Neuroscience Letters, 98 (1989) 280–284
Elsevier Scientific Publishers Ireland Ltd.

NSL 05967

Adenosine, 'pertussis-sensitive' G-proteins, and K^+ conductance in central mammalian neurones under energy deprivation

A. Spuler and P. Grafe

Department of Physiology, University of Munich, Munich (F.R.G.)

(Received 14 October 1988; Revised version received 5 December 1988; Accepted 5 December 1988)

Key words: Hippocampus; Adenosine; Energy deprivation; Potassium conductance; Pertussis-sensitive G-protein

There is a striking similarity between the effects of adenosine and of hypoxia or glucose depletion on membrane potential and conductance of hippocampal neurones in tissue slices of rat brain. Both induce a membrane hyperpolarization by an increase in potassium conductance. It seemed likely, therefore, that a rise in extracellular adenosine concentration during energy deprivation may link neuronal metabolism with membrane K^+ conductance. To test this hypothesis, we have now investigated the effects of hypoxia/glucose deprivation on hippocampal neurones from pertussis toxin-treated rats. In such slices adenosine had no effect on postsynaptic membrane potential and input resistance. Nevertheless, hypoxia or glucose depletion were as effective as in controls. These data provide evidence against adenosine as the main mediator between cell metabolism and potassium conductance.

An early effect of energy deprivation in central mammalian neurones is an increase in membrane K^+ conductance. This has been observed during hypoxia [5–7, 10] as well as during glucose depletion [14]. The controlling substance which links cellular metabolism and K^+ conductance is still subject of discussion. It has been discussed that adenosine may be such a mediator [9] since adenosine induced an increase in neuronal K^+ conductance [8, 12, 13] in electrophysiological studies on mammalian brain slices. Furthermore, the content of this purine increases in brain tissue under energy deprivation [3, 4, 9]. However, so far this hypothesis has not been adequately explored. In our experiments we made use of the knowledge that adenosine receptors are linked to K^+ channels via pertussis toxin (PTX)-sensitive G-proteins [11]. Therefore, hypoxia and glucose deprivation were explored on PTX-treated rats.

The experiments were performed on transverse slices (500 μm thick) or rat (250–300 g) hippocampus. The slices were kept totally immersed in an experimental chamber perfused with an oxygenated solution containing (in mM); NaCl 118, KCl

Correspondence: A. Spuler, Department of Physiology, University of Munich, Pettenkoferstrasse 12, D-8000 Munich 2, F.R.G.

3.0, $NaHCO_3$ 25, NaH_2PO_4 1.2, $MgCl_2$ 1.0, $CaCl_2$ 1.5, glucose 10 (pH 7.4); sometimes tetrodotoxin (0.6 μM) was added. Hypoxia was induced by bubbling the Krebs solution with N_2/CO_2 (95%:5%). Glucose depletion was achieved by perfusing glucose-free solution. The temperature was kept constant at 30°C. Neurones in the CA3 region were impaled with 3 M KCl-filled microelectrodes and current- or voltage-clamped using a single electrode switched clamp circuit (DAGAN 8100); the switching frequency was 1–2 kHz with a duty cycle of 50%. Drugs were applied in fixed concentrations to the bathing solution (bath-exchange time about 60 s) and the data were recorded and stored on a digital oscilloscope (Nicolet 4094) or on a tape recorder. One group of rats received an intraventricular injection of PTX (5 μg in 5 μl Krebs solution) two days prior to recording, as described by Aghajanian [1]. Untreated animals served as controls.

This study is based on recordings from 38 CA3 neurones with stable membrane potentials more negative than -55 mV. The mean resting membrane potential of neurones under current clamp conditions was -66.1 ± 5.9 mV (mean $\pm$ S.D.; $n=15$) for control animals and -63.1 ± 6.1 mV ($n=23$) for PTX-treated animals. Steady-state input resistance measured with hyperpolarizing current pulses (-0.2 nA, 500–800 ms) was 77.0 ± 11.1 MΩ ($n=15$) in controls and 82.7 ± 19.4 MΩ ($n=23$) in pretreated animals.

Fig. 1 shows the effects of hypoxia, glucose-deprivation and adenosine (300 μM) on a hippocampal CA3 neurone of a control animal. All produced a similar membrane hyperpolarization and a decrease in input resistance. It has been shown that

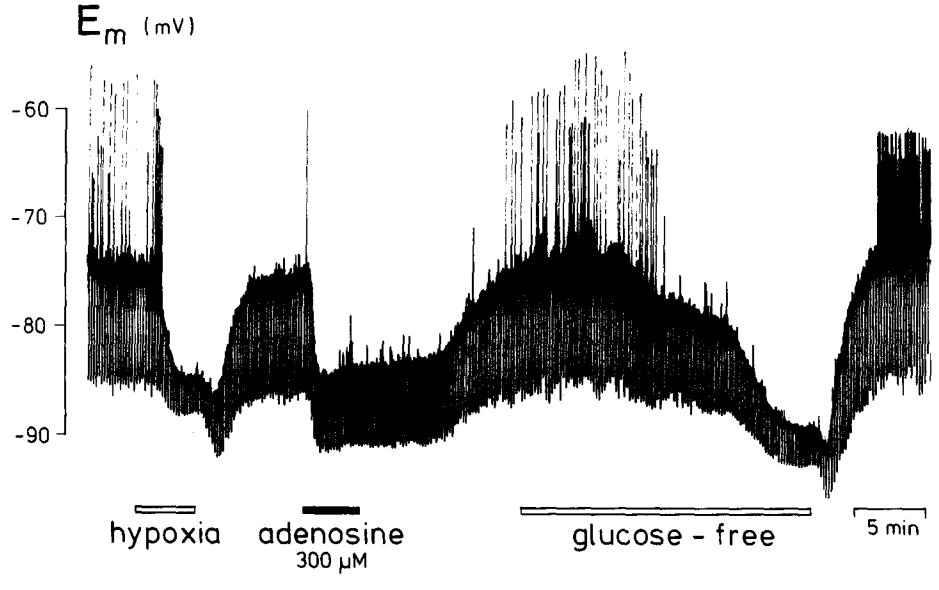

Fig. 1. Effects of energy deprivation and adenosine (300 μM) on membrane potential (E_m) and input resistance of a CA3 neurone in a rat hippocampal slice. Intracellular recording in current-clamp mode was performed with KCl-filled microelectrodes (3 M). For the determination of input resistance, hyperpolarizing current pulses (800 ms, -0.2 nA) were regularly applied via the electrode.

the membrane hyperpolarization under these conditions is due to an enhanced K^+ conductance [5, 8, 10, 14]. In our experiments, however, there was less reduction of membrane resistance for the same hyperpolarization during adenosine as compared to hypoxia. Quantitatively, adenosine (300 μM) reduced input resistance to $59 \pm 11\%$ ($n = 14$) of control, hypoxia to $38 \pm 12\%$ ($n = 17$). This finding indicates that hypoxia has a stronger effect on the membrane K^+ conductance (with an additional rise in extracellular potassium) than adenosine. There was also a difference in the period after application of adenosine and the time after energy deprivation. The latter period was accompanied by a transient membrane hyperpolarization. This event can be explained by a reactivation of the Na^+/K^+ pump [5].

In a second series of experiments, the same experimental protocol was applied to slices prepared from PTX-treated rats. A typical experiment is illustrated in Fig. 2. A CA3 neurone was exposed to hypoxia, adenosine (300 μM), DL-baclofen (5 μM), and glucose-free solution. It was observed that adenosine was unable to hyperpolarize the membrane and to decrease the input resistance. As described by others [2] also the $GABA_B$ receptor agonist baclofen did not induce a change in membrane conductance. Nevertheless, hypoxia or glucose deprivation were as effective as in controls. Both hyperpolarized the cell membrane and reduced input resistance with a time course similar to control slices. The reduction in input resistance during hypoxia was similar in PTX-treated rats (to $35 \pm 16\%$ of control values; $n = 23$) as compared to normal rats ($38 \pm 12\%$; $n = 17$). The transient hyperpolarization during readdition of substrate was also seen. In addition, voltage clamp recordings were performed on slices from pertussis toxin treated rats. Fig. 3 shows V/I curves which were constructed on line by means of a slow (15 s), ramp-shaped voltage command. Three curves are shown which were recorded in control conditions and during application of adenosine (300 μM) or hypoxia. Control curves taken before exposure to adenosine and hypoxia were identical. These experiments revealed that, similar to control slices, energy deprivation opened a K^+ conductance, whereas no effect of adenosine was observed.

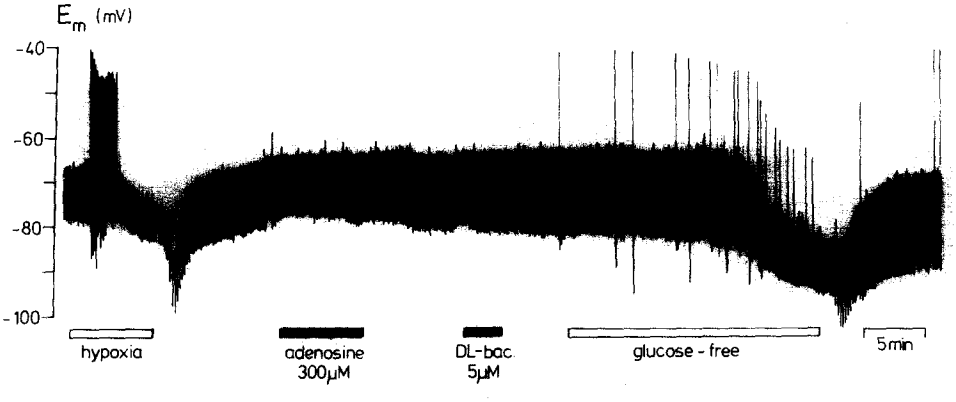

Fig. 2. Effects of energy deprivation, adenosine (300 μM), and DL-baclofen (5 μM) on a CA3 neurone of a rat pretreated with pertussis toxin. Recording conditions were the same as in Fig. 1.

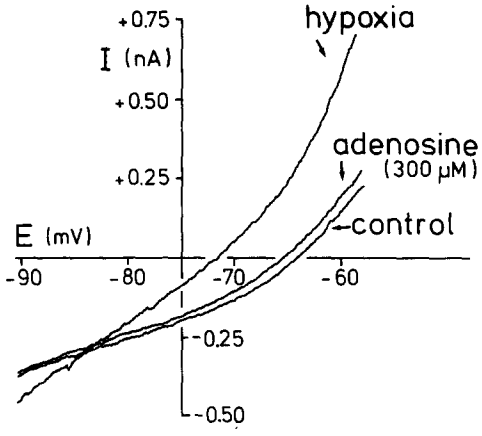

Fig. 3. Hippocampal slice from a rat which was treated with pertussis toxin. Effects of hypoxia and adenosine (300 μM) on the voltage/current (V/I) relationship of a CA3 neurone. Recordings were from the same neurone. Tetrodotoxin (0.6 μM) was present in the bathing solution. The V/I curve was generated with a slow voltage ramp under voltage clamp conditions (see text). Recordings were taken after 4 min of exposure to adenosine and 6 min of hypoxia, respectively.

In summary, our data show that energy failure induces an increase in membrane K^+ conductance. This effect is independent of the function of pertussis toxin sensitive G-proteins. Adenosine, on the other hand, uses this transducing system to enhance K^+ conductance. It is unlikely, therefore, that adenosine is the main mediator between neuronal metabolism and K^+ conductance.

This study was supported by the Deutsche Forschungsgemeinschaft (SFB 220). We would like to thank Mrs. C. Müller for technical and secretarial assistance and W. Endres, J. Haberstroh, and H.L. Haas for helpful discussions.

1 Aghajanian, G.K. and Wang, Y.Y., Pertussis toxin blocks the outward currents evoked by opiate and α_2-agonists in locus coeruleus neurons, Brain Res., 371 (1986) 390–394.
2 Andrade, R., Malenka, R.C. and Nicoll, R.A., A G protein couples serotonin and $GABA_B$ receptors to the same channels in hippocampus, Science, 234 (1986) 1261–1265.
3 Deuticke, B., Gerlach, E. and Dierkesmann, R., Abbau freier Nukleotide in Herz, Skeletmuskel, Gehirn und Leber der Ratte bei Sauerstoffmangel, Pflügers Arch., 292 (1966) 239–254.
4 Fredholm, B.B., Dunwiddie, T.V., Bergman, B. and Lindström, K., Levels of adenosine and adenine nucleotides in slices of rat hippocampus, Brain Res., 295 (1984) 127–136.
5 Fujiwara, N., Higashi, H., Shimoji, K. and Yoshimura, M., Effects of hypoxia on rat hippocampal neurones in vitro, J. Physiol. (Lond.), 384 (1987) 131–151.
6 Glötzner, F., Intracelluläre Potentiale, EEG und corticale Gleichspannung an der sensomotorischen Rinde der Katze bei akuter Hypoxie, Arch. Psychiat. Ztsch. ges. Neurol., 210 (1967) 274–296.
7 Godfraind, J.M., Kawamura, H., Krnjevic, K. and Pumain, R., Actions of dinitrophenol and some other metabolic inhibitors on cortical neurones, J. Physiol. (Lond.), 215 (1971) 199–222.
8 Greene, R.W. and Haas, H.L., Adenosine actions on CA1 pyramidal neurones in rat hippocampal slices, J. Physiol. (Lond.), 366 (1985) 119–127.

9 Hagberg, H., Andersson, P., Lacarewicz, J., Jacobson, I., Butcher, S. and Sandberg, M., Extracellular adenosine, inosine, hypoxanthine, and xanthine in relation to tissue nucleotides and purines in rat striatum during transient ischemia, J. Neurochem., 49 (1987) 227–231.
10 Hansen, A., Hounsgaard, J. and Jahnsen, H., Anoxia increases potassium conductance in hippocampal nerve cells, Acta Physiol. Scand., 115 (1982) 301–310.
11 Isenberg, G., Cerbai, E. and Klöckner, U., Ionic channels and adenosine in isolated heart cells. In E. Gerlach and B.F. Becker (Eds.), Topics and Perspectives in Adenosine Research, Springer, Berlin, 1987, pp. 323–335.
12 Segal, M., Intracellular analysis of a postsynaptic action of adenosine in the rat hippocampus, Eur. J. Pharmacol., 79 (1982) 193–199.
13 Siggins, G.R. and Schubert, P., Adenosine depression of hippocampal neurons in vitro: an intracellular study of dose-dependent actions on synaptic and membrane potentials, Neurosci. Lett., 23 (1981) 55–60.
14 Spuler, A., Endres, W. and Grafe, P., Glucose depletion hyperpolarizes guinea pig hippocampal neurons by an increase in potassium conductance, Exp. Neurol., 100 (1988) 248–252.