J. Physiol. (1987), **384**, *pp.* 131–151
With 9 text-figures
Printed in Great Britain

EFFECTS OF HYPOXIA ON RAT HIPPOCAMPAL NEURONES *IN VITRO*

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(*Received 24 February 1986*)

SUMMARY

1. The effects of hypoxia on the rat hippocampal CA1 neurones in tissue slices of the rat brain were studied *in vitro* by intracellular recording.

2. In response to superfusion of a hypoxic medium equilibrated with 95 % N_2–5 % CO_2, a majority of the neurones showed a hyperpolarization of 5–15 mV in amplitude and 4–12 min in duration. The hyperpolarization was, in turn, followed by a slow depolarization which within 20 min of hypoxic exposure reached a plateau level of about 25 mV above the pre-hypoxic resting potential. Both the initial hyperpolarization and subsequent depolarization were associated with a reduction in membrane resistance.

3. The hyperpolarization reversed in polarity at a membrane potential of −83 mV. There was an almost linear relationship between amplitude of the hyperpolarization and membrane potential. The hyperpolarization was markedly enhanced in potassium-free media and was depressed in high-potassium solutions.

4. The hyperpolarization was not significantly affected by low-chloride or low-sodium medium or by solution containing tetraethylammonium (10 mM), 4-aminopyridine (1·5 mM) or caesium (3 mM). Moreover, intracellular injection of ethyleneglycol-bis-(β-aminoethylether)*N*,*N*-tetraacetic acid (EGTA) did not alter the hyperpolarization. On the other hand, barium (0·5 mM)-containing medium reduced the amplitude of the hyperpolarization by 20–40 %.

5. Superfusion of ouabain (5–7 μM)-containing medium in normoxic conditions produced hyperpolarizing and depolarizing responses similar to those elicited by hypoxic exposure. The slow depolarization was also mimicked by elevation of the extracellular potassium concentration to 10–20 mM.

6. Evoked i.p.s.p.s were abolished within 4 min of hypoxic exposure while evoked e.p.s.p.s were maintained for about 20 min of hypoxic superfusion. Soma spikes of the neurones elicited by a depolarizing pulse were also well preserved. Their threshold was, however, raised, concomitant with a decrease in the peak amplitude.

7. When the slice was reoxygenated after 20–40 min of hypoxic exposure, the neurones immediately began to repolarize and showed a transient hyperpolarization of 5–10 mV in amplitude and 1–2 min in duration. The membrane potential, input

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resistance and action potential returned to the pre-hypoxic levels after 15–20 min of reoxygenation. The amplitude of the reoxygenation-induced hyperpolarization was not significantly changed when the membrane was hyperpolarized or depolarized. The hyperpolarization was eliminated by potassium-free medium or solution containing ouabain (1 μM).

8. In a minority of the neurones the slow depolarization was suddenly followed by a rapid depolarization, after which the neurones showed no functional recovery. Such an abrupt and irreversible depolarization appeared when the slow depolarization reached membrane potentials of -30 to -40 mV.

9. The results suggest that hypoxia-induced hyperpolarization is due to an increase in voltage-independent potassium conductances, that the subsequent depolarization results from an elevation of extracellular potassium concentration brought about by inactivation of the sodium pump, and that the transient hyperpolarization following reoxygenation is caused by reactivation of the pump.

INTRODUCTION

The electrical activity of neurones in the mammalian central nervous system (C.N.S.) is highly sensitive to hypoxic conditions, i.e. electroencephalographic activity attenuates after 10–30 s and cerebral evoked responses are depressed within 1–3 min of hypoxia (cf. Hansen, 1985). Little is known of the mechanism underlying these depressant effects of oxygen lack. It has been reported that in rat hippocampal neurones of *in vitro* slice preparations, hypoxia produces a hyperpolarization associated with a decrease in membrane resistance, which may be involved in the reduction of neurone excitability (Hansen, Hounsgaard & Jahnsen, 1982). On the other hand, membrane depolarization, rather than hyperpolarization, has been suggested as being responsible for the failure of evoked potentials during hypoxia in guinea-pig hippocampal slices (Lipton & Whittingham, 1979). The ionic basis and mechanism of generation of these responses are not known in detail, although it is likely that the hyperpolarization observed at an early stage of hypoxia is due to activation of potassium channels (Hansen *et al.* 1982). In this paper we have characterized some properties of hypoxia-induced potential changes and transmission failure of CA1 neurones in the rat hippocampal slice. The important findings were that in response to superfusion of a hypoxic medium equilibrated with 95 % N_2–5 % CO_2 for 20–40 min, a majority of the neurones showed a transient hyperpolarization followed by a slow depolarization which reached a plateau level of about 25 mV above the pre-hypoxic resting potential, and that evoked e.p.s.p.s were well preserved during hypoxic exposure. These results suggest that low oxygen tension *per se* may not be the major cause of neuronal dysfunction due to hypoxic hypoxia or ischemia.

A preliminary report has been presented elsewhere (Higashi, Yoshimura, Fujiwara & Shimoji, 1986).

METHODS

Male Wistar rats about 12 weeks old were decapitated and the brain removed and placed in chilled (4 °C) Krebs solution of the following composition (mM): NaCl, 117; KCl, 3·6, NaH_2PO_4, 1·2; $CaCl_2$, 2·5; $MgCl_2$, 1·2; glucose, 11; $NaHCO_3$, 25, gassed with 95 % O_2–5 % CO_2. The hippocampi were dissected and sliced with an Oxford Vibratome transverse to their longitudinal axis. The slice

thickness was about 400 μm. The slice was placed on a nylon net in a recording chamber (volume 500 μl) and immobilized by a titanium grid placed gently on the upper surface of the section. The tissue was superfused with oxygenated Krebs solutions, maintained at $37 \pm 0{\cdot}5$ °C, at a rate of 8 ml/min, so that the partial oxygen pressure (P_{O_2}) in the recording chamber was $621{\cdot}4 \pm 16{\cdot}5$ mmHg (mean $\pm$ S.D., $n = 8$), measured by an oxygen micro-electrode. The slices were allowed to recover for at least 2 h prior to starting intracellular recordings (see e.g. Reid, Schurr, Tseng & Edmonds, 1984). The slices were made 'hypoxic' by superfusing with Krebs solution equilibrated with 95 % N_2–5 % CO_2; P_{O_2} in the chamber fell to approximately $39{\cdot}6 \pm 4{\cdot}1$ mmHg ($n = 8$) in 10–15 s after onset of hypoxic superfusion.

Intracellular recordings from CA1 pyramidal cells were made with micropipettes (40–60 MΩ) filled with 3 M-potassium acetate or potassium chloride. Excitatory and inhibitory post-synaptic potentials (e.p.s.p.s and i.p.s.p.s) were elicited by stimulation through monopolar silver electrodes (50 μm diameter, insulated except at the tip) placed in the stratum radiatum. In some experiments, ethyleneglycol-bis-(β-aminoethylether)*N*,*N*-tetraacetic acid (EGTA) was injected intracellularly. This was done using double-barrelled electrodes in which one barrel contained potassium chloride (3 M) and the other potassium EGTA (100–300 M-EGTA in 0·3 M-potassium hydroxide, pH adjusted to 7·7). EGTA was ejected by anodal currents of up to 2 nA amplitude and 100–300 ms duration. Drugs and solutions of different ionic composition were applied by changing from one superfusion solution to another. Drugs used were from Sigma except tetrodotoxin (TTX) from Sankyo. All quantitative results were expressed as mean $\pm$ S.D. of the mean. The number of neurones examined is given in parentheses.

RESULTS

This study is based on recordings of over 120 CA1 pyramidal neurones with stable membrane potentials more negative than -50 mV. The mean resting membrane potential of this sample was $-65{\cdot}8 \pm 7{\cdot}5$ mV($n = 87$) and input resistance was $35{\cdot}4 \pm 12{\cdot}9$ MΩ ($n = 87$) in normal Krebs solution equilibrated with 95 % O_2–5 % CO_2.

Initial changes

Hypoxia-induced hyperpolarization and its ionic mechanism. In the majority of neurones (fifty-nine out of eighty-seven), the initial effect of hypoxia was a membrane hyperpolarization associated with a fall in input resistance, as previously described (Hansen *et al.* 1982). Duration and amplitude of this hyperpolarization increased with increasing duration of exposure to hypoxia (Fig. 1*A*). The minimum effective duration of hypoxic exposure for producing the hyperpolarization was 1–1·5 min. The hyperpolarization occurred very slowly; its latency was $1{\cdot}2 \pm 0{\cdot}3$ min ($n = 59$) and the time-to-peak was $2{\cdot}8 \pm 0{\cdot}8$ min ($n = 59$) from onset of hypoxic exposure. The peak amplitude of the hyperpolarization was $8{\cdot}5 \pm 2{\cdot}3$ mV ($n = 59$) while input resistance at the peak of the hyperpolarization was reduced to $41{\cdot}8 \pm 9{\cdot}3$ % (range 32–61 %, $n = 43$) of the pre-hypoxic values. During longer hypoxic exposure (> 5–12 min), the hyperpolarization was followed by a depolarization, also associated with reduction in input resistance (see later). In all four neurones tested, the hyperpolarization was unaffected by cobalt-containing (2 mM), high-magnesium (20 mM) solution, which completely blocked spontaneous and evoked synaptic potentials (Fig. 1*B*). Similar results were obtained in low-calcium (0·25 mM)–high-magnesium (15 mM) or TTX-containing (0·3 μM) medium in four other cells. These findings indicate that the response was brought about by a direct action of hypoxia on the impaled neurone.

When the solution was changed to one which did not contain potassium ions, the hypoxia-induced hyperpolarization increased in amplitude with only a small increase

in input resistance (Figs. 2*A* and 8*C*). The peak amplitude of the hyperpolarization in normal-potassium (3·6 mM)-containing medium was 8·6±2·6 mV ($n = 6$) while the value in potassium-free medium was 13·4±3·8 mV ($n = 6$). Elevation of the potassium concentration in the medium to 10 and 20 mM suppressed the hypoxic hyperpolarization. The peak amplitudes of the hyperpolarization in these high-

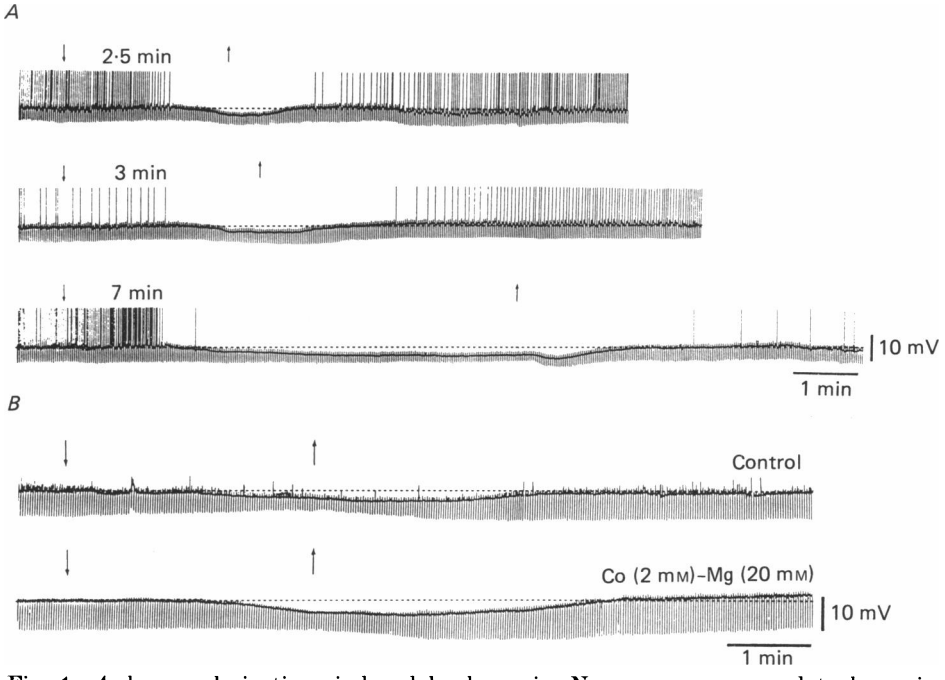

Fig. 1. *A*, hyperpolarizations induced by hypoxia. Neurone was exposed to hypoxic solution between the downward and upward arrows. All records were obtained from the same neurone. Resting potentials before hypoxic exposure were −62, −64 and −62 mV for the upper, middle and lower traces, respectively. In each trace, downward deflexions are hyperpolarizing tonic potentials elicited by anodal current pulses (0·2 nA, 100 ms, 0·2 Hz). *B*, the hypoxia-induced hyperpolarization was unaffected in cobalt-containing medium. The upper trace was recorded in control medium and the lower in 2 mM-cobalt–20 mM-magnesium medium. The cell was depolarized by 4 mV in the latter medium, from −69 to −65 mV. Hyperpolarizing tonic potentials were elicited by anodal current pulses (0·5 nA, 100 ms, 0·2 Hz).

potassium-containing media, 4·9±0·8 mV (10 mM-potassium, $n = 5$) and 1·3±0·5 mV (20 mM-potassium, $n = 4$), were much smaller than their control values (9·6±2·2 mV, $n = 5$ and 10·8±1·9 mV, $n = 4$, respectively) in normal-potassium-containing media. The record in Fig. 2*B* represents one of these results. These increases in potassium ion concentration to 10 and 20 mM caused reductions in membrane potential of 14–20 and 28–33 mV, respectively, and reductions in input resistance of 35·6±8·0% (range 25–48%, $n = 5$) and 50·5±8·6% (range 35–68%, $n = 4$). In all four neurones examined, reduction of the chloride concentration to 40 mM (by substituting sodium isethionate for sodium chloride) did not significantly change the hyperpolarization induced by hypoxia (data not shown). Replacement of

sodium chloride with choline chloride did not significantly affect the hyperpolarization either, in three out of four neurones. In the remaining neurone, the amplitude of the hyperpolarization decreased to about 90% of the control value after 20 min of superfusion with sodium-free medium (data not shown). In addition, the hyperpolarization was unaffected by superfusion with a low concentration (1 μM) of ouabain (Fig. 8*B*). These results suggest that the hypoxia-induced hyperpolarization is probably due to an increase in membrane conductance to potassium ions. The hyperpolarization became progressively smaller and reversed in polarity when the membrane was progressively hyperpolarized (Fig. 2*C*). Fig. 2*D* illustrates graphically the amplitude of the hypoxia-induced hyperpolarization *versus* membrane potential, which was obtained from seven neurones using potassium chloride-filled microelectrodes. The amplitudes varied widely between neurones, and even within the same neurone were different at membrane potentials of -60 and -70 mV, due to anomalous rectification, as described initially by Purpura, Prelevic & Santini (1968). However, the mean amplitude of the hypoxia-induced hyperpolarization was almost linear with changes in the membrane potential throughout the range examined (-60 to -90 mV). Reversal potentials obtained with potassium chloride and potassium acetate electrodes were $-82{\cdot}5 \pm 5{\cdot}4$ mV ($n = 7$) and $-83{\cdot}4 \pm 6{\cdot}2$ mV ($n = 5$), respectively. These reversal potentials were more positive than the reversal potential ($-91{\cdot}5 \pm 4{\cdot}7$ mV, $n = 6$) for post-tetanic hyperpolarizations following bursts of action potentials (see Discussion).

In approximately one-third of the neurones (twenty-eight out of eighty-seven) the hypoxia-induced hyperpolarization was preceded by a transient depolarization (Figs. 1, 2, 3, 5 and 6). This depolarization was associated with a slight increase in input resistance, and was blocked by cobalt-containing (2 mM) or TTX-containing (0·3 μM) Krebs solution (data not shown).

Effects of intracellular injection of EGTA and various potassium-channel blockers. It has been reported that in the mammalian hippocampal CA1 neurones there are at least five different voltage-dependent potassium currents: potassium currents underlying delayed rectification ($I_{K,V}$), A-currents (I_A), M-currents (I_M), calcium-dependent potassium currents ($I_{K,Ca}$) and Q-currents (I_Q) (cf. Halliwell & Adams, 1982; Segal & Barker, 1984). In order to elucidate the type of potassium current(s) associated with the hypoxia-induced hyperpolarization, the effect of various potassium-channel blockers on the hyperpolarization was tested.

Neurones impaled with EGTA-containing electrodes showed, over a period of tens of minutes or hours, a progressive attenuation of a long (several hundred milliseconds) after-hyperpolarization which follows the soma spike. Injection of EGTA into the cell by a series of hyperpolarizing pulses abolished the post-tetanic hyperpolarization following a burst of action potentials, but the hypoxia-induced hyperpolarization was well maintained in all three neurones tested (Fig. 3*A*). Although superfusion with 4-aminopyridine (4-AP, 1·5 mM)-containing medium markedly increased the frequency and amplitude of spontaneous e.p.s.p.s and i.p.s.p.s without affecting membrane potential or input resistance, the hypoxia-induced hyperpolarization in this solution was not significantly different from that obtained in normal Krebs solution for all three cells (Fig. 3*B*). Barium (0·5 mM)- or tetraethylammonium (TEA, 10 mM)-containing medium resulted in a depolarization of a few millivolts.

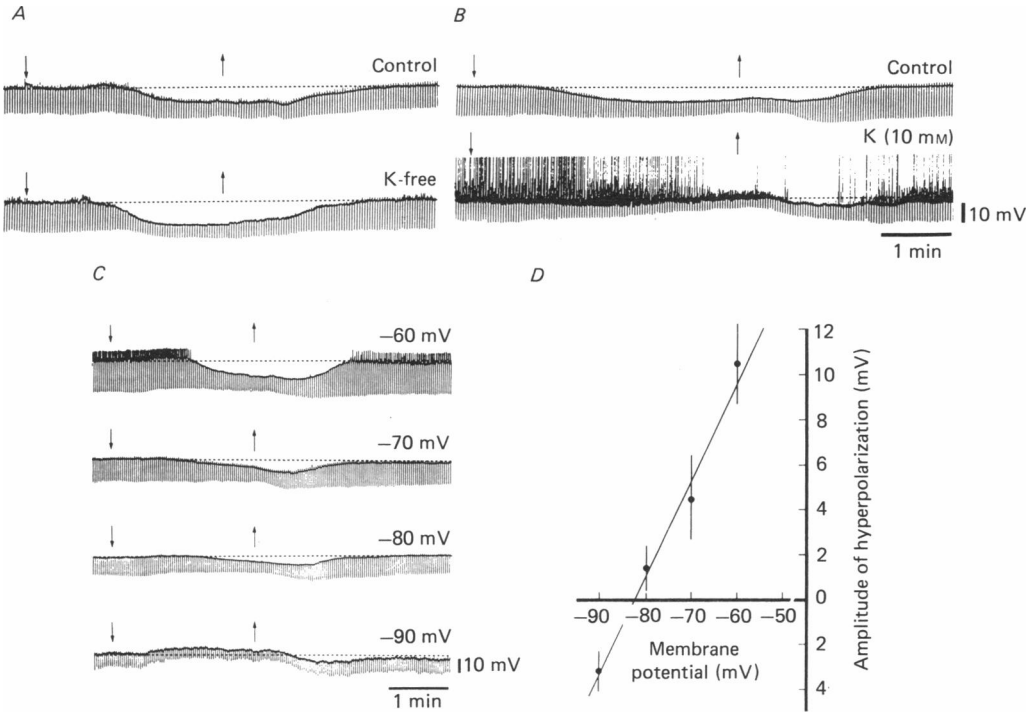

Fig. 2. Effects of changing extracellular potassium concentration on the hypoxia-induced hyperpolarization, and its amplitude as a function of membrane potential. Neurones were exposed to hypoxic solution between arrows. *A*, the hypoxia-induced hyperpolarization was increased in amplitude in potassium-free medium. The upper trace was recorded in control medium and the lower in potassium-free medium. Membrane potential was held at −65 mV before hypoxic exposure. *B*, the hypoxia-induced hyperpolarization was suppressed by high-potassium-containing medium. The upper trace was recorded in control medium and the lower in high-potassium (10 mM) medium. Membrane potential was held at −67 mV before hypoxic exposure. In each record of *A* and *B*, downward deflexions are hyperpolarizing tonic potentials elicited by anodal current pulses (0·3 nA, 100 ms, 0·2 Hz). *C*, reversal of the hypoxia-induced hyperpolarization. Resting potential was −60 mV. In the second, third and fourth records membrane potentials were held at −70, −80 and −90 mV, respectively, by passing anodal d.c. currents. Hyperpolarizing tonic potentials were elicited by anodal current pulses (0·3 nA, 100 ms, 0·3 Hz). Note anomalous rectification at hyperpolarized membrane potentials. See text for the sensitivity of the amplitude and conductance change of hypoxia-induced hyperpolarization to membrane potential. *D*, amplitude of the hypoxia-induced hyperpolarization *versus* membrane potential. Data were obtained from seven neurones using potassium chloride electrodes. Bars indicate S.D. of mean. In each neurone, the peak amplitude of hyperpolarization was measured during hypoxic exposure.

The barium-containing medium reduced the amplitude of the hypoxia-induced hyperpolarization by 20–40% in all four neurones (Fig. 3*C*) whereas the TEA-containing medium did not affect it in any of the three cells tested (Fig. 3*D*). In three out of five neurones, addition of caesium (3 mM) to the extracellular solution slightly depressed the amplitude of the hypoxia-evoked response by 10–20% (Fig. 3*E*). These results, combined with the almost-linear relationship found between amplitude of the

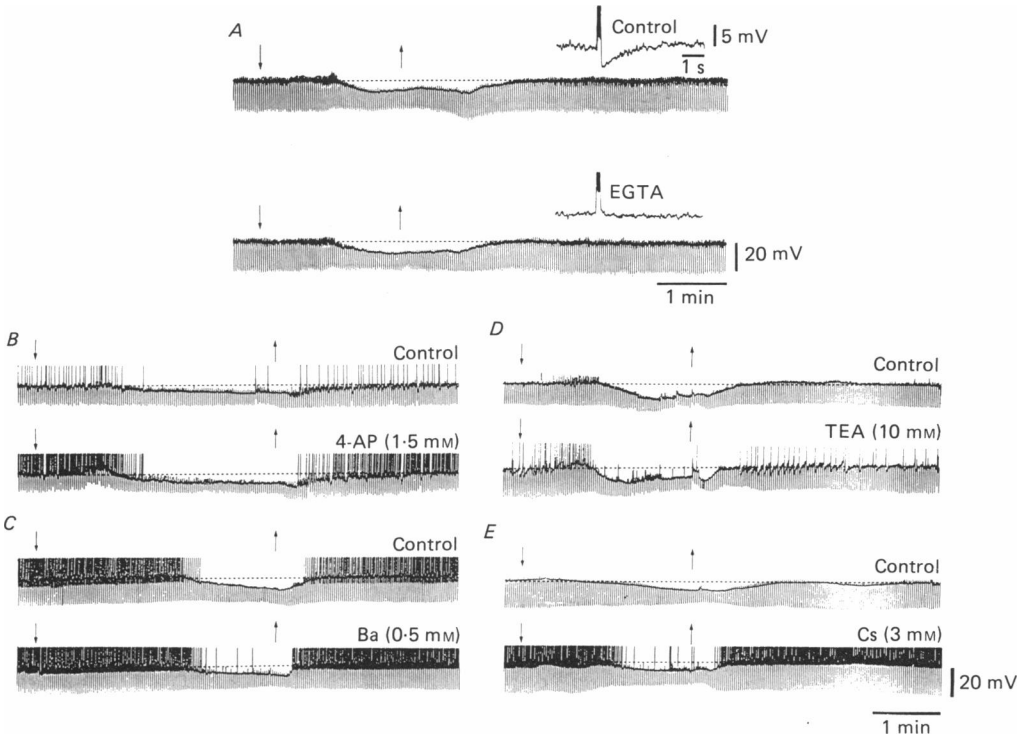

Fig. 3. Hypoxia-induced hyperpolarization is unaffected by intracellular EGTA injection or by potassium-channel blockers applied by superfusion. Neurones were exposed to hypoxic solution between arrows. *A*, upper and lower records were obtained from the same neurone before and 20 min after the beginning of EGTA injection, respectively. The membrane potential prior to EGTA injection was −62 mV. Superimposed hyperpolarizing tonic potentials were elicited by anodal current pulses (0·6 nA, 200 ms, 0·2 Hz). Insets are post-tetanic hyperpolarizations evoked by ten pulses (30 Hz) before and 18 min after EGTA injection, respectively. Note that post-tetanic hyperpolarization is abolished by EGTA injection whereas hypoxia-induced hyperpolarization is not. *B–E*, effect of 4-AP (1·5 mM), barium (0·5 mM), TEA (10 mM) or caesium (3 mM) on the hypoxia-induced hyperpolarization. Upper and lower records in *B–E* were, respectively, obtained before and 5–10 min after starting application of a potassium-channel blocker. Note that the hypoxia-induced hyperpolarization was reduced slightly by barium or caesium, while it was not appreciably altered by 4-AP or TEA. All these blockers increased both spontaneous e.p.s.p. and firing, and except for 4-AP, decreased the resting potential (−62 mV in *B*, −60 mV in *C*, −62 mV in *D* and −60 mV in *E*) by a few millivolts. In each record, hyperpolarizing tonic potentials were elicited by anodal current pulses (0·25 nA, 100 ms, 0·2 Hz). *B–E* were recorded from four different neurones.

hypoxia-induced hyperpolarization and membrane potential, as shown in Fig. 2*D*, suggest that the hyperpolarization is due to an increase in a voltage-independent potassium conductance (see also Discussion).

Active membrane properties during hypoxia. The spontaneous spike activity disappeared when the membrane started to hyperpolarize (Fig. 1), while the soma spike was still elicited by a depolarizing pulse for up to 20 min of hypoxic superfusion. During the first 5 min of hypoxic exposure, the peak amplitude of soma spikes was

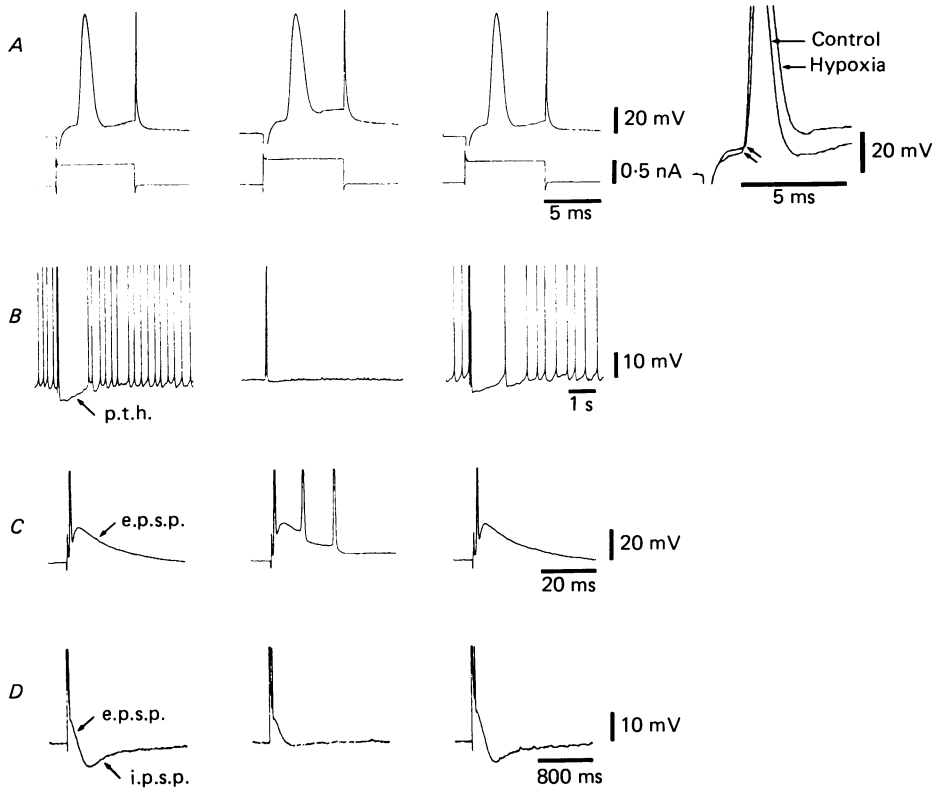

Fig. 4. Effect of hypoxia on soma spikes, post-tetanic hyperpolarizations and synaptic potentials. All records in the left, centre and right columns were taken before, 4 min after starting hypoxic exposure and 8 min after reoxygenation, respectively. Since the neurones hyperpolarized after 4 min of hypoxic exposure, all traces in the middle column were recorded after the membrane potential was restored to the control level by passing d.c. currents. *A*, action potentials (upper trace) were elicited by depolarizing current pulses (lower trace). Superimposition of the control spike and that recorded during hypoxic exposure reveals that the threshold for spike generation (indicated by small, oblique arrows) is shifted in a depolarizing direction and the spike after-hyperpolarization is depressed following hypoxia. Control membrane potential, −70 mV. *B*, the post-tetanic hyperpolarizations (p.t.h.) evoked by five depolarization pulses (30 Hz) were almost abolished after 4 min of hypoxia. Control membrane potential, −60 mV. *C*, using a monopolar silver electrode (50 μm diameter, insulated except at its tip), a constant rectangular pulse (200 μs) was applied to the stratum radiatum. The e.p.s.p. evoked by a submaximal stimulation was well maintained after 4 min of hypoxia. Control membrane potential, −72 mV. *D*, the e.p.s.p. and subsequent i.p.s.p. were evoked by a supramaximal stimulation. The i.p.s.p. was completely eliminated while the e.p.s.p. was preserved after 4 min of hypoxia. Control membrane potential, −64 mV.

unaltered or slightly decreased, but the duration increased in association with a decrease in the amplitude of a brief (several tens of milliseconds) after-hyperpolarization of soma spikes, as shown in Fig. 4*A*. Furthermore, in four out of six neurones, the threshold for spike generation (indicated with small, oblique arrows on the superimposition in Fig. 4*A*) was shifted in the depolarizing direction by 3–5 mV

after 4 min of hypoxic exposure. On the other hand, the post-tetanic hyperpolarization progressively decreased in amplitude shortly after starting hypoxic superfusion, and was eliminated after 4 min of hypoxic exposure (Fig. 4*B*). Longer exposures (> 5 min) to hypoxia raised the threshold level markedly, concomitant with a decrease in the peak amplitude of soma spikes. Thus the threshold was shifted in the depolarizing direction by 6–12 mV and the peak amplitude was reduced to 79·6 ± 9·2 % (range 63–89 %, $n = 5$) of the control value after 20 min of hypoxic exposure.

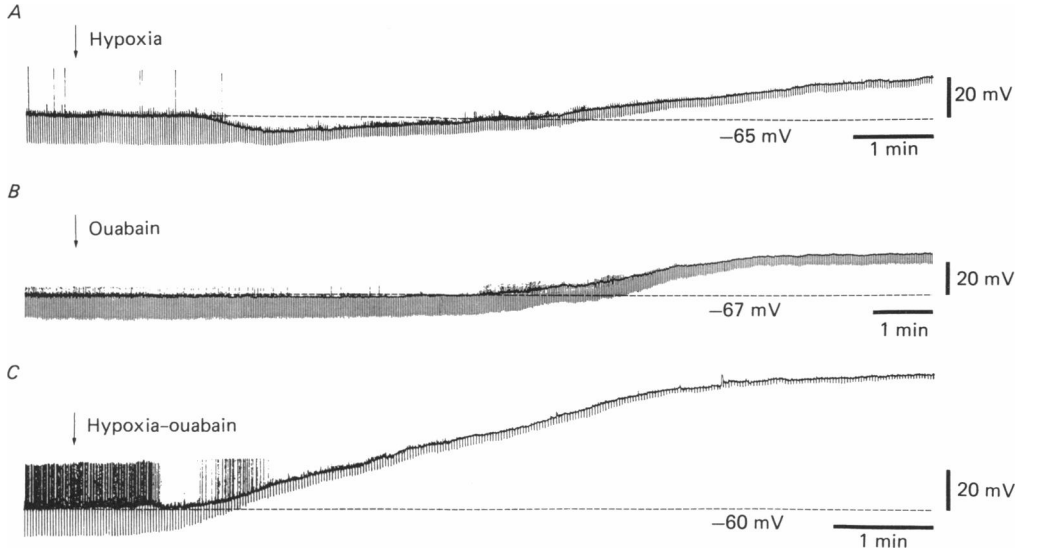

Fig. 5. Effects of hypoxia and ouabain on membrane potential. *A*, prolonged application of hypoxic solution caused an initial hyperpolarization and a subsequent depolarization. Hyperpolarizing tonic potentials were elicited by anodal current pulses (0·4 nA, 100 ms, 0·2 Hz). Control membrane potential, −65 mV. *B*, prolonged application of ouabain (5 μM) produced a potential change similar to that induced by hypoxia. Note that an initial small hyperpolarization was followed by a long-lasting depolarization of approximately 25 mV in amplitude. Hyperpolarizing tonic potentials were elicited by anodal current pulses (0·3 nA, 100 ms, 0·2 Hz). Control membrane potential, −67 mV. *C*, application of hypoxic solution with ouabain (7 μM) caused a progressive depolarization. Membrane potential became 0 mV after 7·5 min of superfusion. Hyperpolarizing tonic potentials were elicited by anodal current pulses (0·5 nA, 100 ms, 0·2 Hz). Control membrane potential, −60 mV.

Synaptic potentials during hypoxia. When the membrane potential was held to the pre-hypoxic level by a depolarizing d.c. current, the e.p.s.p.s evoked by a submaximal stimulation were well preserved (Fig. 4*C*) whereas the i.p.s.p.s evoked by a supramaximal stimulation were completely abolished by 4 min of exposure to hypoxia in all seven neurones (Fig. 4*D*). Subsequently the amplitude of the e.p.s.p.s gradually decreased, although in four other neurones it was maintained at about 30–40 % of the pre-hypoxic value 20 min after the onset of hypoxia. In a majority of the neurones in which spontaneous e.p.s.p.s could be observed, the frequency of spontaneous e.p.s.p.s markedly increased when the membrane started to depolarize after approximately 4–8 min of exposure to hypoxia (Figs. 5 and 6).

Late changes

Hypoxia-induced slow depolarization and the effect of ouabain. With longer exposures to hypoxic solution (> 5–12 min), a depolarization followed the initial hyperpolarization. The time-to-onset of the depolarization varied with each neurone; the mean was 6·9 ± 3·5 min (range 3·0–14·3 min, $n = 22$). As shown in Fig. 5*A*, the depolarization developed gradually, and the rate of decrease of membrane potential was approximately 2 mV/min. 20 min after the onset of hypoxia, the membrane potential remained at a level 25·1 ± 3·8 mV ($n = 18$) more positive than the pre-hypoxic resting potential. Input resistance at the plateau phase of depolarizations, measured while the membrane potential was transiently restored to the control level was 45·1 ± 10·5 % (range 30–71 %, $n = 18$) of the pre-hypoxic value. Next we attempted to examine the effects of low-sodium (26·2 mM), low-chloride (40 mM) or cobalt-containing (2 mM) medium on the slow depolarization. It was not possible, however, to convincingly compare the depolarizations in these media with those in normal Krebs solution in the same neurone because of changes in tip resistance of the recording electrodes. Nevertheless, both amplitude and time-to-onset of the depolarization of the neurones in these media (*i.e.* three neurones in low-sodium, four in low-chloride and three in cobalt-containing medium) were not significantly different from those in normal Krebs solution.

The changes in membrane potential observed during hypoxia were similar to those observed during prolonged (> 6 min) application of ouabain at relatively high concentrations in normoxic medium. Thus, in response to ouabain (5–7 μM) superfusion, six out of the ten neurones tested showed an initial hyperpolarization of a few millivolts, which was followed by a depolarization (Fig. 5*B*). The remaining four neurones showed a simple depolarization without any pre-hyperpolarization. The initial hyperpolarization and subsequent depolarization were associated with a fall in membrane resistance. The amplitude of the hyperpolarization produced by ouabain was, however, consistently smaller than that of the hyperpolarization elicited by hypoxia, the ouabain-induced hyperpolarization being 1·8 ± 0·3 mV (range 1·5–2·2 mV, $n = 6$). Approximately 15–20 min after the onset of ouabain application, the depolarization reached a plateau level which was 23·5 ± 4·8 mV (range 18–29 mV, $n = 10$) more positive than the resting potential. When membrane potential was restored to the control level, the input resistance was decreased to 48·4 ± 3·3 % (range 42–53 %, $n = 10$) of the control value. The results suggest that inactivation of the Na^+–K^+-ATPase (sodium pump) is likely to be involved in the generation of the depolarization during hypoxia. In most cells, application of hypoxic solution with a high concentration of ouabain (7 μM produced a depolarization not preceded by hyperpolarization. The membrane depolarized continuously and membrane potentials became 0 mV after 8–20 min of hypoxia, as shown in Fig. 5*C*.

Hypoxia-induced rapid depolarization. When the hypoxic period was sufficiently prolonged (> 15 min), three types of responses were produced. In about one-fifth of the neurones (six out of twenty-eight) the plateau phase of the slow depolarization was followed by a phase characterized by a much faster rate (3–5 mV/min) of depolarization, and the resting potential was reduced to 0 mV after 15–30 min of hypoxic superfusion, as shown in Fig. 6*A*. Input resistance during the rapid

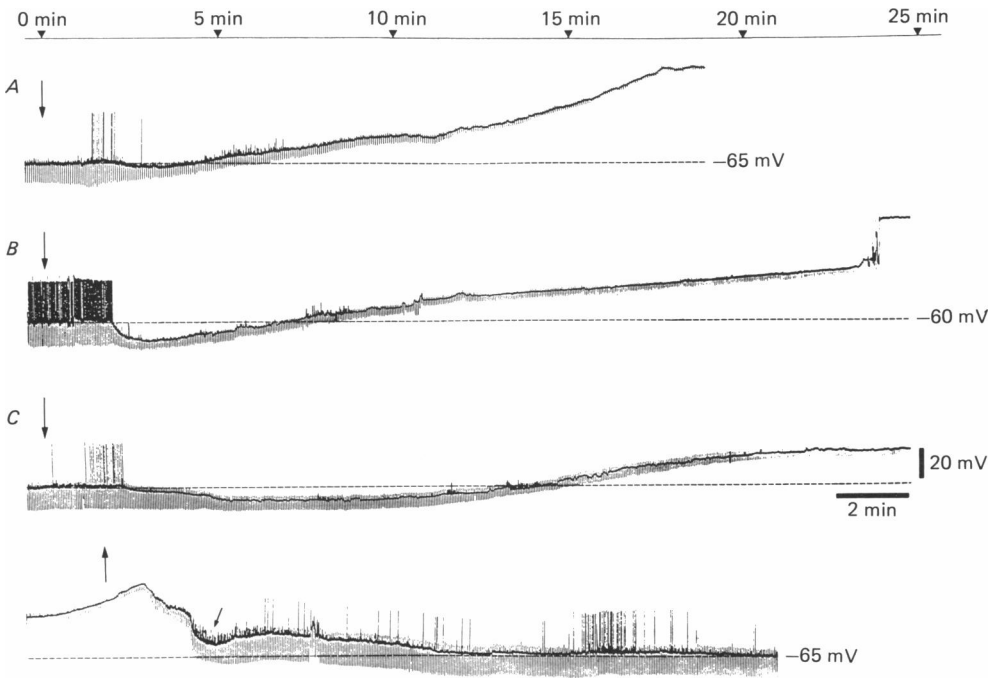

Fig. 6. Variation in the membrane potential changes induced by hypoxia. *A*, in response to hypoxia, this neurone showed an initial hyperpolarization followed by a slow depolarization. The slow depolarization was followed by a much faster depolarization after 12 min of hypoxia, and the membrane potential was reduced to 0 mV after 16 min of hypoxic superfusion. Hyperpolarizing tonic potentials were elicited by anodal current pulses (0·4 nA, 200 ms, 0·2 Hz). Control membrane potential, −65 mV. *B*, in this neurone, the intracellular recording electrode suddenly came out of the cell without any tissue movement after 24 min of hypoxia. Hyperpolarizing tonic potentials were elicited by anodal current pulses (0·4 nA, 200 ms, 0·3 Hz). Control membrane potential, −60 mV. *C*, the hypoxia-induced depolarization in this neurone maintained its plateau level at approximately 25 mV from the resting potential till 36 min after starting hypoxic superfusion. The depolarization then showed a relatively fast increase. After 40 min of hypoxia, the neurone was reoxygenated (indicated by the upward arrow in bottom trace). The membrane was repolarized, concomitant with a marked increase in the frequency of spontaneous e.p.s.p.s. In addition, an abrupt hyperpolarizing hump (indicated by a small, oblique arrow) appeared during the early phase of repolarization. Hyperpolarizing tonic potentials were elicited by anodal current pulses (0·4 nA, 200 ms, 0·3 Hz). Control membrane potential, −65 mV.

depolarization phase became immeasurably low. When the slice was reoxygenated during the period of rapid depolarization, the neurones did not repolarize, but abruptly depolarized further, and eventually showed no functional recovery. In all neurones of this type, the rapid depolarization began at membrane potentials of −30 to −40 mV. In a second group of neurones (ten out of twenty-eight) the intracellular recording electrode suddenly came out of the impaled neurone, without obvious tissue movement, during the slow depolarizing phase (Fig. 6*B*). It is likely, although not proven, that swelling of the cell is the cause of the displacement of the neurone relative

to the recording electrode. It should be noted that most of the neurones appeared to rapidly recover pre-hypoxic characteristics when the slice was reoxygenated during this phase (*i.e.* after 20–40 min of the hypoxic exposure); within 15 min after reoxygenation the neurones could be impaled without any technical difficulties and had stable resting membrane potentials, input resistances and synaptic potentials,

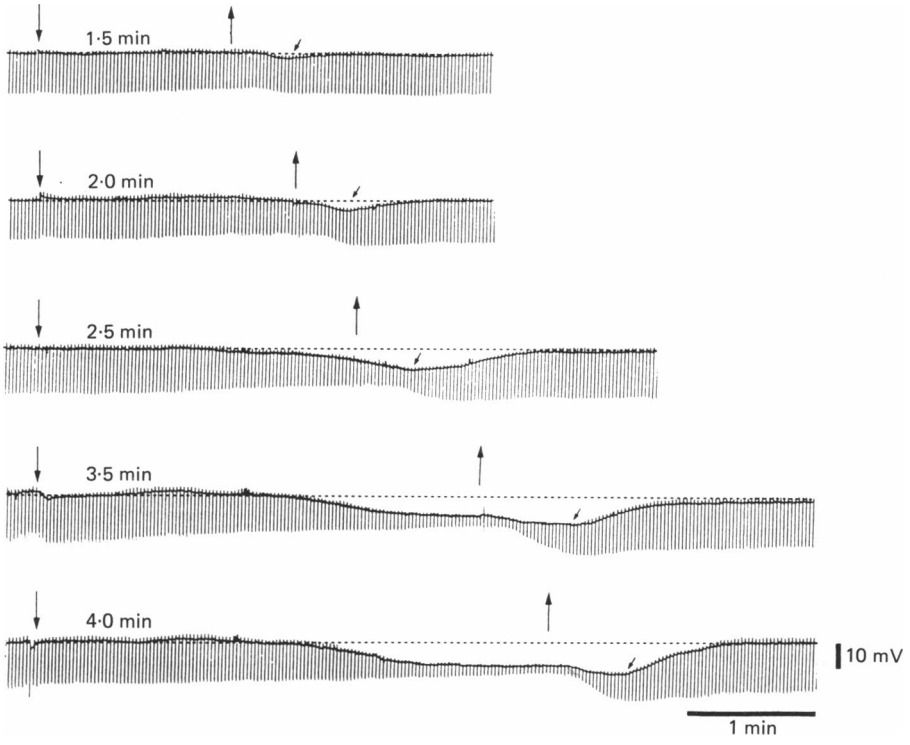

Fig. 7. Reoxygenation-induced hyperpolarization (indicated by small, oblique arrows). Neurones were exposed to hypoxic solution between large arrows. All records were obtained from the same neurone. Resting potential prior to each hypoxic exposure was held at −70 mV. Hyperpolarizing tonic potentials were elicited by anodal current pulses (0·2 nA, 100 ms, 0·6 Hz).

with values not different from those observed in the pre-hypoxic control. A third group (twelve out of twenty-eight) were somewhat resistant to the hypoxic environment for 40 min. As shown in Fig. 6*C*, the membrane of these neurones initially hyperpolarized and then depolarized, maintaining a potential approximately 25 mV positive to the resting potential. After 40 min of hypoxia, the slice was reoxygenated. After a transient depolarization, the membrane repolarized, concomitant with a marked increase in the frequency of spontaneous e.p.s.p.s (lower trace in Fig. 6*C*). In addition, an abrupt hyperpolarizing hump (indicated by a small, oblique arrow on the lower trace in Fig. 6*C*) consistently appeared during the early phase of repolarization. The membrane potential and input resistance returned to pre-hypoxic levels approximately 20 min after reoxygenation.

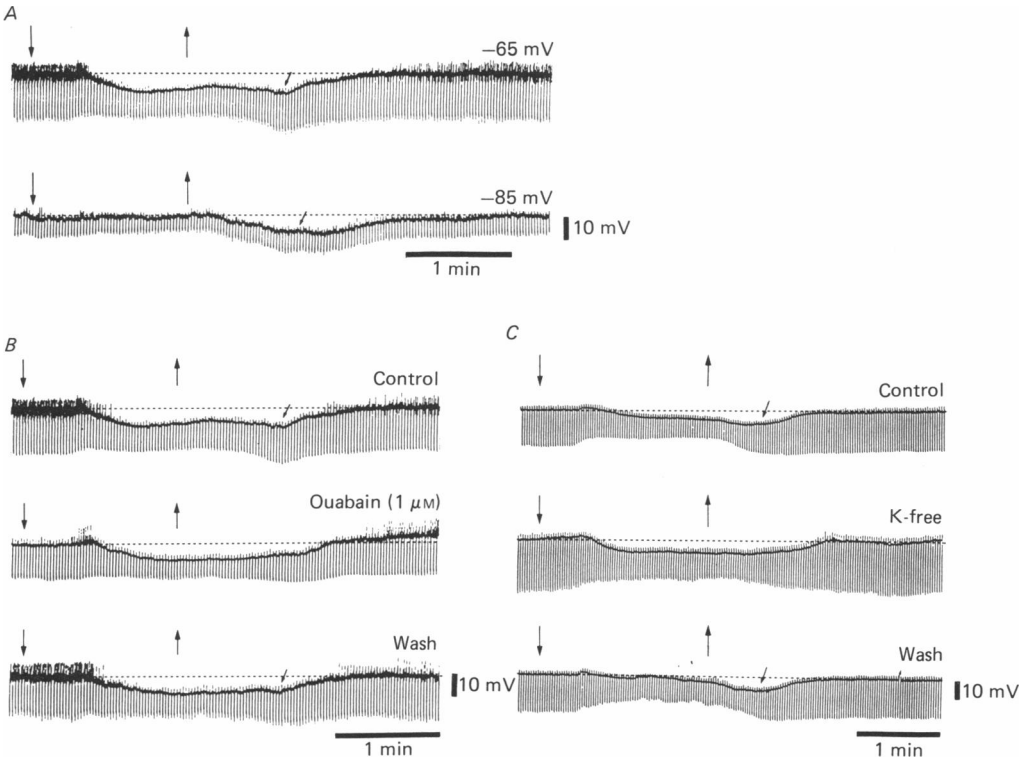

Fig. 8. *A*, effect of changing membrane potential on reoxygenation-induced hyperpolarization. Upper trace is control in which the hypoxic response was initiated at the resting potential (−65 mV). Lower trace shows the hypoxic response which was initiated at a hyperpolarized membrane potential (−85 mV). Note that the hyperpolarization during hypoxic exposure was almost nullified at −85 mV, while the reoxygenation-induced hyperpolarization (indicated by small, oblique arrow) was not altered in amplitude but prolonged in duration. Hyperpolarizing tonic potentials were elicited by anodal current pulses (0·3 nA, 200 ms, 0·2 Hz). *B*, effect of ouabain (1 μM) on reoxygenation-induced hyperpolarization. Upper, middle and lower traces were obtained before, 15 min after application of ouabain and 20 min after washing, respectively. The cell was hyperpolarized by 3 mV in ouabain-containing medium (from −61 to 64 mV). The reoxygenation-induced hyperpolarization (indicated by the small, oblique arrow) was eliminated by ouabain. Hyperpolarizing tonic potentials were elicited by anodal current pulses (0·4 nA, 200 ms, 0·2 Hz). *C*, effect of potassium-free medium on reoxygenation-induced hyperpolarization. Upper, middle and lower records were taken before, 15 min after superfusion with potassium-free solution and 10 min after washing, respectively. Membrane potential was held at −69 mV before each hypoxic exposure. Hyperpolarizing tonic potentials were elicited by anodal current pulses (0·5 nA, 200 ms, 0·2 Hz). The hyperpolarization induced by reoxygenation was practically abolished in potassium-free medium.

Reoxygenation

Reoxygenation-induced hyperpolarization and its mechanism of generation. When the tissue was reoxygenated after 20–40 min of hypoxic exposure, a majority of the neurones immediately began to repolarize, showing a transient hyperpolarization, and the membrane potential, input resistance and synaptic potentials returned to the pre-hypoxic levels 15–20 min after reoxygenation, as described above.

The amplitude of this reoxygenation-induced hyperpolarization increased when the hypoxic exposure was prolonged (Fig. 7). The minimum effective duration of hypoxia required to cause this response was 1–1·5 min, a duration which was coincident with that required to produce the initial hyperpolarization during hypoxia. Moreover, when the duration of hypoxic exposure was too short to produce

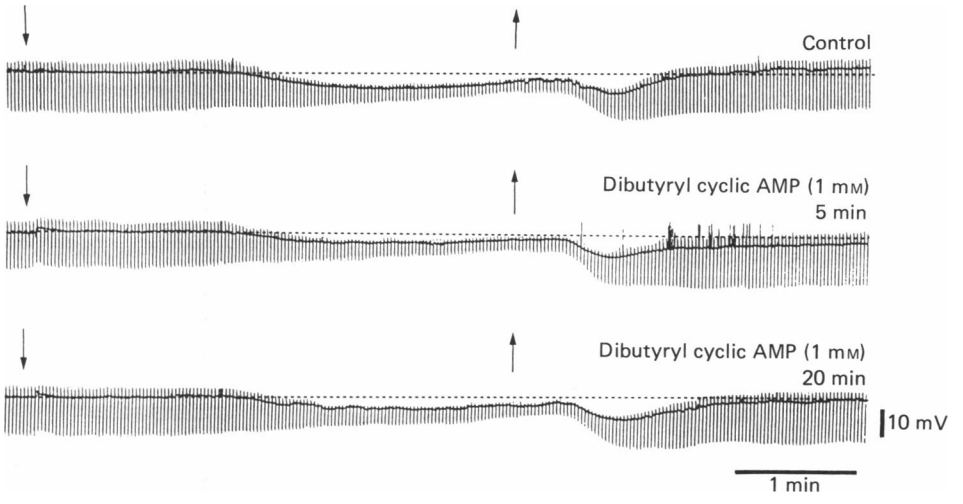

Fig. 9. Effect of dibutyryl cyclic AMP on hyperpolarizing responses during and after hypoxia. Upper, middle and lower traces were recorded before, and during treatment for 5 and 20 min, respectively. The peak amplitudes of the hypoxia-induced hyperpolarization after 5 and 25 min of treatment with dibutyryl cyclic AMP (1 mM) were decreased to approximately 70 and 90% of the control value, respectively. On the other hand, the reoxygenation-induced hyperpolarization was not significantly affected. Resting potential was −69 mV. Hyperpolarizing tonic potentials were elicited by anodal current pulses (0·4 nA, 200 ms, 0·2 Hz). Since 5 min of application of dibutyryl cyclic AMP caused a membrane hyperpolarization of 4 mV, the membrane potential was restored to the control level by passing d.c. current before hypoxic exposure. Note that the input resistance 5 min after starting application of dibutyryl cyclic AMP was slightly decreased, compared to that in control medium. The membrane potential and input resistance after 25 min of superfusion of dibutyryl cyclic AMP returned almost to the control values (see text).

changes in membrane potential and input resistance, reoxygenation did not elicit the hyperpolarization. The amplitude of this hyperpolarization was not altered significantly when the membrane was hyperpolarized (Fig. 2) or depolarized (data not shown). Thus, when the membrane potential was shifted from −65 to −85 mV, as shown in Fig. 8*A*, the reoxygenation-induced hyperpolarization at −85 mV was not altered in amplitude but prolonged in duration (also see Fig. 2). Similar results were obtained with three other neurones examined. In all four neurones tested, ouabain at a low concentration (1 μM) reversibly depressed the reoxygenation-induced hyperpolarization without affecting the hypoxia-induced hyperpolarization (Fig. 8*B*). The hyperpolarization was virtually eliminated 15–20 min after switching from the normal Krebs solution to potassium-free medium in all three neurones tested (Figs. 2*A* and 8*C*). These results indicate that the reoxygenation-induced hyperpolarization may be brought about by reactivation of an electrogenic sodium pump, activity of which was depressed during the period of hypoxia.

Effect of cyclic AMP. It has been proposed that the effects of hypoxia in the C.N.S. result from protein phosphorylation processes (e.g. Hansen, 1985), because anoxia increases the tissue cyclic AMP levels (Schmidt, Schmidt & Robinson, 1973; Winn, Rubio & Berne, 1981; Whittingham, Lust & Passonneau, 1984). We examined, therefore, the influence of dibutyryl cyclic AMP on the resting membrane properties of the neurones and on the hyperpolarizations induced by hypoxia or reoxygenation. In three out of five neurones, neither membrane potential nor input resistance was affected by superfusion of dibutyryl cyclic AMP (1 mM). Moreover, neither the hypoxia-induced hyperpolarization nor the reoxygenation-induced hyperpolarization was altered significantly by dibutyryl cyclic AMP. In the remaining two neurones, application of dibutyryl cyclic AMP caused a membrane hyperpolarization of 3–5 mV, which was associated with a decrease in input resistance. In these neurones, the peak amplitudes of the hypoxia-induced hyperpolarization were decreased to 68 and 75 % of the control values after 5 min of superfusion of dibutyryl cyclic AMP. After 25 min of continuous application, the amplitudes, however, returned to 87 and 91 % of the control values, respectively (Fig. 9).

TABLE 1. Changes in membrane potential induced by hypoxia and reoxygenation

	Time-to-onset (min)	Duration (min)	Peak amplitude (mV)	Change in input resistance
Hypoxia-induced initial depolarization	0·5–1	0·5	3–5	Increased
Hypoxia-induced hyperpolarization	1–1·5	5–6	5–10	Decreased
Hypoxia-induced slow depolarization	7	> 20	20–30	Decreased
Reoxygenation-induced hyperpolarization	0·5–1	1–2	5–10	Unclear

All values are obtained from neurones with resting potentials of −60 to −70 mV. Changes in input resistance during reoxygenation-induced hyperpolarization are unclear because of the coexistence of reoxygenation-induced and hypoxia-induced hyperpolarizations. The former are inseparable from the latter.

DISCUSSION

The present study demonstrates that in response to hypoxic exposure, a majority of CA1 pyramidal cells showed a transient hyperpolarization which was followed by a slow depolarization, and that in a minority of neurones the hyperpolarization was preceded by a transient depolarization. In addition, reoxygenation produced a transient hyperpolarization. The membrane potential changes induced by either hypoxia or reoxygenation are summarized in Table 1. Another finding of the present study is that evoked i.p.s.p.s were abolished within 3 min of hypoxic exposure while evoked e.p.s.p.s were maintained for about 20 min of hypoxic superfusion. The mechanisms underlying these phenomena produced by hypoxia and the hypoxic environment surrounding the impaled neurones will be discussed in the following sections.

Transient depolarization at initial stage of hypoxia

The initial depolarization was associated with a slight increase in input resistance, and was blocked by cobalt (2 mM)- or TTX (0·3 μM)-containing medium. This suggests that either the depolarization is due to depression of the spontaneous i.p.s.p.s, or that it is brought about by a direct action such as inactivation of $I_{K,Ca}$ in the impaled neurone. Since the post-tetanic hyperpolarization (probably due to $I_{K,Ca}$) was not significantly altered 0·5–1 min after the onset of hypoxia, inactivation of the voltage-dependent current is not likely to contribute to the generation of the initial depolarization. It has been reported that even in slice preparations, CA1 neurones are under a tonic inhibitory control exerted by spontaneously active basket cell interneurones (cf. Alger & Nicoll, 1980; Buckle & Hass, 1982), and that the reversal potentials for chloride-dependent and potassium-dependent i.p.s.p.s of CA1 neurones are −71 and −91 mV, respectively, which are more negative than the resting membrane potential (Newberry & Nicoll, 1985). Thus the initial depolarization may be due to suppression of the spontaneous i.p.s.p.s resulting from depression of the spontaneous firing in the basket cells following hypoxic exposure.

Transient hyperpolarization at early stage of hypoxia

The transient hyperpolarization was accompanied by a decrease in input resistance. The results showing that the hyperpolarization was markedly enhanced in potassium-free medium, depressed in high-potassium solutions and reversed in polarity at a membrane potential of −83 mV, suggest that the hyperpolarization probably involves an increase in potassium conductance.

We considered the possibility that the activation of voltage-dependent potassium channels may contribute to the generation of the hypoxia-induced hyperpolarizations, as has been suggested previously (Hansen *et al.* 1982). Of the variety of voltage-dependent potassium currents, $I_{K,Ca}$ might play a main role in producing the hyperpolarization, since hypoxia would cause an increase in intracellular calcium concentration by blocking the ATP-dependent sodium pump, and processes such as ATP-dependent calcium extrusion and sodium–calcium exchange driven by the sodium gradient and calcium uptake systems located in the mitochondria and other organelles. Moreover, the acidification of neurones during hypoxia (Whittingham *et al.* 1984) might also contribute to the increase in intracellular calcium concentration via proton–calcium exchange in mitochondria. Indeed, it has been reported that in hippocampal slices intracellular ATP decreased by 15 % after about 2 min of hypoxia (Lipton & Whittingham, 1982) and that intracellular pH decreased by 0·7 after 10 min of hypoxia (Kass & Lipton, 1982). The present results, however, showed that intracellular injection of EGTA did not affect the hypoxia-induced hyperpolarization. These data therefore conflict with the possibility that calcium acts as a messenger for activating potassium channels in hypoxia (e.g. Krnjević, 1975). Moreover, the result that the post-tetanic hyperpolarization was completely abolished by 4 min of hypoxic exposure suggests that $I_{K,Ca}$ may be inhibited by acidification of the cytoplasmic membrane surface, even if hypoxia caused an increase in intracellular calcium concentration. $I_{K,V}$ would be also depressed by intracellular acidification, since 4 min of hypoxic exposure increased the duration of action potentials and

decreased the amplitude of the after-hyperpolarization. These results are consistent with the studies on the depressant effects of pH or $I_{K,Ca}$ of pancreatic B cells (Cook, Ikeuchi & Fujimoto, 1984) and on $I_{K,V}$ of myelinated nerve fibres (Hille, 1968; Drouin & The, 1969).

I_Q is not likely to contribute to the hyperpolarization because the response was not significantly affected by the caesium-containing or sodium-free medium, in which I_Q is strikingly reduced in hippocampal neurones (Halliwell & Adams, 1982; Segal & Barker, 1984). The present result that the hyperpolarization was insensitive to 4-AP contrasts with a previous observation (Hansen *et al.* 1982). It has been reported that in rat hippocampal neurones, I_A activates rapidly, reaching a peak within 3–5 ms, and inactivates quickly (< 30 ms; Segal & Baker, 1984). Therefore, even if 4-AP could selectively depress I_A, it is unlikely that I_A is linked to the hypoxia-induced hyperpolarization. Barium depressed the hypoxia-induced hyperpolarization. However, it has been suggested that external barium has actions other than on I_M (Schwindt & Crill, 1981). Moreover, I_M has been reported to be inactivated at membrane potentials negative to −62 mV (Halliwell & Adams, 1982). Thus, we would argue that its contribution seems to be minimal. From these results, and in view of the almost linear relationship between the amplitude of the hyperpolarization and the membrane potential, at least over the range −50 to −100 mV, it is most likely that the hypoxia-induced hyperpolarization is produced mainly by activation of voltage-independent potassium channels. Patch-clamp techniques reveal ATP-dependent potassium channels in cardiac muscle (Noma, 1983; Kakei & Noma, 1984) and in pancreatic B cells (Cook & Hales, 1984). Treatment with cyanide or perfusion of the cytoplasmic surface of the membrane with ATP-free solutions activates these channels (Noma, 1983; Kakei & Noma, 1984), which are insensitive to membrane potential and intracellular pH (Cook & Hales, 1984). The characteristics of ATP-dependent potassium currents seem to be qualitatively similar to those of the voltage-independent potassium currents which result in the hypoxia-induced hyperpolarization.

Although the hypoxia-induced hyperpolarization was reversed in polarity at a membrane potential of −83 mV, i.e. 9 mV more positive than the potassium equilibrium potential estimated from the reversal potential for post-tetanic hyperpolarization, the hyperpolarization was not significantly affected by low-chloride, low-sodium or cobalt-containing media. In addition, the reversal potential for the hyperpolarization obtained using potassium chloride electrodes was very similar to that obtained using potassium acetate electrodes. These results suggest that the involvement of ions other than potassium ions in the hyperpolarization is, if anything, minimal. Thus, the difference between the reversal potentials may be due to an inactivation of the electrogenic sodium pump during the hypoxic hyperpolarization.

Since it has been reported that tissue cyclic AMP levels in hippocampal slices rise during hypoxia (Whittingham *et al.* 1984) and that application of cyclic AMP causes either inhibition of spontaneous activity or hyperpolarization of hippocampal cells (Segal & Bloom, 1974; Segal, 1981), it is possible that the hypoxia-induced hyperpolarization is mediated by intracellularly produced cyclic AMP. Our results agreed with these findings, dibutyryl cyclic AMP causing a membrane hyperpolarization in two out of five neurones, and suppressing the hypoxia-induced hyperpolarization.

However, the inhibitory effect of dibutyryl cyclic AMP was transient, i.e. the amplitude of the hypoxia-induced hyperpolarization returned to the pre-hypoxic value following a prolonged application of dibutyryl cyclic AMP. It has been suggested that both cyclic AMP and dibutyryl cyclic AMP can cause inhibitory effects in the hippocampus by an action at extracellular adenosine receptors (Dunwiddie & Hoffer, 1980; Madison & Nicoll, 1986) and that intracellular injection of cyclic AMP causes a small depolarization accompanied by an increase in input resistance (Madison & Nicoll, 1986). Thus it is most unlikely that cyclic AMP is involved in the generation of the hypoxia-induced hyperpolarization.

Depolarizations during long-term exposure of hypoxia

Our experiments show that following prolonged application of hypoxic solution, ouabain at relatively high concentrations or high-potassium medium, the membrane was depolarized and membrane potential was maintained at levels 20–30 mV positive to the pre-hypoxic resting potential. These results suggest that depression of sodium pump activity is the main cause of the depolarization, i.e. a decrease in the electrogenic pump activity and the resultant elevation of extracellular potassium concentration is involved in the slow depolarization. The accumulation of intracellular sodium ions may not be a major factor in the depolarization because a low sodium-ion permeability, i.e. a P_{Na}/P_K of 0·02, has been reported in olfactory cortex cells *in vitro* (Scholfield, 1978). Since the slow depolarization was not significantly altered by either low-sodium or low-chloride medium, or cobalt-containing solutions, the contribution of ions other than potassium seems to be rather small.

In one-fifth of the neurones tested, the hypoxia-induced slow depolarization was followed by a much faster depolarization. Since this rapid depolarization began at membrane potentials of -30 to -40 mV, and the neurones did not recover to the normoxic condition when the slice preparation was reoxygenated during the period of the rapid depolarization, a marked influx of calcium ions probably triggers irreversible processes, as described previously (Hass, 1981; Siesjö, 1981; Kass & Lipton, 1982). It has been reported that blockage of synaptic transmission by a high concentration of extracellular magnesium ions, low extracellular calcium concentration or TTX can prevent hypoxic damage in cultured hippocampal neurones (Rothman, 1983). This effect is also assumed to result from the lack of calcium influx activated by synaptic responses and by action potentials. On the other hand, formation of superoxide free radicals and lipid peroxidation during and after prolonged hypoxia has been reported (Chan & Fishman, 1980). This could also be involved in the depolarization after reoxygenation and the irreversible cell dysfunction. Although of great interest, such mechanisms need direct experimental evidence.

Suppression of i.p.s.p.s at early stage of hypoxia

In most cells the membrane was hyperpolarized by about 5 mV following 3–4 min of hypoxic exposure, and the i.p.s.p.s evoked by supramaximal stimuli were completely abolished. Restoration of the membrane potential to the pre-hypoxic level by cathodal currents could not reverse the blockade of i.p.s.p.s. Since the input resistance during the hyperpolarization was decreased to 40–50 % of the control value,

suppression of the i.p.s.p.s could be partially explained by the shunting effect on the synaptic potentials. Other possible explanations are, for example: (1) depression of the spike generation in the basket cells, (2) blocking effects on impulse invasion into the presynaptic nerve terminals, (3) reduction of transmitter release from the presynaptic sites, (4) depression of sensitivity of the receptors, and (5) blocking actions on the channels linked with the receptors. The finding that the initial depolarization could result from depression of spontaneous firing in the basket cells seems to support indirectly the first and/or second explanation. In addition, it has been demonstrated previously that in hippocampal pyramidal neurones an increase in chloride conductance induced by GABA is well maintained after 25 min of hypoxic exposure (Hansen *et al.* 1982). Therefore, the depression of i.p.s.p.s is presumably due to a presynaptic mechanism, but further work is necessary to elucidate the mechanisms involved.

The transient hyperpolarization induced by reoxygenation

The present study demonstrates that the reoxygenation-induced hyperpolarization did not reverse polarity at the reversal potential for the post-tetanic hyperpolarization, and that the hyperpolarization was blocked by removal of potassium ions or by addition of ouabain to the extracellular solution. These results suggest that the hyperpolarization is brought about by reactivation of the electrogenic sodium pump. The reoxygenation-induced hyperpolarization at the resting potential (approximately -65 mV) had a shorter duration than that measured at the membrane potential close to the potassium equilibrium potential (-80 to -90 mV). The difference could be explained by the coexistence of the potential shift due to inactivation of the voltage-independent potassium currents with the reoxygenation-induced hyperpolarization at the resting membrane potential. This potential shift might mask the true duration of the post-hypoxic hyperpolarization.

Hypoxic environment surrounding the impaled neurone

The present result reveals that in the majority of CA1 neurones (twenty-two out of twenty-eight), changes in the membrane potential, input resistance and synaptic potentials following 20–40 min of hypoxic exposure reversed to reach pre-hypoxic levels 15–20 min after reoxygenation. It has been reported that there is almost no recovery of the evoked population spike following 10 min exposure to 95 % N_2–5 % CO_2 in hippocampal slices from adult rats (Kass & Lipton, 1982). On the other hand, it has been demonstrated that the hypoxic suppression of the population e.p.s.p.s and spikes of CA1 neurones in the guinea-pig hippocampal slice is fully reversible after 30 min of exposure to 95 % N_2–5 % CO_2 (Hansen *et al.* 1982) and that the CA1 population spikes in some rat hippocampal slices can be maintained during 20–40 min exposure to 75 % N_2–20 % O_2–5 % CO_2 (Reid *et al.* 1984). The hypoxic condition in our experiments was very close to that used in the experiments of Kass & Lipton (1982). The CA1 neurones impaled in the present study were located at approximately 100 μm depth from the surface of the slice. Direct measurements of P_{O_2} in the olfactory cortex showed that P_{O_2} fell to zero in mid-slice if the thickness exceeded 430 μm, for conditions approximating two-side perfusion with a solution saturated with 95 % O_2 (Fujii, Baumgartl & Lubbers, 1982). Ganfield, Nair & Whalen

(1970) measured P_{O_2} in the cat cerebral cortex slab, 1000 μm thick, with a surface P_{O_2} of 250 mmHg, and found that the critical depth where P_{O_2} was zero was 108 μm. From these data, we estimate that during hypoxic exposure in our experiments, P_{O_2} in the interstitial space at 100 μm below the slice surface would be 0 mmHg. This speculation seems to be supported by the direct measurement of P_{O_2} in the hippocampal slice 100–200 μm below the surface following exposure to 95% N_2–5% CO_2 (see Fig. 2 in Hansen *et al.* 1982).

It has been shown that intracellular lactate levels do not rise during *in vitro* ischemia, even though intracellular pH exhibited a decrease similar to that following decapitation and *in vivo* ischemia, and that the lack of increased lactate concentrations probably results from the continued media flow during that period (Whittigham *et al.* 1984). It is well known that in *in vivo* rat brain cortex, the extracellular potassium concentration gradually increases for the first 1 min of hypoxia, suddenly rising steeply from 10 to 60 mM after 2 min of hypoxia, and subsequently increasing to almost 80 mM during the next few minutes (Hansen, 1977). Such a drastic change in extracellular potassium concentration is not apparent in the *in vitro* slice preparation, judging from the membrane potential changes of the neurones in the present experiments. Thus, the continuous superfusion of the slices with normal potassium medium may prevent accumulation of extracellular potassium ions, and consequently cause such a small depolarization and resultant small calcium influx into the cytoplasm, that the neurones are protected from irreversible damage. Following application of hypoxic solution, the membrane potential of CA1 neurones was maintained at a level 20–25 mV more positive than the resting membrane potential, while the membrane potential under hypoxic conditions in the high-ouabain medium declined continuously and eventually reached 0 mV. These results suggest that the activity of the sodium pump may not be completely depressed by 40 min of hypoxic exposure. Alternatively, the activity of the sodium pump could be partially sustained by ATP generated through anaerobic metabolism.

We are grateful to Drs S. Nishi and C. Polosa for helpful reviews of the manuscript and Mr Y. Sato for technical assistance. This study was supported by a Grant-in-Aid for Scientific Research of Japan.

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