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Action of adenosine receptor antagonists on hypoxia-induced effects in the rat hippocampus *in vitro*

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1 We have studied three hypoxia-induced phenomena in the CA1 stratum pyramidale of the rat hippocampal slice: (a) the increase in extracellular potassium ion concentration ($[K^+]_e$) measured with ion-sensitive microelectrodes, (b) the intracellularly-recorded pyramidal cell hyperpolarization and (c) the extracellularly-recorded depression of the synaptically-evoked field potential recorded in stratum pyramidale.

2 The extracellular potassium ion concentration ($[K^+]_e$) rose from 3 mM to 4.1–4.4 mM at a time when the pyramidal cells hyperpolarized by about 6 mV and neurotransmission was virtually abolished.

3 Presumed glial cells depolarized in response to hypoxia. The shape and time course of this response was remarkably similar to the rise in $[K^+]_e$ so induced. This is consistent with findings that glial cell membrane potential is dependent on transmembrane K^+ gradient.

4 We investigated the effects of theophylline (100 μM) and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, 0.1 μM) on these effects. We have found that these compounds attenuated by about half the hypoxia-induced increase in $[K^+]_e$; however, they did not reduce the hypoxia-induced hyperpolarization. We have confirmed that they dramatically reduced the suppression of excitatory transmission caused by the hypoxia.

We conclude that adenosine A_1 receptors may be involved in the alteration of K^+ homeostasis in the hippocampal slice during hypoxia.

Keywords: Hippocampus; hypoxia; potassium ions; adenosine receptor antagonists; glia

Introduction

Electrical activity within the brain is rapidly lost under conditions of oxygen deprivation; this is seen as a suppression of electroencephalographic activity *in vivo* (e.g. Pulsinelli & Brierley, 1979) and as a depression of synaptically evoked potentials *in vivo* and *in vitro* (Anderson 1960; Hansen *et al.*, 1982). While the exact mechanism of these changes is unclear, some studies have attributed this failure to lack of energy supply (Lipton & Whittingham, 1982). In the hippocampus, a structure known to be highly sensitive to oxygen deprivation (e.g. Pulsinelli & Brierley, 1979) a number of ionic and electrophysiological changes are known to occur. Extracellular K^+ concentration ($[K^+]_e$) begins to increase soon after the onset of hypoxia or anoxia; this has been demonstrated both in rats *in vivo* (Hansen, 1977) and in slices maintained *in vitro* (Hansen *et al.*, 1982). The mechanism(s) behind this increase are currently obscure. It certainly begins at a time when the ATP level is still relatively high (Ekholm *et al.*, 1992) and, therefore, is unlikely simply to reflect failure of Na^+/K^+-ATPase activity. Intracellular recording studies (both *in vivo* and *in vitro*) have revealed that CA1 pyramidal neurones initially hyperpolarize during oxygen deprivation, and if the insult is severe or protracted, they will eventually depolarize with the membrane potential approaching 0 mV. The hyperpolarization has been shown to be mediated by an increase in K^+ conductance of the cell, although the identity of the potassium channel(s) remains to be determined (Hansen *et al.*, 1982; Fujiwara *et al.*, 1987; Leblond & Krnjevic, 1989; Xu & Pulsinelli, 1994). It has been suggested that the efflux of cellular K^+ underlying this hyperpolarization may be in part responsible for the observed increase in $[K^+]_e$ (see Martin *et al.*, 1994). As mentioned, electrically-evoked excitatory synaptic potentials are depressed and eventually abolished during hypoxia or anoxia in slices. Available evidence points to a presynaptic locus for transmission failure as presynaptic Ca^{2+} currents, and therefore neurotransmitter release, are inhibited during anoxia at a time when postsynaptic cell responsiveness to excitatory neurotransmitters is largely unaffected (Young & Somjen, 1992; Hershkowitz *et al.*, 1993).

Adenosine is known to be a potent inhibitor of neuronal activity throughout the central nervous system (see Stone, 1981; Dunwiddie, 1985). The rat hippocampus contains high concentrations of adenosine A_1 receptors, especially in the CA1 region (Fastbom *et al.*, 1987) and it is these receptors that are thought to mediate the inhibitory actions of adenosine (Reddington *et al.*, 1982). Activation of these receptors is known to reduce presynaptic calcium influx in the terminal region of hippocampal CA3 pyramidal cells where they innervate CA1 pyramidal cells (Wu & Saggau, 1994) and to hyperpolarize CA1 pyramidal cells (Greene & Haas, 1985). These effects of adenosine are relevant as a possible explanation for the synaptic transmission failure and cellular hyperpolarization that occurs during hypoxia and anoxia, for it is well known that such insults lead to elevated extracellular adenosine concentrations both *in vivo* and *in vitro* (Zetterström *et al.*, 1982; Fredholm *et al.*, 1984). Indeed, studies have revealed that adenosine A_1 receptor antagonism can delay the hypoxia-induced depression of synaptically evoked potentials (Fredholm *et al.*, 1984; Fowler, 1989; Katchman & Hershkowitz, 1993).

In order to clarify the role of adenosine in these changes, we have monitored three parameters: the effects of hypoxia on (1) the increase in $[K^+]_e$, (2) the pyramidal cell hyperpolarization and (3) the depression of excitatory synaptic transmission in the CA1 stratum pyramidale of rat hippocampal slices. We have confirmed that adenosine receptor antagonists inhibit the depression of excitatory synaptic transmission and that A_1 receptor activation seems unlikely to contribute to hypoxia-induced cellular hyperpolarization. Additionally, we report here that A_1 receptor activation may contribute to the increase in $[K^+]_e$ seen in the CA1 pyramidal cell layer of the hippocampal slice during hypoxia.

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A preliminary account of this study has been published (Croning *et al.*, 1994).

Methods

Male Sprague-Dawley rats (70–110 g, Harlan Olac, Bicester, U.K.) were anaesthetized with pentobarbitone (440 mg kg^{-1}) and killed by decapitation. Transverse hippocampal slices (350 μm) were cut at 4°C and transferred to a holding chamber where they remained at room temperature for 1–2 h before the experiment. Slices were transferred and submerged in a chamber (volume 0.5 ml) which was perfused (2–3 ml min^{-1}, 30°C) with an aqueous solution containing (mM): NaCl 125, KCl 2, KH_2PO_4 1, $MgSO_4$ 1, glucose 5, $NaHCO_3$ 25, $CaCl_2$ 2.5 gassed with 5% CO_2 in O_2 under normoxic conditons, or 5% CO_2 in N_2 to induce hypoxia. Slices were superfused with normoxic medium for at least 30 min before experimental measurements were taken.

Near-maximal field potentials were evoked every 30 s by a concentric bipolar stimulating electrode (2 mA, 100 μs) placed in the CA3 stratum radiatum, the evoked field potential being recorded in the CA1 stratum pyramidale by a single-barrelled micropipette filled with the aqueous superfusate (3–10 MΩ). The evoked field potentials were displayed on a digital storage oscilloscope and subsequently printed to a chart recorder. Evoked potentials were quantified by measuring the distance between the positive and negative peaks of the population spike. Slices were subjected to further experimentation only if the evoked field potential was free of secondary spikes.

Intracellular recordings of CA1 pyramidal cell membrane potentials were made with 3M KCl-filled microelectrodes (55–110 MΩ). The apparent input resistance of cells was measured by passage of hyperpolarizing current pulses (0.2–0.3 nA, 150 ms every 5 s). Experiments were performed only if the cells had stable membrane potentials and action potentials >85 mV. Occasionally, we made recordings from presumed glial cells: they did not produce action potentials upon injection of depolarizing current, they had a very low apparent membrane resistance and they had more negative membrane potentials (ca.–90 mV).

In separate experiments, $[K^+]_e$, evoked field potential, and extracellular d.c. potential were measured in the CA1 stratum pyramidale by ion-selective microelectrodes based on the neutral ionophore valinomycin. Micropipettes were formed in a vertical puller from double-barrelled borosilicate capillary glass. One barrel of the micropipette was silanized by exposure to N,N-dimethyltriethyl-silylamine vapour for 1 h. Micropipettes were placed in an oven (180°C) for 18 h. Micropipette tips were broken under microscopic control to diameters of 5–8 μm. The tip of the silanized barrel of the pipette was back-filled with K^+ ion-selective resin (Fluka Potassium Ionophore I – Cocktail A) giving a column 2–3 mm high. The remainder of the barrel was filled with 150 mM KCl. The reference barrel was back-filled with 150 mM NaCl. The potential difference between the barrels showed a logarithmic relationship to the extracellular potassium ion concentration. Electrodes were calibrated in the recording chamber by the addition of known concentrations of KCl to the superfusate. Only electrodes responding with >55 mV per $\log_{10}$ unit increase in $[K^+]$ were used in the experiments: a change of 60 mV is predicted by the Nernst equation. These electrodes were highly selective for K^+ (over Na^+ and Ca^{2+}) and had rapid response times (<5 s) as previously reported (Ammann *et al.*, 1987). They could be stored for up to a week at 4°C and could be used repeatedly. The potential difference between the ion-selective and reference barrels (indicating $[K^+]_e$) and the potential difference between the reference barrel and the bath electrode (called here the extracellular d.c. potential) were displayed on a dual-pen chart recorder. Evoked field potentials were recorded via the reference barrel of the ion-selective microelectrode and displayed and printed as above. The extracellular K^+ concentration ($[K^+]_e$) was calculated from the calibration line relating the potential difference between the barrels and $[K^+]_e$ determined before and after each experiment. A schematic representation of a K^+ ion-selective microelectrode and recording apparatus is shown in Figure 1.

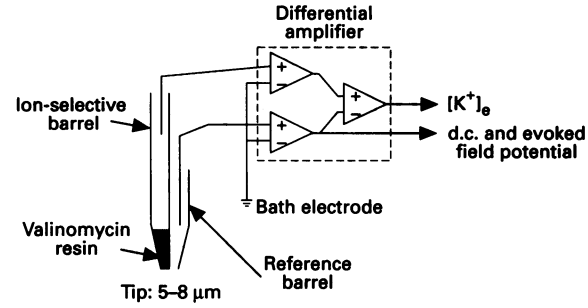

Figure 1 Schematic diagram of the equipment used to measure the extracellular potassium ion concentration ($[K^+]_e$). The double-barrelled ion selective microelectrode has the tip of one barrel filled with the potassium ionophore valinomycin, the remainder of this barrel is filled with 150 mM KCl. The reference barrel is filled with 150 mM NaCl. $[K^+]_e$ can be inferred from the potential difference between the barrels. The extracellular d.c. potential and evoked field potential were recorded between the reference barrel and the bath electrode.

Each slice was subjected to a single period of hypoxia (5 min) with or without antagonist pretreatment (30 min). To confirm that the superfused concentrations of the adenosine receptor antagonists had been effective in reducing adenosine-induced responses, in some experiments, following recovery from hypoxia, we measured the hyperpolarization or suppression of evoked field potential induced by adenosine in the presence or absence of these compounds.

Data are presented as median (range, number), or individual observations, and a significant effect was considered as $P<0.05$ in the Mann-Whitney U-test, (unpaired).

Theophylline and adenosine were obtained from Sigma Chemical Co. Ltd. (Poole, U.K.). N,N-dimethyltriethyl-silylamine and Potassium Ionophore I – Cocktail A were obtained from Fluka Chemicals Ltd. (Gillingham, U.K.); 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) and (5R, 10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclo-hepten-5,10-imine hydrogen maleate [(+)-MK-801] from Research Biochemicals Incorporated (Semat Technical (UK) Ltd., St. Albans, U.K.). DPCPX was initially dissolved in ethanol and diluted 1 in 10000 into the superfusate. All other chemicals used were Analar reagent grade from BDH, Merck Ltd. (Lutterworth, U.K.).

Results

Effect of hypoxia and adenosine on the intracellularly recorded membrane potential

Under control conditions pyramidal cells had a membrane potential of ca.–70 mV and an apparent input resistance of ca. 50 MΩ. After switching to hypoxic medium and a delay of 1–2 min, all cells hyperpolarized by ca. 6 mV with a reduction in input resistance (see Figure 2a and Table 1). On reperfusing normoxic medium, there was a further hyperpolarization of 6 mV (2 to 9 mV, $n=15$) accompanied by a gradual increase in input resistance. After a period of ca. 20 min of 'reoxygenation', the membrane potential recovered to near control levels. In 8 out of 15 cells, a slight depolarization of 1 to 3 mV, often associated with action potential firing, was seen just prior to the hypoxia-induced hyperpolarization. Two out of 15 cells hyperpolarized during hypoxia, then depolarized strongly, before the end of the hypoxic insult (i.e. >10 mV more positive than the membrane potential prior to hypoxia).

We examined the action of adenosine in some experiments, when the membrane potential had stabilized for 20–30 min

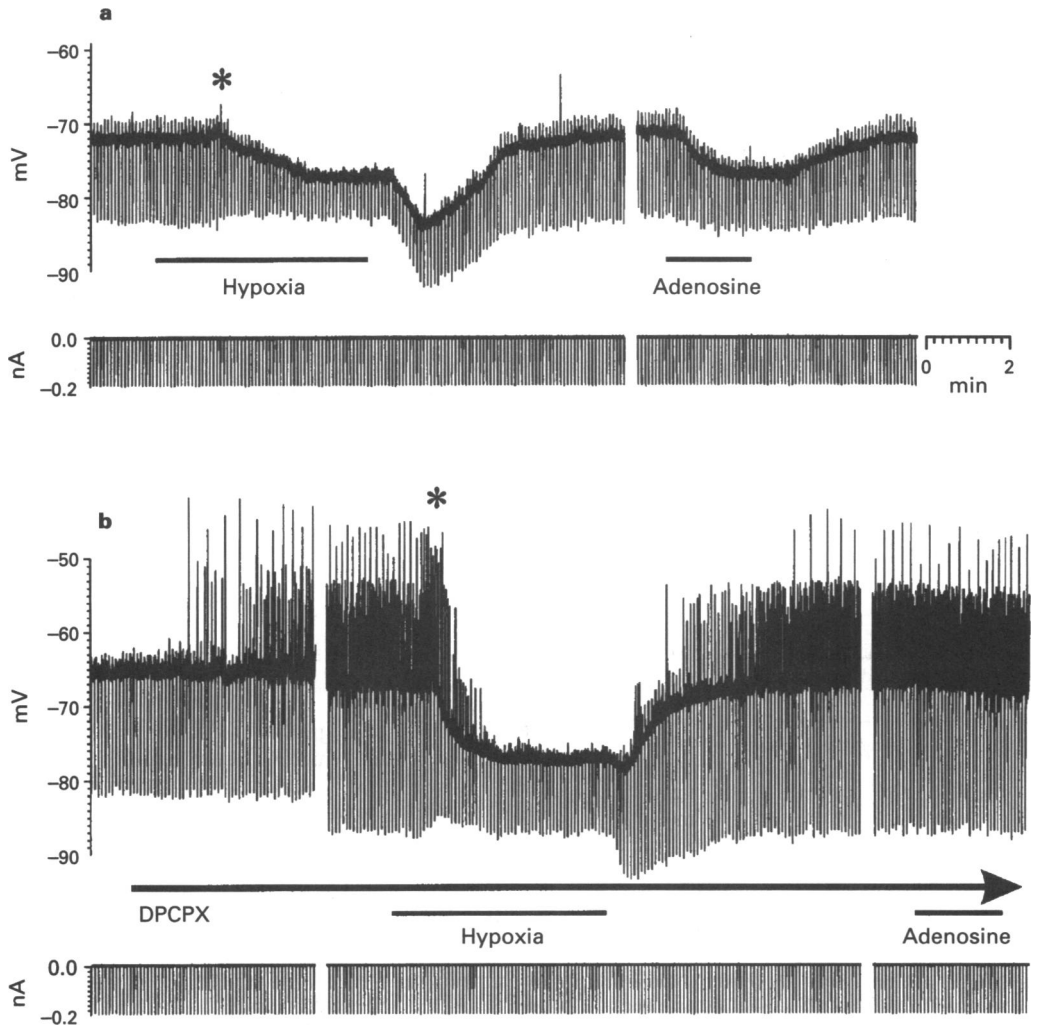

Figure 2 Hypoxia and adenosine-induced membrane potential changes in hippocampal CA1 pyramidal cells. (a) An untreated cell; (b) a cell treated for 30 min with DPCPX (100 nM). Downward deflections of membrane potential (on upper traces) are electrotonic potentials produced by current pulses (150 ms every 5 s) the amplitudes of which are shown on lower traces. The amplitude of the electrotonic potential indicates the apparent input resistance which is reduced during the hypoxia-induced hyperpolarization. Note the further hyperpolarization on returning to normoxic medium. Following recovery from hypoxia (20–30 min), adenosine (30 μM) was superfused. Large upward deflections in b are truncated action potentials, the largest of these are bursts of action potentials. *Indicates transient depolarization seen just prior to hypoxia-induced hyperpolarization. In Figure 2(b) this depolarization is masked by a concomitant increase in action potentials. It can be seen that DPCPX abolishes the adenosine-induced hyperpolarization but does not significantly reduce the hypoxia-induced hyperpolarization.

after the hypoxic insult. The superfusion of adenosine (30 μM, 2 min) reproducibly induced a hyperpolarization of ca. 7 mV which reversed following washout (see Figure 2a and Table 2).

Effect of adenosine receptor antagonists on the hypoxia- and adenosine-induced changes in membrane potential

As previously reported (Alzheimer *et al.*, 1987) the continuous superfusion with theophylline (100 μM) or 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, 100 nM) induced epileptiform activity within the slice and often induced a small depolarization (3–8 mV). The epileptiform activity peaked after 10–15 min and then the frequency of activity tended to fall, reaching a steady state by ca. 25 min. In spite of these effects the pooled membrane potentials of treated cells were not significantly different from untreated controls prior to the hypoxic insult (see Table 1). Pooled apparent input resistances of DPCPX-treated cells were not significantly different from controls; however, those of the theophylline-treated group were. The latter finding is consistent with reports that theophylline can block K^+ channels (e.g. Munakata & Akaike, 1993). Neither treatment significantly reduced the hyperpolarization or the reduction in apparent input resistance induced by hypoxia (see Figure 2b and Table 1). The hyperpolarization which occurred following the reperfusion with normoxic medium was significantly reduced by these antagonists. In 4 out of 8 cells in the presence of theophylline a 'reoxygenation' hyperpolarization measuring 5 mV (2 to 6, $n=4$) was observed. In the presence of DPCPX this hyperpolarization was 2 mV (1 to 3, $n=6$). In 3 of the cells treated with theophylline a pronounced depolarization occurred before the end of the hypoxic insult (not shown) which could have masked the 'reoxygenation' hyperpolarization. This depolarization did not occur in the presence of DPCPX. In the presence of either antagonist, the depolarization preceding the hypoxia-induced hyperpolarization occurred more frequently (13/14 treated cells cf. 8/15 control cells). This depolarization was often accompanied by an increase in epileptiform burst frequency, and as the cells hyperpolarized, this activity ceased (see Figure 2b). Both theophylline and DPCPX significantly reduced the amplitude of the hyperpolarization induced by adenosine (see Figure 2b and Table 2).

Table 1 The actions of adenosine receptor antagonists on the hypoxia-induced effects recorded in the rat hippocampal slice

	Before hypoxia		*Hypoxia-induced effect*			
Antagonists	V_m (mV)	R_m (MΩ)	ΔV_m (mV)	$\downarrow R_m$ (%)	$\downarrow$*Field pot.* (%)	$\uparrow[K^+]_e$ (mM)
Control	−71	50	−6	45	100	1.3
	(−55 to −76)	(20 to 65)	(−3 to −11)	(26 to 80)	100	(1.1 to 1.4)
	$n=15$	$n=15$	$n=15$	$n=15$	100	$n=5$
Theophylline	−65	65*	−10[a]	55	22*	0.6*
(100 μM)	(−58 to −74)	(50 to 85)	(−8 to −14)	(33 to 80)	18	(0.4 to 0.9)
	$n=8$	$n=8$	$n=8$	$n=8$	25	$n=5$
DPCPX	−68	49	−10	57	24*	0.6*
(100 nM)	(−58 to −77)	(43 to 75)	(−4 to −12)	(13 to 71)	15	(0.5 to 0.9)
	$n=6$	$n=6$	$n=6$	$n=6$	16	$n=5$

The intracellularly recorded membrane potential (V_m) and apparent input resistance (R_m) of cells in slices perfused with control medium, theophylline or DPCPX before hypoxia are shown. The hypoxia-induced hyperpolarization of membrane potential (ΔV_m), percentage reduction in apparent input resistance ($\downarrow R_m$), depression of the electrically evoked population spike ($\downarrow$Field pot.) and increase in extracellular potassium ion concentration ($\uparrow[K^+]_e$) are also tabulated. The intracellular measurements of V_m and R_m were carried out in separate experiments from those of field potential and $[K^+]_e$. Data are median (range) and number of experiments. Individual observations are shown for $n=3$. *Significant difference of group from control, $P<0.05$ by Mann–Whitney U-test. [a]$P=0.05$ versus control.

Table 2 The actions of adenosine receptor antagonists on adenosine-induced effects recorded in the rat hippocampal slice

	Adenosine-induced effects		
Antagonists	ΔV_m[a] (mV)	$\downarrow$*Field pot.*[b] (%)	$\uparrow[K^+]_e$[c] (mM)
Control	−7	80	No
	(−6 to −9)	(74 to 92)	observed
	$n=7$	$n=4$	effect ($n=4$)
Theophylline (100 μM)	−4*	4*	Not
	(−2 to −4)	(0 to 7)	determined
	$n=5$	$n=4$	
DPCPX (100 nM)	0*	2*	Not
	(0 to −3)	(0 to 4)	determined
	$n=6$	$n=4$	

The hypopolarization of membrane potential (ΔV_m), depression of the electrically evoked population spike ($\downarrow$ Field pot.) and increase in extracellular potassium ion concentration ($\uparrow[K^+]_e$) caused by adenosine are shown in slices perfused with control medium, theophylline or DPCPX. Concentrations of adenosine used were [a]10 μM, [b]30 μM and [c]10–100 μM. The intracellular measurement of V_m was carried out in separate experiments to those of field potential and $[K^+]_e$. Data are median (range) and number of experiments (or individual observations). *Indicates significant difference of group from control, $P<0.05$ by the Mann–Whitney U-test.

Effect of hypoxia and adenosine on $[K^+]_e$

The basal level of $[K^+]_e$ in the CA1 stratum pyramidale during normoxia was equal to that of the superfusing medium, i.e., 3 mM. After a delay of 1–2 min, hypoxia gradually increased $[K^+]_e$ by about 40% (see Table 1). On the return to normoxic superfusing medium, $[K^+]_e$ rapidly fell to below baseline level by 1.0 mM (0.7 to 1.2, $n=5$) and then gradually returned to the basal level (see Figure 3a). There was no change in extracellular d.c. potential in any experiment as a result of hypoxia.

We considered whether adenosine could increase $[K^+]_e$. The superfusion of adenosine at concentrations which evoked maximal effects on membrane potential and synaptic transmission (10–100 μM) had no effect on $[K^+]_e$. Small increases in $[K^+]_e$ (0.2 to 0.6 mM) could only be evoked with a much higher concentration of adenosine (500 μM, $n=3$).

Effect of hypoxia and adenosine on the evoked field potential

Hypoxia caused a gradual reduction in the evoked field potential amplitude which began at a similar time to the start of the increase in $[K^+]_e$ and resulted in complete abolition of the evoked field potential. Return to normoxic medium resulted in a gradual recovery of the evoked field potential, reaching a level similar to that seen prior to hypoxia after ca. 5 min (see Figure 3a and Table 1).

In some experiments, following recovery from hypoxia, adenosine was superfused in order to determine its effect on evoked field potential. Adenosine (10 μM) reduced evoked field potential amplitude by ca. 80% (see Table 2). This inhibition was rapidly reversed upon washout.

It is possible that the rise in $[K^+]_e$ could contribute to the depression of the evoked field potential induced by hypoxia, so in two experiments, the effect of raising $[K^+]$ in the superfusate from 3 to 5 mM was investigated. In both cases primary population spike amplitude was increased by ca. 15%.

Effect of adenosine receptor antagonists on hypoxia-induced changes in $[K^+]_e$ and evoked field potential

As stated above, the superfusion of theophylline (100 μM) or DPCPX (100 nM) resulted in epileptiform activity in the slices. In these experiments, this was recorded as population spikes using the reference barrel signal of the ion-selective electrode, often with coincident transient increases in $[K^+]_e$ of 0.1–0.2 mM; however, the basal level of $[K^+]_e$ was unaffected by these antagonists. Such epileptiform activity tended to peak within 10–15 min of the start of antagonist treatment, and then fell in frequency, reaching a steady level before the start of the hypoxic insult. As previously reported (Alzheimer *et al.*, 1989), the antagonists also had a facilitatory effect on the evoked field potential, increasing primary spike amplitude sometimes with the introduction of additional population spikes (cf. Figure 3a and 3b).

Pretreatment with either theophylline (100 μM) or DPCPX (100 nM) significantly reduced the hypoxia-induced increase in $[K^+]_e$ to about half of control value (see Figure 3b and Table 1). Often during hypoxia, there was an increase in epileptiform spike frequency during the first 1–2 min of the insult and then this activity was rapidly abolished.

Both antagonists, significantly reduced the fall in evoked field potential amplitude seen during hypoxia, so that at the end of the 5 min period it was reduced only by ca. 20% (see Table 1). The compounds also markedly decreased the in-

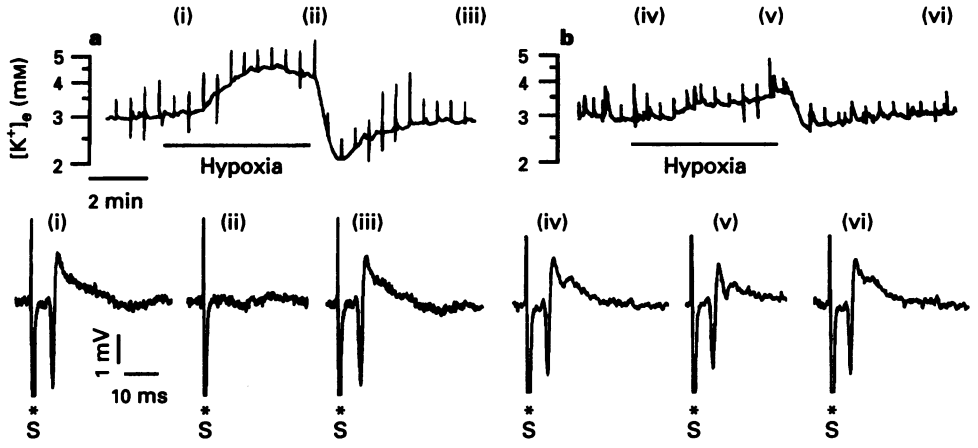

Figure 3 Hypoxia-induced increase in extracellular potassium ion concentration ($[K^+]_e$) and depression of the electrically evoked population spike (lower traces) recorded in the CA1 stratum pyramidale. (a) An untreated slice, (b) a slice treated for 30 min with DPCPX (100 nM). The calibration scales for $[K^+]_e$ are logarithmic. Evoked field potentials ((i)–(vi)) were recorded at the times indicated on the $[K^+]_e$ traces. Stimulus artefacts are marked with (*S). Vertical deflections on the $[K^+]_e$ traces are either stimulus artefacts (at 30 s intervals) or spontaneous increases in $[K^+]_e$ due to antagonist-induced epileptiform activity in the slice. Note the 'undershoot' below baseline of $[K^+]_e$ following return to normoxic medium.

hibition of evoked field potentials by adenosine so that reduction in amplitude in the presence of adenosine (10 μM) was less than 10% (see Table 2).

Effect of (+)-MK-801 on these hypoxia-induced effects

It has been shown that NMDA receptor activation can induce the release of adenosine in the rat hippocampus *in vitro* (Manzoni *et al.*, 1994). We therefore tested the possibility that our hypoxic insult caused the release of glutamate which activated NMDA receptors to release adenosine. We found that pretreatment of the slices with the NMDA receptor antagonist (+)-MK-801 (1 μM, 30 min) failed to alter significantly the pyramidal cell hyperpolarization ($P=0.36$, $n=5$), the rise in $[K^+]_e$ ($P=0.26$, $n=4$) or the inhibition of evoked field potential ($P>0.9$, $n=4$) induced by hypoxia. We therefore conclude that NMDA receptor activation is unlikely to be involved in these phenomena *in vitro*.

Effect of hypoxia on presumed glial cells

In five experiments we recorded from presumed glial cells with membrane potentials of –92 mV (–90 to –93). Hypoxia induced a reversible depolarization of 8 mV (6 to 11) of these cells as previously reported (Leblond & Krnjevic, 1989). Following return to normoxic medium, these cells hyperpolarized to membrane potentials more negative than before hypoxia, and then gradually recovered. The shape and time course of these effects was remarkably similar to that of the hypoxia-induced change in $[K^+]_e$ (cf. Figures 3a and 4). Given that the resting membrane potential of this type of cell depends almost exclusively on the transmembrane K^+ gradient (Kuffler, 1967), it is possible to estimate $[K^+]_e$ increase during hypoxia from the magnitude of depolarization of the glial cells during this insult. The Nernst equation was applied twice: first to estimate intracellular K^+ concentration (from glial resting membrane potential and K^+ concentration in the superfusate) and secondly, to infer the $[K^+]_e$ increase during hypoxia. Using this method we estimated a median rise in $[K^+]_e$ of 1.0 mM (range 0.8 to 1.5) which was not significantly different from the increase in $[K^+]_e$ determined with the ion-selective microelectrode technique. This can be viewed as independent corroboration of the results obtained with ion-selective microelectrodes.

Discussion

We have studied three hypoxia-induced phenomena in the CA1 stratum pyramidale of the rat hippocampal slice: (1) the increase in $[K^+]_e$, (2) the pyramidal cell hyperpolarization and (3) the depression of excitatory synaptic transmission, and we have investigated the possible role of adenosine receptor activation in these changes. We have found that adenosine receptor antagonists, probably via an A_1 receptor mechanism, can attenuate hypoxia-induced increase in $[K^+]_e$; however, they do not attenuate hypoxia-induced hyperpolarization. Additionally, we can confirm that adenosine receptor antagonists prevent (or delay) hypoxia-induced transmission failure.

In agreement with previous studies, in the CA1 stratum pyramidale of the rat hippocampal slice (e.g. Hansen *et al.*, 1982), hypoxia increases $[K^+]_e$ which falls below baseline level upon reoxygenation. This 'undershoot' has been attributed to stimulation of Na^+/K^+-ATPase activity by raised extracellular K^+ (Roberts & Sick, 1987). The increase in $[K^+]_e$ seen during

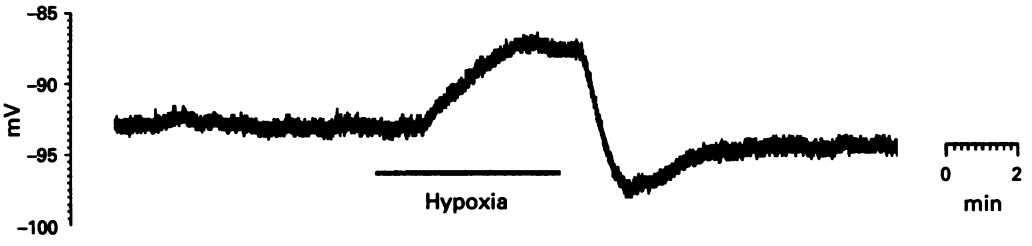

Figure 4 Hypoxia induces a delayed depolarization of a presumed glial cell in the stratum pyramidale of the CA1 region of the hippocampal slice. Note the prominent hyperpolarization on reperfusing normoxic medium. Compare the time-course of these responses to the increase in extracellular potassium concentration shown in Figure 3a.

oxygen deprivation both *in vivo* and *in vitro* is known to be biphasic, the initial increase in $[K^+]_e$ occurs gradually over a period of minutes and the level rises to ca. 10 mM at which point there is an explosive increase to >60 mM. This latter stage (conventionally referred to as phase 2 of the disturbance of ionic homeostasis) coincides with a large negative shift in extracellular d.c. potential (see Hansen, 1985; Jing *et al.*, 1993) which was not observed in the present study, indicating that the hypoxic insult was not of sufficient severity to elicit these changes. Therefore, the $[K^+]_e$ increase seen in the CA1 stratum pyramidale in the current study may correspond to the phase 1 changes observed *in vivo* (see Hansen, 1985; Zetterström *et al.*, 1995). It is possible that the superfusion of the slice tended to limit the increase of $[K^+]_e$ due to removal of excess K^+ and/or the lowered temperature tended to attenuate hypoxia-induced changes (Reid *et al.*, 1988). These findings are different from those reported in studies where interface chambers have been used in which gaseous nitrogen can be directly applied to the slice, as under such insult phase 2 ionic changes are rapidly elicited (e.g. Jing *et al.*, 1993).

The increase in $[K^+]_e$ as a result of hypoxia was attenuated by adenosine receptor antagonists by about half. This attenuation was seen with both theophylline, a non-selective adenosine receptor antagonist (Bruns *et al.*, 1986) and DPCPX, a compound previously shown to be highly A_1 receptor-selective (Lohse *et al.*, 1987). Taken together, this suggests that the adenosine receptor involved in the inhibition of hypoxia-induced increase in $[K^+]_e$ is the A_1 receptor subtype (Fredholm *et al.*, 1994). The reduction of the $[K^+]_e$ increase occurs at a time when the hypoxia-induced K^+-mediated neuronal hyperpolarization was not reduced. This suggests that the rise in $[K^+]_e$ may originate from another cell type and/or these antagonists may increase the clearance of K^+ from the extracellular space. The prevalence of glial cells and their involvement in K^+ homeostasis suggests they could be involved in this effect. Alternatively, these xanthine derivatives could facilitate water movement out of cells (neurones or glia) which could effectively reduce the rise in $[K^+]_e$, possibly by a mechanism similar to their effect in the kidney tubules which is also considered to be mediated by adenosine receptor blockade (Kuan *et al.*, 1993). As mentioned, hypoxia is known to elevate extracellular adenosine levels in the hippocampal slice (Fredholm *et al.*, 1984), therefore, we investigated whether superfusion of adenosine could mimic the increase in $[K^+]_e$ seen during mild hypoxia. However, only a high concentration of adenosine (500 μM) could elicit a small rise in $[K^+]_e$ and this was less than 50% of that seen during hypoxia in the present study.

The changes in CA1 pyramidal cell membrane potential and apparent input resistance were consistent with previous studies (Hansen *et al.*, 1982; Fujiwara *et al.*, 1987; Leblond & Krnjevic, 1989) i.e. the predominant change was a sustained hyperpolarization (preceded by a transient depolarization in ca. 50% of cells) which was followed by a further hyperpolarization on reperfusion with normoxic medium. This latter hyperpolarization probably represents stimulation of the Na^+/K^+-ATPase by increased $[K^+]_e$ as it can be blocked by ouabain (Fujiwara *et al.*, 1987).

In contrast to the finding that theophylline and DPCPX can attenuate hypoxia-induced increase in $[K^+]_e$, the hypoxia-induced pyramidal cell hyperpolarization was not inhibited by these adenosine receptor antagonists. This suggests that cell hyperpolarization during hypoxia is not the result of G-protein-linked adenosine A_1 receptors opening K^+ channels. These results are in agreement with the studies of Leblond & Krnjevic (1989) who found that caffeine, a weak, non-selective adenosine antagonist (Bruns *et al.*, 1986) did not attenuate hypoxia-induced hyperpolarization and Spuler & Grafe (1989) who found that pertussis toxin treatment (which abolished the hyperpolarization induced by application of exogenous adenosine) did not block the hyperpolarization during anoxia.

The fall in evoked field potential amplitude seen during hypoxia is consistent with those of previous studies (e.g. Lipton & Whittingham, 1982). The adenosine receptor antagonists, theophylline and DPCPX greatly reduced the inhibition in evoked field potential seen during hypoxia, confirming previous reports of the role of A_1 receptor activation in this inhibition (Fredholm *et al.*, 1984; Fowler, 1989; Katchman & Hershkowitz, 1993). In order to confirm that the increase in $[K^+]_e$ did not inhibit evoked field potential *per se* (as they occur concurrently), we investigated the effect of increasing $[K^+]$ in the superfusate from 3 to 5 mM. Rather than inhibit transmission, an increase in evoked field potential amplitude was seen.

In summary, the increase in $[K^+]_e$ cell hyperpolarization and reduction in hippocampal cell excitability are consistent with previous studies of hippocampal slices maintained *in vitro* and are similar to those elicited *in vivo* by mild hypoxia. The findings that antagonists of the A_1 receptor can reduce inhibition of evoked field potential during hypoxia and that such antagonists do not reduce hypoxia-induced hyperpolarization are consistent with earlier studies. In addition, the ability of A_1 receptor antagonists to reduce hypoxia-induced increase in $[K^+]_e$ in the CA1 stratum pyramidale suggest a modulatory role for A_1 receptor activation in K^+ homeostasis during hypoxia. It remains to be investigated whether a similar effect occurs in the rat hippocampus *in vivo*.

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