the action of almitrine potentiating hypoxic chemoreception (Lagneaux, 1994) can be satisfactorily explained regardless of whether BK participates in the genesis of membrane potential because rat chemoreceptor cells can generate action potentials during hypoxic stimulation (Peers and Buckler, 1995).

The data presented here indicate that almitrine behaves as a selective blocker of BK in rat chemoreceptor cells and that the inhibitory effect of almitrine is dependent on the intracellular Ca2+ levels, being less prominent at higher Ca2+ concentrations (Fig. 7). Although the mechanism of this blockade has not been characterized, it probably involves a direct interaction of almitrine with the channel, because the role of intracellular mediators can be excluded in the inside-out configuration. Regarding the tissue-specificity of the effect, we found that almitrine at 10 μM also inhibits BK in GH3 cells (Fig. 7), although a detailed characterization of this inhibition is lacking. Due to the variability of expression of BK in rabbit chemoreceptor cells (see above), we have not studied the effect of almitrine on this channel in this species, but our preliminary results in GH3 cells make conceivable that almitrine would have comparable effects. In the rabbit CB chemoreceptor cells, BK currents are not O2 sensitive (Ganfornina and López-Barneo, 1991). However, contrary to those in rat, rabbit chemoreceptor cells generate action potentials at rest and after hypoxic stimulation (López-López et al., 1989; Montoro et al., 1996), so BK currents, when present, should contribute to action potential repolarization, and their inhibition by almitrine would lead to increased Ca2+ entry and consequent activation of the cell. Moreover, considering that chemoreceptor cells are electrically coupled (Abudara and Eyzaguirre, 1994), cell activation could spread to adjacent cells and ultimately to the entire CB.

This hypothesis is consistent with previous data showing that almitrine both promotes the release of neurotransmitters from rabbit CB and potentiates the secretory response induced by hypoxia (Almaraz et al., 1992).

In addition to its chemostimulant actions, almitrine improves ventilation/perfusion matching and increases the oxygenation of arterial blood through potentiation of the hypoxic pulmonary vasoconstriction (Chardon et al., 1980; Saadjian et al., 1994). This therapeutically important effect of almitrine also could be accounted for by the inhibition of BK because it is well documented that BK plays a very important role in regulation of pulmonary artery tone (Weir and Archer, 1995). Thus, the findings reported in the current work might represent the description of a common cellular mechanism generating the therapeutic effects of almitrine.

Based on the ubiquitous distribution of BK in the organism, it seems that almitrine would produce a wide spectrum of unwanted effects; however, this is not the case in laboratory animals or humans, indicating that at clinically useful doses, the function of most cells is not altered by almitrine. How this specificity is achieved remains unknown. It could be related to the tissue specificity of the properties of BK (Bolton and Beech, 1982; Tseng-Crank et al., 1994) or, more likely, to the conjunctive action of additional cell mechanisms that, as targets for almitrine, amplify the effects of BK inhibition. In fact, almitrine produces other cellular effects (Leverve et al., 1994) that might contribute to sharpen the specificity of almitrine actions at the level of CB chemoreceptors and pulmonary artery smooth muscle cells, minimizing simultaneously its actions in other tissues.

In conclusion, we demonstrate that almitrine at therapeutically useful doses inhibits the O2-sensitive high-conductance Ca2+-dependent K+ channel in rat CB chemoreceptor cells without affecting other ionic currents. This effect of almitrine could represent an important mechanism underlying the chemostimulant action of almitrine.

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References


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