Oxygen sensitivity in the sheep adrenal medulla: role of SK channels

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Keating, Damien J., Grigori Y. Rychkov, and Michael L. Roberts. Oxygen sensitivity in the sheep adrenal medulla: role of SK channels. Am J Physiol Cell Physiol 281: C1434–C1441, 2001.—The hypoxia-evoked secretion of catecholamines from the noninnervated fetal adrenal gland is essential for surviving intrauterine hypoxemia. The ion channels responsible for the initial depolarization that leads to catecholamine secretion have not been identified. Patch-clamp studies of adrenal chromaffin cells isolated from fetal and adult sheep revealed the presence of a Ca2+-dependent K+ current that was reduced by hypoxia. Apamin, a blocker of small-conductance K+ (SK) channels, reduced the Ca2+-dependent K+ current, and the sensitivity of the channels to apamin indicated that the channels involved were of the SK2 subtype. In the presence of apamin, the hypoxia-evoked change in K+ currents was largely eliminated. Both hypoxia and apamin blocked a K+ current responsible for maintaining the resting potential of the cell, and the depolarization resulting from both led to an influx of Ca2+. Simultaneous application of hypoxia and apamin did not potentiate the increase in cytosolic Ca2+ concentration beyond that seen with either agent alone. Similar results were seen with curare, another blocker of SK channels. These results indicate that closure of SK2 channels would be the initiating event in the hypoxia-evoked catecholamine secretion in the adrenal medulla.

hypothesis; potassium channels; calcium

In the adrenal medulla of the sheep fetus and rat newborn, before the development of a functional innervation, hypoxia acts directly on the chromaffin cells to stimulate catecholamine secretion (4, 5, 21). This direct response of the adrenal medullary cells to hypoxia results in a redirection of blood to the heart, brain, and adrenal glands and is crucial for the survival of the fetus (18). Once innervation has developed (by 130 days of gestation in the sheep), the direct response of the adrenal medullary cells to low Po2 disappears (4); however, it is restored upon denervation, as demonstrated by the hypoxia-evoked secretion of catecholamines from the perfused whole adrenal gland isolated from the adult sheep (1).

It is believed that the mechanism of oxygen sensing in the adrenal chromaffin cells is similar to that in the glomus cells of the carotid body, the major chemoreceptor cells in the adult mammals, where it involves oxygen-sensitive K+ (KO2) channels. These channels close in hypoxic conditions, leading to depolarization, opening of voltage-dependent Ca2+ channels, and exocytosis of neurotransmitters subsequent to the elevation of the cytosolic Ca2+ concentration ([Ca2+]i) (reviewed in Ref. 19). In both the carotid body and the adrenal medulla, there is debate over what types of KO2 channels are responsible for the initial hypoxia-evoked depolarization of the cell membrane.

In adrenal chromaffin cells of the sheep, a current through Ca2+-dependent K+ (KO2) channels was shown to be reduced during hypoxia (20), but the types of KO2 channels involved were not fully identified. Large-conductance K+ (BK) channels are present in the adrenal chromaffin cells of many species (13, 14), and at least part of the oxygen-sensitive current in the sheep adrenal medulla is through BK channels (20). Closure of these channels is unlikely to cause the hypoxia-evoked depolarization, however, because BK channels are strongly voltage dependent and normally are closed at resting potential (11). KO2 channels that are open at resting potential and might be responsible for the sensitivity of the chromaffin cells to hypoxia are intermediate-conductance K+ (IK) channels and the small-conductance K+ (SK) channels. IK channels are not present in the human adrenal medulla (8) and do not appear to have been described in the adrenal of other species. Voltage-independent SK channels have been found in bovine and rat chromaffin cells (13, 14), and mRNA for SK2 channels, a subtype of the SK channel family, has been isolated from the rat adrenal medulla (9). Recently, SK channels were suggested to play the role of the oxygen sensor in rat adrenal chromaffin cells (10).

In this report, the results of patch-clamp studies and intracellular Ca2+ measurements show that more than one channel type is involved in the response of the sheep adrenal medulla to hypoxic stimuli, with the SK channel being responsible for the initial depolarization.

MATERIALS AND METHODS

Isolation of adrenal chromaffin cells. Pregnant Border Leiceste- ter × Merino cross ewes between 137 and 142 days of...
gestation (term: 147 ± 3 days) were used for these experiments, which were approved by the Adelaide University Animal Ethics Committee. Ewes were killed with intravenous pentobarbitone (8.1 g), and the fetus was removed through a laparotomy incision and decapitated. The fetal adrenal gland was removed, and the medulla, dissected free of the cortex, was minced and incubated at 37°C for 45 min in a Ca²⁺-free Locke's solution consisting of (in mM) 154 NaCl, 5.6 KCl, 3.6 NaHCO₃, 5.6 glucose, and 5.0 HEPES, pH 7.4, supplemented with collagenase (Worthington type II, 0.1%) and deoxyribonuclease (type IV, 100 U/ml; Sigma). Repeated pipetting mechanically dispersed the tissue, and the cells were washed twice in Ca²⁺-free Locke's solution. The cells were then resuspended in DMEM containing 10% charcoal-absorbed fetal bovine serum (Trace Biosciences, Sydney, Australia), 100 U/ml penicillin, and 0.5 mg/ml streptomycin, plated on glass coverslips, and maintained in culture. Adult chromaffin cells were isolated by the same technique, with the only modification being the doubling of the collagenase concentration to 0.2%.

Electrophysiology. Whole cell recordings were conducted on cells 1–3 days after plating, always at room temperature. Pipettes with a resistance between 2 and 4 MΩ were used, and series resistance was 80–95% compensated. Currents were recorded with an EPC-9 amplifier (HEKA), and data acquisition and analysis were performed on an IBM-compatible computer using Pulse and Pulsefit software (v8.11; HEKA). Corrections for liquid junction potential between the bath and electrode solutions were estimated by using JPCalc (HEKA). Images were captured with confocal software (F900e, Optiscan, Notting Hill, Australia) scanning at a peak of 488 nm. Images were captured with confocal software (F900e, v1.6; Optiscan) and analyzed with Scion Image (Scion, Frederick, MD). Laser intensity was reduced to 1% of maximum by using neutral density filters, and the number of scans per cell was kept to a minimum to avoid photobleaching.

Changes in intracellular Ca²⁺ levels were taken as the ratio of the increase in the mean pixel value of the whole cell compared with that in the control period, where the pixel values are a gray scale ranging from 0 to 255. All values of increased fluorescence were taken 60 s after the start of application of either hypoxic solution or specific channel antagonists through the perfusion system. This ratio is calculated according to the equation R = (F – Fmin)/Fmin, where R is the ratio of Ca²⁺ fluorescence change, Fmin is the mean fluorescence intensity level during the control period, and F is the mean fluorescence intensity level after 1 min of stimulation. This ratio was then multiplied by 100 to express any fluorescence changes as a percentage of the resting fluorescence level.

Drugs. Apamin extracted from bee venom, d-tubocurarine, TEA-Cl, NPPB, amphotericin, and DMEM were obtained from Sigma. Fluo 3-AM and Pluronic F-127 were supplied by Molecular Probes (Eugene, OR).

Statistics. Results are expressed as means ± SE. The effects of individual treatments, such as hypoxia, were tested by the Student’s paired t-test, and the significance of differences between treatments was tested by an ANOVA and Tukey’s post hoc test. P < 0.05 was taken as the minimum level of significance.

RESULTS

Oxygen-sensitive K⁺ currents in fetal adrenal chromaffin cells. Of the 40 adrenal chromaffin cells tested from 137- to 142-day fetal sheep, in 32 cells the outward current was suppressed significantly by acute hypoxia. The current-voltage (I-V) relationship in these cells showed a local maximum at about +40 mV, a characteristic of Ca²⁺-dependent K⁺ currents, and hypoxic suppression was significant in the region of 60–60 mV (P < 0.05), the region where the Ca²⁺-dependent current is obvious (Fig. 1A). The peak current in these cells was reduced by 36 ± 7% by hypoxia at 40 mV (n = 11). This hypoxic suppression of current was reversible on return to normoxic conditions, and these cells were classed as oxygen sensitive. In perforated-patch recording mode, similar currents were present, and hypoxia produced a similar reduction in the outward current that was reversed on return to normoxic conditions (results not shown).

Effect of hypoxia on voltage-dependent Ca²⁺ current. The reduction in the Ca²⁺-dependent K⁺ current by hypoxia could be through a direct action on the K⁺ channels or secondary to a reduced Ca²⁺ influx. The I-V relationship of Ca²⁺ current in fetal adrenal chromaffin cells was not shifted by hypoxia, and at none of the voltage steps was a significant difference seen between the Ca²⁺ currents in control and hypoxic conditions. The peak current amplitude of Ca²⁺ current observed when stepping from a holding potential of –80 to 10 mV in control conditions was −395 ± 36 pA (Fig. 1B, n = 7), which was not significantly different from the current amplitude at 10 mV of −391 ± 36 pA seen in hypoxia (n = 7).

Contribution of different types of K⁺ channels to the oxygen-sensitive current. Apamin, an inhibitor of SK channels, reduced the outward current in fetal adrenal chromaffin cells (Fig. 2A). This reduction was significant at voltages from 0 to 60 mV, and at 40 mV, 200 nM apamin reduced the current by 36 ± 8% (n = 9). Comparison of the effects of different concentrations of apamin on the K⁺ current showed that 1, 10, and 200
nM all caused significant decreases in whole cell current from 20 to 60 mV ($P < 0.05$), but at no potential were the effects of these three concentrations different from each other (Fig. 2D).

In the presence of apamin, the absolute reduction of $K^+$ currents by hypoxia was much smaller, indicating that SK channels in the adrenal chromaffin cells of fetal sheep are sensitive to oxygen concentration. Application of 200 nM apamin did not eliminate oxygen-sensitive currents in these cells completely; an oxygen-sensitive component persisted at voltages ranging from 0 to 50 mV ($P < 0.05$) (Fig. 2A). The combination of apamin and hypoxia resulted in an absolute reduction to 40 $\pm$ 7% of the control at 40 mV. This level of current suppression was less than the sum of that caused by hypoxia (36%) or apamin (36%) separately, indicating an overlap in the channels that are blocked by apamin and hypoxia. The outward current remaining in the presence of apamin was almost completely blocked by application of 1 mM TEA in the external solution. Dependence on external $Ca^{2+}$ (not shown) and complete block by relatively low concentrations of TEA were consistent with this current being mediated by BK channels. In the presence of both apamin (200 nM) and TEA (1 mM), no oxygen-sensitive current remained (Fig. 2C). These results indicate that the adrenal chromaffin cells of fetal sheep possess at least two types of $Ca^{2+}$-dependent $K^+$ channels, SK and BK channels, and that both are oxygen sensitive.

The availability of fetal sheep adrenal tissue is seasonal. Adult chromaffin cells are also capable of oxygen sensitivity once the influence of innervation has been removed (1, 10). Consequently, the adult cells have been used for the later parts of this project, with initial experiments to confirm the identity of the channels responsible for the hypoxia-evoked reduction on $K^+$ currents in fetal and adult chromaffin cells.

Apamin (1 nM) significantly reduced $Ca^{2+}$-dependent $K^+$ currents in the adult adrenal chromaffin cells in the range from 0 to 60 mV ($P < 0.05$) (Fig. 3). Hypoxia produced a reduction of a similar magnitude that was significant over the same range of membrane potentials ($P < 0.05$). The application of hypoxia and apamin together gave a larger reduction in $K^+$ currents than was seen with apamin alone across a voltage range from 0 to 40 mV ($P < 0.05$). There was overlap in the populations of channels blocked by hypoxia and apamin, because the reduction produced by the combined treatment (39 $\pm$ 6% at 30 mV) was less than the sum of the effects of hypoxia (28 $\pm$ 5%) and
apamin (27 ± 5%) alone. Thus in the adult, as in the fetus, the adrenal chromaffin cells have two classes of oxygen-sensitive K⁺ currents, with SK2 channels accounting for a significant proportion.

Measurement of reversal potential. For Koa channels to participate in initiation of the hypoxic response, closure of those channels must first depolarize the cell membrane to stimulate Ca²⁺ influx and catecholamine secretion. A ramp protocol from -120 to +60 mV over 100 ms from a holding potential of -80 mV was used to investigate the effect of hypoxia, apamin, and curare on the reversal potential (Erev) of the membrane current of the adult adrenal chromaffin cells; Erev can be used as a measure of the resting membrane potential. While the most obvious effect of all three treatments was seen at positive membrane potentials, each of them also altered Erev (Fig. 4, A–C). Erev in control conditions was -55.1 ± 3.0 mV (n = 18), and this shifted to more positive potentials during exposure to hypoxia (-46.4 ± 4.4 mV, n = 5, P < 0.05), apamin (-43.4 ± 5.1 mV, n = 5, P < 0.05), or curare (-44.7 ± 5.2 mV, n = 8, P < 0.05). These shifts in Erev indicate that SK channels contribute to the resting potential of chromaffin cells and that closure of these channels by hypoxia or either of the blocking agents can cause depolarization of the cell membrane. In contrast to apamin and curare, 1 mM TEA failed to shift the Erev of the I-V plot of the adrenal chromaffin cells (n = 4), despite the significant block of the K⁺ currents at more positive potentials (Fig. 4D).

**Fig. 2.** Contribution of apamin- and TEA-sensitive channels to oxygen sensitivity in fetal sheep adrenal chromaffin cells. A: I-V relationship in control conditions (normoxia) (n = 9), in the presence of 200 nM apamin (n = 9), and during hypoxia in the presence of apamin (apamin + hypoxia) (n = 8). B: example trace of a whole cell current evoked by a single-step pulse from -80 to +40 mV during control, in the presence of 200 nM apamin, and during hypoxia with apamin. C: I-V relationship for K⁺ current in control conditions (normoxia), in the presence of both apamin (200 nM) and TEA (1 mM) (TEA + apamin), and during hypoxia in the presence of both apamin (TEA + apamin + hypoxia) (n = 3). D: sensitivity of fetal adrenal chromaffin cells to varying apamin concentrations. I-V relationship in control conditions and in the presence of apamin at 1, 10, and 200 nM (n = 4) is shown. In all I-V plots, cells were held at -80 mV and stepped to potentials ranging from -40 to +60 mV for 200 ms.

**Fig. 3.** Contribution of apamin-sensitive channels to oxygen sensitivity in adult sheep adrenal chromaffin cells. I-V relationship in control conditions, during hypoxia, in the presence of 1 nM apamin, and during hypoxia in the presence of apamin (n = 5 for all) is shown. Cells were held at -80 mV and stepped to potentials ranging from -40 to +60 mV for 200 ms.
**Ca^{2+} imaging.** The ability of membrane depolarization subsequent to closure of SK channels to stimulate Ca^{2+} influx in sheep fetal adrenal chromaffin cells was investigated with the use of fluo 3 (Fig. 5A). Fluorescence intensity was increased by treatment with hypoxia (27 ± 4%, $n = 10; P < 0.05$) or 200 nM apamin (24 ± 2%, $n = 7; P < 0.05$). The increase in [Ca^{2+}]_{i}, as indicated by the change in fluorescence, was not sig-

![Fig. 4](image)

**Fig. 4.** Example traces of the effect of hypoxia (A), 200 nM apamin (B), 200 μM curare (C), and 1 mM TEA (D) on whole cell currents in adult chromaffin cells occurring when cells are held at −60 mV and ramped from −120 to +60 mV over 100 ms. Traces shown are the average of 10 ramps under each condition. Insets: magnification of current traces from −90 to −30 mV showing that reversal potentials ($E_{rev}$) shifted due to hypoxia, apamin, and curare but not due to TEA.

![Fig. 5](image)

**Fig. 5.** Effects of hypoxia and $K^+$ channel blockers on intracellular Ca^{2+} in fetal (A) and adult adrenal chromaffin cells (B). Fluorescence of fluo 3 in each cell, measured 1 min after the application of various treatments, was compared with fluorescence in the control normoxic period. C: effect of hypoxia, apamin, and curare on Ca^{2+} influx was blocked by application of 200 μM Cd^{2+}. Concentrations of drugs used in all graphs were 200 nM apamin, 200 μM curare, and 10 mM TEA. The number of cells used for each treatment is shown in parentheses.

*Value for this group is significantly greater than control ($P < 0.05$) but not different from other groups marked with a single asterisk.

**Value for this group is significantly different from control and from all groups marked with a single asterisk ($P < 0.05$).
significantly different between cells treated with hypoxia or apamin. Simultaneous treatment with apamin and hypoxia produced an increase in fluorescence of \(21 \pm 5\%\) (\(n = 5\)), which was not significantly different from that produced by either treatment alone.

Similar results were seen in adult chromaffin cells (Fig. 5B) with equivalent increases in fluorescence produced by hypoxia (22 \(\pm\) 4\%, \(n = 12\)), 200 nM apamin (24 \(\pm\) 5\%, \(n = 7\)), or apamin and hypoxia simultaneously (21 \(\pm\) 6\%, \(n = 5\)). The levels of fluorescence in these experimental conditions were all significantly greater than the fluorescence level in the control period (\(P < 0.05\)) but were not different from each other. Curare (200 \(\mu\)M), another blocker of SK channels, caused a fluorescence increase of 23 \(\pm\) 5\% (\(n = 7\)), whereas treatment with both curare and hypoxia caused a 26 \(\pm\) 6\% increase (\(n = 7\)). There was no significant difference among the responses to curare, hypoxia, or both of these treatments combined.

In view of the presence of an oxygen-sensitive \(Ca^{2+}\)-dependent \(K^+\) current in sheep adrenal chromaffin cells that can be blocked by TEA, as demonstrated in this study and previously (20), we investigated the possible role of these channels in initiating the hypoxic response. TEA (10 mM) applied externally to both fetal and adult cells caused no significant increase in cell fluorescence (\(n = 7\)). When TEA was applied along with apamin, the increase in cell fluorescence (43 \(\pm\) 3\%, \(n = 5\)) was significantly greater than that observed with apamin alone. Hence, it appears that although closure of TEA-sensitive channels cannot depolarize the cell membrane to induce \(Ca^{2+}\) influx, it can modulate the cell response initiated by closure of apamin-sensitive \(K^+\) channels.

To investigate the source of the increased \([Ca^{2+}]_i\) seen during acute hypoxia and upon the blockade of SK channels, we applied \(Cd^{2+}\) (200 \(\mu\)M) to the bath solution to block \(Ca^{2+}\) channels in the plasma membrane. In the presence of \(Cd^{2+}\), exposure to hypoxia, apamin, or curare did not induce a significant change in the fluorescence level, which indicates that normally these treatments stimulate \(Ca^{2+}\) entry from the extracellular space but do not cause \(Ca^{2+}\) release from the intracellular \(Ca^{2+}\) stores (Fig. 5C).

**DISCUSSION**

The ability of the fetal adrenal medulla to secrete catecholamines in response to hypoxemia implies that the adrenal chromaffin cells possess an oxygen sensor. In the glomus cells of the carotid body, a major chemoreceptor in adult mammals, the role of the oxygen sensor is played by \(K_O2\) channels that close in hypoxic conditions. It is well established that closure of \(K_O2\) channels in glomus cells causes depolarization of the cell membrane, resulting in \(Ca^{2+}\) influx into the cytosol and exocytosis of neurotransmitters (reviewed in Ref. 19). There are a number of different types of \(K^+\) channels in glomus cells of different species that are inhibited by hypoxia. Moreover, a certain degree of variation exists even between different preparations within the same species. For example, in isolated glomus cells of the rat, closure of TASK-1-like \(K^+\) channels by hypoxia was found to be responsible for the cell depolarization, and 10 mM TEA was ineffective in mimicking the hypoxic response (3). In thin slices of rat carotid body, however, a \(Ca^{2+}\)-dependent BK channel was implicated as an oxygen sensor, and, in contrast to the previous study, application of 5 mM TEA depolarized the cell membrane and caused release of catecholamines (16). In the rabbit carotid body, it has been suggested that closure of human ether-à-go-go-related gene (HERG)-like channels that regulate resting potential may be responsible for the initial depolarization (19) and that an oxygen-sensitive transient \(K^+\) current modulates the response (7, 15). In PC-12 cells, derived from a tumor of the rat adrenal medulla but often used as a model of the glomus cell, 1 mM TEA stimulated the secretion of catecholamines apparently through membrane depolarization due to block of \(K^+\) channels (23). The \(K_O2\) channel in this cell type has been identified as Kv1.2 from the Shaker subfamily of voltage-gated \(K^+\) channels (6).

The carotid body and the adrenal medulla share a common embryological origin in the neural crest, and, not surprisingly, \(K_O2\) channels have been proposed to act as the oxygen sensor in chromaffin cells of the adrenal medulla of the rat (24) and sheep (20). Previous work in this laboratory (20) has shown that the adrenal chromaffin cells of the fetal sheep possess \(K_O2\) channels, and application of 5 mM \(Co^{2+}\) revealed that all of this oxygen-sensitive current is \(Ca^{2+}\)-dependent. \(Ca^{2+}\)-dependent \(K_O2\) channels, BK and SK, were found in rat adrenal chromaffin cells. Thompson and Nurse (25) reported that in neonatal rat chromaffin cells, BK channels account for the major portion of the hypoxic suppression of outward current; however, block of BK channels by iberiotoxin failed to induce membrane depolarization and did not affect the depolarization caused by hypoxia. Studies on the adult rat chromaffin cells implicated SK channels as the oxygen sensor (10). Apamin, a selective inhibitor of SK channels, blocked all of the oxygen-sensitive \(K^+\) current in these cells and caused depolarization of the cell membrane. The effects of hypoxia and apamin were not additive.

In the present work, we have found that oxygen-sensitive BK and SK channels are both present in the adult and fetal sheep adrenal chromaffin cells. In the presence of high concentrations of apamin (200 nM) that would block all SK channels, some oxygen-sensitive current remained, indicating that SK are not the only \(K_O2\) channels in these cells. The fact that this remaining oxygen-sensitive current was \(Ca^{2+}\)-dependent and was completely blocked by application of 1 mM TEA indicates that the channel mediating this current is the BK channel. Complete block of all oxygen-sensitive currents by simultaneous application of apamin and TEA strongly suggests that SK and BK channels account for all of the oxygen-sensitive \(K^+\) current in sheep adrenal chromaffin cells.

Of the two subtypes of apamin-sensitive SK channels, SK2 has a higher affinity for apamin (IC\(_{50}\) = 60
pM) than does SK3 (IC_{50} = 1 nM) (8, 9). In the study on the adult rat adrenal chromaffin cells in which the presence of an oxygen-sensitive SK current was reported (10), the very high concentrations of apamin (400 nM) used did not allow identification of the particular channel subtype involved. In the fetal sheep adrenal chromaffin cells, 1 nM apamin produced a maximal effect, indicating that SK2 is most likely to be responsible for the apamin-sensitive current. This conclusion is supported by the analysis of mRNA in rat adrenal chromaffin cells where the mRNA for SK2, but not for SK3, has been found (9).

Oxygen sensitivity of the K^+ currents by itself does not prove that they are involved in initiating the hypoxic response; for hypoxia to stimulate catecholamine secretion, closure of K_Ca channels must depolarize the cells and open voltage-dependent Ca^{2+} channels. In adult sheep chromaffin cells, hypoxia caused a significant shift in E_{vrev} to more positive potentials. Similar results were seen with the application of either apamin or curare, indicating that the closure of SK channels is capable of depolarizing the cell membrane and initiating the hypoxic response. In contrast, TEA did not shift membrane potential of the sheep adrenal chromaffin cell, suggesting that BK channels are closed at resting potential in these cells and are not involved in the membrane depolarization caused by hypoxia.

These results were supported by measurements of [Ca^{2+}]_i in both the fetal and adult adrenal chromaffin cells. Because the increase in [Ca^{2+}]_i produced by the SK blockers apamin and curare was the same as that produced by hypoxia, and because the effects on [Ca^{2+}]_i of either of these blockers in conjunction with hypoxia were not additive, closure of SK channels alone could explain the change in membrane potential resulting from hypoxia. Although TEA itself did not induce Ca^{2+} influx, the combination of TEA and apamin resulted in a much greater increase in [Ca^{2+}]_i than that seen when apamin was applied alone. This observation, presumably, was a result of the prolongation of action potential duration when TEA-sensitive channels were blocked. This has been seen to occur in dorsal vagal neurones of the rat where blockade of SK channels causes increased action potential firing frequency, whereas BK channel block results in increased action potential duration (17).

The reduction in Ca^{2+}-dependent K^+ current during exposure to hypoxic conditions could result from a hypoxia-evoked decline in Ca^{2+} influx, while on the other hand, direct increase of the Ca^{2+} currents by hypoxia would explain catecholamine release. Although there is some evidence from the carotid body for a reduction in voltage-dependent Ca^{2+} currents as a result of hypoxia (12), more recent work has shown an increase in such currents in this tissue (22). In the present experiments, voltage-dependent Ca^{2+} currents were not affected by hypoxia, suggesting that the reduction in Ca^{2+}-dependent K^+ current was not secondary to a reduced [Ca^{2+}]_i. At the same time, this observation ruled out the possibility that the hypoxic response in these cells is initiated by the increase of the amplitude of Ca^{2+} currents, the leftward shift, or their voltage dependence.

In summary, our results show that the direct response to hypoxia in chromaffin cells isolated from sheep adrenal medulla, characterized by membrane depolarization and a subsequent influx of Ca^{2+} through voltage-dependent Ca^{2+} channels, is initiated by closure of SK2 channels. BK channels in these cells are also oxygen sensitive, but their likely role is to modulate the action potentials that result from closure of SK2 channels.

Involvement of K_Ca channels in depolarization of cell membrane due to hypoxia seems paradoxical, because increase in Ca^{2+} influx should activate more K_Ca channels and cause cell hyperpolarization. However, these events are separated in time, with membrane depolarization, Ca^{2+} influx, and catecholamine release happening first; a subsequent increase in Ca^{2+}-dependent K^+ currents due to an increase in (Ca^{2+})_i may provide an important negative feedback mechanism that protects chromaffin cells from Ca^{2+} overload in long periods of hypoxia.

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