Mediation by Intracellular Calcium-Dependent Signals of Hypoxic Hyperpolarization in Rat Hippocampal CA1 Neurons In Vitro

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INTRODUCTION

Short-term (2–4 min) oxygen deprivation induces a hyperpolarization (hypoxic hyperpolarization) in rat hippocampal CA1 neurons (Fujiwara et al. 1987; Leblond and Krnjević 1989). There is general agreement that the hypoxic hyperpolarization is most likely mediated by an enhanced potassium conductance ($G_K$) (Fujiwara et al. 1987; Hansen et al. 1982; Leblond and Krnjević 1989). The precise nature of the $G_K$ activation has not yet been elucidated. Two major contending hypotheses ascribe the hyperpolarization to either activation of an ATP-dependent potassium conductance ($G_{KATP}$) (Fujimura et al. 1997) or to elevation of the calcium-dependent potassium conductance ($G_{KCa}$) (Leblond and Krnjević 1989). Because anoxia or hypoxia sooner or later leads to both a rise in intracellular calcium concentration ([Ca$^{2+}$]$_i$) (Dubinsky and Rothman 1991; Duchen 1990; Kass and Lipton 1982; Siesjo 1981) and a reduction in cytosolic ATP ([ATP]$_c$) (Lipton and Whittingham 1982; Siesjo 1981), there is, as pointed out by Zhang and Krnjević (1993), no a priori reason for considering one rather the other as the primary signal.

The calcium hypothesis is favored by the depression of the hyperpolarization (or corresponding outward current during voltage clamp) by blockers of Ca$^{2+}$ release from internal stores, such as dantrolene, heparin, thapsigargin, ruthenium red, and procaine (Belousov et al. 1995; Krnjević and Xu 1989). Nevertheless, the effects of intracellular Ca$^{2+}$ chelators are variable and inconclusive. (cf. Fujiwara et al. 1987; Leblond and Krnjević 1989).

Thus the main aim of this study was to investigate the involvement of activation of Ca$^{2+}$-dependent K$^+$ channels in hypoxic hyperpolarization. Attempts were made to examine whether or not hypoxic hyperpolarization is dependent on external Ca$^{2+}$ concentrations and able to be depressed by either intracellular Ca$^{2+}$ chelators or inhibitors of release from intracellular Ca$^{2+}$ stores. In addition, the intracellular Ca$^{2+}$-dependent signal transduction systems contributing to the generation of hypoxic hyperpolarization were also examined.

METHODS

The methods have been previously described (Fujimura et al. 1997). Briefly, rats were killed under deep ether anesthesia by severing the great vessels of the chest. The brain was removed and a block of tissue that contained the hippocampus was sectioned with a Vibratome (400 μm). The tissue slice was submerged in a flowing (6–8 ml/min) physiological saline that contained (in mM) 117 NaCl, 3.6 KCl, 2.5 CaCl$_2$, 1.2 MgCl$_2$, 1.2 Na$_2$HPO$_4$, 25 NaHCO$_3$, and 11 glucose, saturated with 95% O$_2$-5% CO$_2$, preheated to 36 ± 0.5°C. Intracellular recordings were made from the pyramidal layer in the hippocampal CA1 region with the use of electrodes that contained potassium chloride (2 M), with electrode resistances of 60–90 MΩ. The membrane potential was determined
by the baseline level recorded from an X-Y recorder with low-pass filter.

Slice preparations were made "hypoxic" by superfusing medium equilibrated with 95% N₂-5% CO₂ (hypoxic medium). When switching the superfusing medium, there was a delay of 15–20 s before the new medium reached the chamber, because of the volume of the connecting tubing.

Drugs used were tolbutamide (Research Biochemicals International), procaine (Sigma), N-[(6-amino-hexyl]-5-chloro-1-naphthalenesulfonylamine hydrochloride (W-7), N-[(6-aminoxyethyl]-1-naphthalenesulfonylaminde hydrochloride (W-5), 1-[N-O-bis(1,5-isoquinolinesulfonyl) N-methyl-1-tyrosyl]-4-phenyl-piperazine (KN-62), 1-(5-iodonaphthalene-1-sulfonyl)-1H-hexa-hydro-1,4-diazepine hydrochloride (ML-7), N-[2-(p-bromocinnamyl-ami-no)ethyl]-5-isoquinolinesulfonamide (H-89, Seikagaku Kogyo), bis-(o-aminophenoxy)-N,N,N′,N′-tetraacetic acid (BAPTA)-AM (Dojin), and ryanodine (Calbiochem).

All quantitative results are expressed as means ± SD. The number of neurons examined is given in parentheses. The unpaired Student’s t-test was used to compare data, with P<0.05 considered significant unless specified otherwise.

RESULTS

This study was based on recordings from 100 CA1 pyramidal neurons of adult rats with stable membrane potentials more negative than –60 mV. The resting membrane potential and the apparent input resistance were –69.3 ± 2.7 mV and 51.4 ± 8.2 MΩ (n = 100), respectively. In this study, unless specified otherwise, the membrane potential was depolarized to –60 mV before hypoxic exposure by passing depolarizing DC current through the recording electrode.

**Ca²⁺-dependent K⁺ conductance is involved in the hypoxic hyperpolarization**

Brief applications of hypoxic media (2–4 min) induced hyperpolarizations with a mean amplitude of 10 ± 4 mV (n = 70), which were accompanied by a reduction in the apparent input resistance to 56 ± 12% (n = 70) of the prehypoxic control. Readmittance of oxygen produced a transient hypoxic hyperpolarization and then restored the membrane potential to the prehypoxic level. To analyze the conductance change of the hypoxic hyperpolarization, hyperpolarizing and subsequent depolarizing ramp command currents were applied before and during the hyperpolarization (Fig. 1A).

The resultant steady-state current-voltage curves showed that the membrane conductance during the hypoxic hyperpolarization was increased compared with that of the control (Fig. 1B), and that the net outward current induced by hypoxia had an outward rectification when the membrane potential was made more positive (Fig. 1B, inset). The net outward current was markedly depressed by tolbutamide (100 µM) (Fig. 1C, inset). In the presence of tolbutamide (100 µM), Ca²⁺ (0.25 mM), Mg²⁺ (8 mM), and Co²⁺ (2 mM), exposure to hypoxic medium shifted the membrane potential from –60 mV to approximately –40 mV and markedly decreased the slope conductance (n = 4, Fig. 1D). The net inward current was reduced at hyperpolarizing membrane potentials, but its polarity was not reversed at membrane potentials between –50 and –95 mV (Fig. 1D, inset). These results suggest that hypoxic hyperpolarization is mediated by activation of both ATP-sensitive K⁺ (K₅ATP) channels and Ca²⁺-dependent K⁺ channels.

**Effects of extracellular Ca²⁺ concentration on the hypoxic hyperpolarization**

To investigate the contribution of Ca²⁺-dependent K⁺ channels to the hypoxic hyperpolarization, the extracellular Ca²⁺ concentration ([Ca²⁺]₀) was varied. Superfusion with low-Ca²⁺ (0.25 or 1.25 mM) medium produced a depolarization –5–10 mV in amplitude, but the apparent input resistance was not significantly changed (n = 8). In 0.25 and 1.25 mM-Ca²⁺ media, the amplitude of the hypoxic hyperpolarization was significantly reduced to 5.2 ± 2 mV (n = 4, P < 0.01) and 5.8 ± 2 mV (n = 4, P < 0.01), respectively (Fig. 2, A and B). Superfusion with high-Ca²⁺ (5.0 or 7.5 mM) medium produced a hyperpolarization –2–4 mV in amplitude (n = 10). The apparent input resistance was significantly reduced in Ca²⁺ (7.5 mM) containing medium but not significantly changed in 5.0 mM-Ca²⁺-containing medium. The apparent input resistance was decreased from 48.7 ± 4.6 MΩ in 2.5 mM-Ca²⁺-containing medium to 44.7 ± 2.8 MΩ in 7.5 mM-Ca²⁺-containing medium (n = 5, P < 0.01). In 5.0 and 7.5 mM-Ca²⁺ media, the amplitude of the hypoxic hyperpolarization was reduced to 4.8 ± 1 mV (n = 5, P < 0.01) and 1.6 ± 1 mV (n = 5, P < 0.01), respectively (Fig. 2B). These results indicate that there is an optimal [Ca²⁺], required to produce the hypoxic hyperpolarization.

**Effects of intracellular Ca²⁺ on the hypoxic hyperpolarization**

To investigate whether internal Ca²⁺ may be important in the generation of the hypoxic hyperpolarization, the effects of a Ca²⁺ chelator and an inhibitor of Ca²⁺ release from intracellular stores on the hypoxic hyperpolarization were examined. The membrane-permeable Ca²⁺ chelator BAPTA-AM (50–100 µM, Fig. 3A), or the ryanodine receptor agonist ryanodine (10 µM), reduced the peak amplitude of the hypoxic hyperpolarization without affecting the posthypoxic hyperpolarization, whereas the inhibitor of Ca²⁺ release from intracellular stores, procaine (300 µM), reversibly depressed both the hypoxic and posthypoxic hyperpolarizations (Fig. 3B). Table 1 summarizes effects of these drugs on the relative amplitude of the hypoxic hyperpolarization and the reduction of neuronal input resistance during hypoxic hyperpolarization. BAPTA-AM, procaine, and ryanodine significantly reduced the peak amplitude of the hypoxic hyperpolarization and reversed the ratio of the reduction of the neuronal input resistance during the hypoxic hyperpolarization.

**Contribution of intracellular signal transduction systems in the generation of hypoxic hyperpolarization**

To investigate the role of intracellular Ca²⁺-dependent signal transduction systems, the effects of antagonists for the protein kinases and calmodulin (CaM) were examined. The CaM antagonist W-7 (50 µM) completely blocked hypoxic hyperpolarizations and reversed their polarity; the membrane was depolarized during exposure to the hypoxic me-
FIG. 1. ATP-sensitive K⁺ (K<sub>ATP</sub>) channel blockers and a reduction in external Ca<sup>2+</sup> depress the net outward current produced by hypoxia in hippocampal CA1 neurons. In this and subsequent figures, hypoxic medium was applied between the downward and upward arrows and, in each trace, the dotted line indicates the preexposure level of the membrane potential, unless specified otherwise. A: pairs of traces show current (top) and potential (bottom) recordings under current-clamp condition. Slow hyperpolarizing and depolarizing DC ramp currents (1–2 mV/s) were passed through the recording electrode to obtain steady-state current-voltage relationships before and during hypoxic exposure in the control condition (Control). B–D: steady-state current-voltage curves were obtained before (Control) and during hypoxic exposure (Hypoxia) in the control condition (B); in the presence of tolbutamide (100 µM) (C); and in the presence of tolbutamide (100 µM), low Ca<sup>2+</sup> (0.25 mM), high Mg<sup>2+</sup> (8 mM), and Co<sup>2+</sup> (2 mM) (D). Inset: net outward current produced by hypoxia, which were obtained by subtraction of the steady-state current-voltage relation at preexposure level from that during hypoxic exposure. For subtraction, steady-state current-voltage relations, which were obtained by passing ramp currents through the recording electrode (Fig. 5), were used. The net outward current induced by hypoxia in the control condition (B, inset) was markedly depressed by tolbutamide (C, inset), and the response to hypoxia was reversed in polarity, and a net inward current was obtained, in tolbutamide (100 µM), low Ca<sup>2+</sup> (0.25 mM), high Mg<sup>2+</sup> (8 mM), and Co<sup>2+</sup> (2 mM) medium (D, inset). A–D: same neuron.
Ca^{2+}\text{ dependency of the hypoxic hyperpolarization

In the presence of the K_{ATP} channel blocker tolbutamide (100 \mu M), hypoxic hyperpolarizations were markedly depressed, whereas in the presence of Krebs solution containing low Ca^{2+} and Co^{2+} and tolbutamide, the hyperpolarizations were not only completely suppressed, but the polarity of the response was also reversed, causing a membrane depolarization. These results suggest that both K_{ATP} and Ca^{2+}-dependent K^{+} conductances contribute to the generation of the hypoxic hyperpolarization. Fujiwara et al. (1987) have shown that electrogenic Na^{+}-K^{+} pump activity is depressed during hypoxia. It is therefore possible that the hypoxic depolarization in tolbutamide and low-Ca^{2+}-containing medium may be due to suppression of electrogenic Na^{+}-K^{+} pump activity.

If the Ca^{2+}-dependent K^{+} conductance were only activated by Ca^{2+} influx from the extracellular environment, the hypoxic hyperpolarization should be increased in high-Ca^{2+} media. As described above, the optimal [Ca^{2+}]_{o} for generation of the hypoxic hyperpolarization was 2.5 mM, a finding that suggests that the hypoxic hyperpolarization may not be simply dependent on Ca^{2+} influx, but may also involve other mechanisms for the regulation of [Ca^{2+}], such as intracellular Ca^{2+}-dependent signal transduction systems. The BAPTA-AM-reduced amplitude of the hypoxic hyperpolarization suggests that intracellular BAPTA may chelate Ca^{2+} that had flowed into the impaled neuron from the extracellular space and/or been extruded from intracellular stores. This reduction is comparable with the inhibitory effect of the Ca^{2+} chelator ethylene glycol-bis(\beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) on the hypoxic hyperpolarization in the majority of hippocampal CA1 neurons (Leblond and Krnjevic 1989).

FIG. 2. Effects of various extracellular Ca^{2+} concentrations ([Ca^{2+}]_{o}) on the hypoxic hyperpolarization. Downward deflections in voltage recordings under current-clamp mode are hyperpolarizing electrotonic potentials elicited by anodal current pulses (0.5 nA applied for 200 ms every 3 s). A: hypoxic hyperpolarization in control medium (2.5 mM Ca^{2+}) (top trace), after pretreatment with low-Ca^{2+} (1.25 mM) medium for 10 min (middle trace), and after washing out the low-Ca^{2+} medium for 10 min (bottom trace). B: changes in the amplitude of the hypoxic hyperpolarization at various [Ca^{2+}]_{o}. The amplitude in various [Ca^{2+}]_{o} was normalized with that of the control medium (2.5 mM Ca^{2+}). Error bars: mean ± SD. Note that a rise or fall in [Ca^{2+}]_{o} decreased the amplitude of the hypoxic hyperpolarization.

FIG. 3. Effects of a Ca^{2+} chelator, intracellular Ca^{2+} release inhibitors, and calmodulin inhibitors on the hypoxic hyperpolarization. Downward deflections in voltage recordings under current-clamp mode are hyperpolarizing electrotonic potentials elicited by anodal current pulses (in the range of 0.2–0.4 nA, applied for 200 ms every 3 s). A: hypoxic hyperpolarization before treatment (top trace); after pretreatment with 50 \mu M (2nd trace), 75 \mu M (3rd trace), and 100 \mu M (4th trace) bis-(\beta-aminophenoxy)-N,N,N',N'-tetraacetic acid (BAPTA)-AM for 10 min; and after washing out of the drug for 30 min (bottom trace). B: hypoxic hyperpolarization before treatment (top trace), after pretreatment with procaine (300 \mu M) for 10 min (middle trace), and after washing out of the drug for 10 min (bottom trace). C: hypoxic hyperpolarization before treatment (top trace), after pretreatment with W-7 (50 \mu M) for 10 min (middle trace), and after washing out of the drug for 30 min (bottom trace).
Intracellular signal transduction systems involved in the generation of the hypoxic hyperpolarization

The present study shows that the CaM inhibitors W-7 and trifluoperazine depressed the hypoxic hyperpolarization. Involvement of CaM in the generation of the hypoxic hyperpolarization therefore seems likely. W-7 reversed the polarity of the response to hypoxia; that is to say, it caused a hypoxic depolarization that was associated with a fall in the apparent input resistance. W-7 is an inhibitor of CaM, phosphodiesterase, and myosin light chain kinase. The value of the half-maximum inhibition for these enzyme activities is \( \sim 30-50 \mu M \) (Cafoules et al. 1982; Hidaka et al. 1981).

Neither the protein kinase A inhibitor H-89 nor the myosin light chain kinase inhibitor ML-7 affected the hypoxic hyperpolarization, suggesting that the involvement, if any, of phosphodiesterase or myosin light chain kinase in the hypoxic hyperpolarization is minimal. It is therefore likely that the hypoxic depolarization in W-7-containing medium is due to inactivation of the electrogenic \( \text{Na}^+ - \text{K}^+ \) pump and the concomitant accumulation of intracellular \( \text{K}^+ \).

CaM activates various protein kinases, including myosin light chain kinase, phosphorylase kinase, and Ca\(^{2+}\)/CaM-dependent protein kinases I, II, and III (CaM kinase I, II, III). Immunohistochemical studies show that CaM kinase II is quite plentiful in the hippocampus (Erondu and Kennedy 1985; Ouisse et al. 1984). The selective inhibitor for CaM kinase II, KN-62, depressed the hypoxic hyperpolarization. This suggests that the increased internal Ca\(^{2+}\) binds to CaM and the Ca\(^{2+}\)/CaM complex activates CaM kinase II, which may phosphorylate and open K\(^+\) channels. CaM kinase II can be phosphorylated at low concentrations (3-500 \( \mu M \)) of [ATP], (Lai et al. 1986; Miller and Kennedy 1986). In guinea pig hippocampal neurons in vitro, the [ATP], could be >1 mM, and the [P], is decreased by \( \sim 15-2 \) min after exposure to hypoxic medium (Lipton and Whittingham 1982). Thus the remaining [ATP], during hypoxia would be enough to sustain CaM kinase II phosphorylation of potassium channels.

**TABLE 1. Effects of BAPTA-AM, procaine, and ryanodine on the relative amplitude of hypoxic hyperpolarization and the reduction in apparent input resistance during the hypoxic hyperpolarization**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>50 ( \mu M )</th>
<th>75 ( \mu M )</th>
<th>100 ( \mu M )</th>
<th>300 ( \mu M )</th>
<th>10 ( \mu M )</th>
</tr>
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<tr>
<td><strong>Amplitude of hypoxic hyperpolarization, mV</strong></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Control</td>
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<tr>
<td>BAPTA-AM</td>
<td>6.8 ± 1.8 (25)</td>
<td>5.6 ± 1.0 (5)*</td>
<td>3.9 ± 1.0 (5)†</td>
<td>2.6 ± 0.5 (5)†</td>
<td>3.1 ± 0.5 (5)†</td>
<td>4.7 ± 0.8 (5)†</td>
</tr>
<tr>
<td>Procaine</td>
<td>46 ± 2 (25)</td>
<td>65 ± 2 (5)†</td>
<td>71 ± 1 (5)†</td>
<td>74 ± 1 (5)†</td>
<td>99 ± 1 (5)†</td>
<td>92 ± 3 (5)†</td>
</tr>
<tr>
<td>Ryanodine</td>
<td>1.8 (33)</td>
<td>3 (33)</td>
<td>2 (5)</td>
<td>2 (5)</td>
<td>2 (5)</td>
<td>2 (5)</td>
</tr>
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</table>

Values are means ± SD, with values of \( n \) in parentheses. Bottom row: ratio of reduction in apparent input resistance during the hypoxic hyperpolarization.

**TABLE 2. Effects of antagonists for the Ca\(^{2+}\)-dependent protein kinases and calmodulin on the relative amplitude of hypoxic hyperpolarization and the reduction in apparent input resistance during the hypoxic hyperpolarization**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>W-7, 50 ( \mu M )</th>
<th>Trifluoperazine, 50 ( \mu M )</th>
<th>W-5, 50 ( \mu M )</th>
<th>KN-62, 10 ( \mu M )</th>
<th>ML-7, 10 ( \mu M )</th>
<th>H-89, 1 ( \mu M )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amplitude of hypoxic hyperpolarization, mV</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>8.1 ± 1.8 (33)</td>
<td>None*</td>
<td>1.8 ± 0.6 (5)†</td>
<td>8.0 ± 1.6 (5)</td>
<td>4.9 ± 1.8 (6)†</td>
<td>7.9 ± 2.0 (5)</td>
<td>8.1 ± 1.9 (7)</td>
</tr>
<tr>
<td>W-7, 50 ( \mu M )</td>
<td>50 ( \mu M )</td>
<td>1.8 ± 0.6 (5)†</td>
<td>8.0 ± 1.6 (5)</td>
<td>4.9 ± 1.8 (6)†</td>
<td>7.9 ± 2.0 (5)</td>
<td>8.1 ± 1.9 (7)</td>
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<tr>
<td>W-5, 50 ( \mu M )</td>
<td>50 ( \mu M )</td>
<td>1.8 ± 0.6 (5)†</td>
<td>8.0 ± 1.6 (5)</td>
<td>4.9 ± 1.8 (6)†</td>
<td>7.9 ± 2.0 (5)</td>
<td>8.1 ± 1.9 (7)</td>
<td></td>
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<tr>
<td>KN-62, 10 ( \mu M )</td>
<td>50 ( \mu M )</td>
<td>1.8 ± 0.6 (5)†</td>
<td>8.0 ± 1.6 (5)</td>
<td>4.9 ± 1.8 (6)†</td>
<td>7.9 ± 2.0 (5)</td>
<td>8.1 ± 1.9 (7)</td>
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<tr>
<td>ML-7, 10 ( \mu M )</td>
<td>50 ( \mu M )</td>
<td>1.8 ± 0.6 (5)†</td>
<td>8.0 ± 1.6 (5)</td>
<td>4.9 ± 1.8 (6)†</td>
<td>7.9 ± 2.0 (5)</td>
<td>8.1 ± 1.9 (7)</td>
<td></td>
</tr>
<tr>
<td>H-89, 1 ( \mu M )</td>
<td>50 ( \mu M )</td>
<td>1.8 ± 0.6 (5)†</td>
<td>8.0 ± 1.6 (5)</td>
<td>4.9 ± 1.8 (6)†</td>
<td>7.9 ± 2.0 (5)</td>
<td>8.1 ± 1.9 (7)</td>
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</table>

Values are means ± SD, with values of \( n \) shown in parentheses. Bottom row: ratio of reduction in apparent input resistance during the hypoxic hyperpolarization.
Ca\textsuperscript{2+} SIGNAL TRANSDUCTION AND HYPOXIC HYPERPOLARIZATION

sensitive hypoxic hyperpolarization, Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channels partially contribute to the generation of the hypoxic hyperpolarization. Thus the ratio for the contribution of Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channels and K\textsubscript{ATP} channels to the hypoxic hyperpolarization is different from neuron to neuron. Two major mechanisms underlying the hypoxic hyperpolarization have been proposed in hippocampal neurons; activation of Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channels via a rise in [Ca\textsuperscript{2+}], (Belousov et al. 1995; Katchman and Hershkowitz 1993; Krnjević and Xu 1989; Leblond and Krnjević 1989) and activation of K\textsubscript{ATP} channels by depletion of [ATP], (Godfraind and Krnjević 1993; Grigg and Anderson 1989). Because hypoxia leads to a significant depletion of [ATP], as well as a rise of [Ca\textsuperscript{2+}], (Biscoe et al. 1988; Hansen 1985; Higashi et al. 1990; Nishimura 1986; Siesjö 1978), both effects seem equally likely, and both could contribute toward the outward K\textsuperscript{+} currents. An important consideration is changes in the proportions of K\textsubscript{ATP} channels and Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channels due to aging of experimental animals, because the density of K\textsubscript{ATP} channels has been shown to increase with age (Xia et al. 1993).

The present finding that BAPTA-AM depressed the hypoxic hyperpolarization is comparable with the previous report that EGTA depressed the hypoxic hyperpolarization in the majority of hippocampal CA1 neurons (Leblond and Krnjević 1989). In our previous study, however, we found that intracellular EGTA injection did not affect the hypoxic hyperpolarization, but markedly depressed a slow afterhyperpolarization following spikes in hippocampal CA1 neurons (Fujiwara et al. 1987). It is possible that the amount of injected EGTA was not enough to chelate the increased cytosolic Ca\textsuperscript{2+} that activates the Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channel, because the chelating action of BAPTA is more potent than that of EGTA (Tsien 1981). Alternatively, the activation of the K\textsubscript{ATP} channel may be predominant in the EGTA-insensitive neurons.

A protein kinase A inhibitor decreases K\textsubscript{ATP} Channel activity in pancreatic β-cells (Ribalet et al. 1989). It has been reported that activity of cloned fly Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channels is augmented by phosphorylation of an A kinaselike protein (Esguerra et al. 1994). In rat cortical neurons, ATP, forskolin, and dibutyryl adenosine 3',5'-cyclic monophosphate stimulated Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channel activity,
whereas H-7, a peptide inhibitor of protein kinase A, diminished the activity (Lee et al. 1995). These results suggest that the activation of Ca\(^{2+}\)-dependent K\(^+\) channels would be augmented by phosphorylation of A kinase. In contrast, our findings that the A kinase inhibitor H-89 did not affect the hypoxic hyperpolarization suggest that the involvement, if any, of an A kinase-mediated phosphorylation in the generation of the hypoxic hyperpolarization is minimal.

In conclusion, the present study indicates that the hypoxic hyperpolarization is due to activation of both K\(_{ATP}\) channels and Ca\(^{2+}\)-dependent K\(^+\) channels. The Ca\(^{2+}\)-dependent K\(^+\) channels may be activated by the increased [Ca\(^{2+}\)], that results from the Ca\(^{2+}\)-induced Ca\(^{2+}\) release from intracellular stores during exposure to hypoxic medium. Furthermore, activation of CaM and Ca\(^{2+}\)/CaM kinase II may be involved in the activation of Ca\(^{2+}\)-dependent K\(^+\) channels. Study of the isolated individual currents is, however, required to further elucidate the pharmacological characteristics of the channels involved.

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