ATP-sensitive K\(^+\) channel activation provides transient protection to the anoxic turtle brain

MARTA PÉK-SCOTT AND PETER L. LUTZ
Department of Biological Sciences, Florida Atlantic University, Boca Raton, Florida 33431

Pék-Scott, Marta, and Peter L. Lutz. ATP-sensitive K\(^+\) channel activation provides transient protection to the anoxic turtle brain. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R2023–R2027, 1998.—There is wide speculation that ATP-sensitive K\(^+\) (K\(_{\text{ATP}}\)) channels serve a protective function in the mammalian brain, being activated during periods of energy failure. The aim of the present study was to determine if K\(_{\text{ATP}}\) channels also have a protective role in the anoxia-tolerant turtle brain. After ouabain administration, rates of change in extracellular K\(^+\) were measured in the telencephalon of normoxic and anoxic turtles (Trachemys scripta). The rate of K\(^+\) efflux was reduced by 50% within 1 h of anoxia and by 70% at 2 h of anoxia, and no further decrease was seen at 4 h of anoxia. The addition of the K\(_{\text{ATP}}\) channel blocker glibenclamide or 2,3-butanedione monoxime prevented the anoxia-induced decrease in K\(^+\) efflux during the first hour of anoxia, but the effect of these blockers was diminished at 2 h of anoxia and was not seen after 4 h of anoxia. This pattern of change in K\(_{\text{ATP}}\) channel blocker sensitivity can be related to a previously established temporary fall and subsequent recovery of tissue ATP during early anoxia. We suggest that activated K\(_{\text{ATP}}\) channels are involved in the downregulation of membrane ion permeability (channel arrest) during the initial energy crisis period but are switched off when the full anoxic state is established and tissue ATP levels have been restored. We also found that, in contrast to those in mammals, K\(_{\text{ATP}}\) channels are not a major route for K\(^+\) efflux in the energy-depleted turtle brain.

When deprived of oxygen, the vertebrate brain rapidly goes into energy failure, which results in a loss of ionic gradients, consequent depolarization, and eventual neuronal death (16). The freshwater turtle (Trachemys scripta) is such an outstanding exception to this breakdown, having a brain that can survive at least 48 h of anoxia at 25°C (17), that it serves as a standard against which less-tolerant neural tissues are compared (3). The most important compensation that the turtle brain makes to survive anoxia is lowering its energy consumption to such a degree (70–80%) that brain energy needs can be fully met by anaerobic glycolysis (17). As a result, the turtle brain is able to maintain ATP levels and ionic gradients during anoxia and thus avoid the fatal consequences of energy failure (16).

Because >50% of the brain’s energy costs are devoted to maintaining and restoring ionic gradients, a reduction in membrane ion leakage (channel arrest) can provide important energy savings (7, 9, 17). Several studies indicate that channel arrest is initiated in the anoxic turtle brain. During anoxia, K\(^+\) flux is significantly reduced (4, 12, 20) and there is a decrease in the density of voltage-gated Na\(^+\) channels (22). Anoxia produces a decrease in turtle brain N-methyl-D-aspartate receptor activity and an associated decrease in Ca\(^{2+}\) permeability (2), but the mechanisms behind channel arrest remain elusive.

In the mammalian brain, much attention has been given to the protective role of the ATP-dependent K\(^+\) (K\(_{\text{ATP}}\)) channels during the early stages of ischemia (1, 3). K\(_{\text{ATP}}\) channels are activated by the fall in ATP that occurs when the brain goes into energy imbalance. The resulting increase in K\(^+\) conductance causes membrane hyperpolarization (1, 3). However, there may be regional differences in specifics. In hippocampal neurons, for example, Ca\(^{2+}\)-activated K\(^+\) channels are thought to mediate anoxia-induced hyperpolarization (14). Hyperpolarization would reduce the duration of the action potential, diminish energy consumption, and attenuate the anoxia-induced depolarization (6). Hyperpolarization has also been associated with a decrease in excitatory neurotransmitter release (6, 27). Modulation of the K\(_{\text{ATP}}\) channel is therefore thought to play an important, albeit short-term, protective role during ischemia/anoxia (1, 3, 6). However, over the longer term, by allowing a continuing increase in extracellular K\(^+\), the open K\(_{\text{ATP}}\) channels cause a gradual depolarization of the cell (12, 14). The K\(_{\text{ATP}}\) channels are also thought to be a major route for the rapid K\(^+\) efflux that occurs during anoxic depolarization (12, 23, 25).

We have very little information on K\(_{\text{ATP}}\) channels in the turtle brain. Glibenclamide (a specific K\(_{\text{ATP}}\) channel antagonist) receptor binding sites have been detected in the turtle brain at levels that are ~10–30% of the adult rat brain (see Fig. 6 of Ref. 12), a level that corresponds to the difference in metabolic intensity between their respective brains (16). J. Jang et al. (12) showed that adding glibenclamide to the anoxic turtle brain stem produced no discernible change in extracellular K\(^+\) levels. However, such a lack of effect might be expected because, like the other turtle brain regions, the turtle brain stem has a depressed K\(^+\) permeability and anoxia produces little or no change in extracellular K\(^+\) (12).

The aim of the present study was to determine if K\(_{\text{ATP}}\) channels have a protective function in the anoxia-tolerant turtle brain. We measured rates of change of extracellular K\(^+\) in the telencephalon of the turtle T. scripta after ouabain administration. The measurements were made during normoxia and prolonged anoxia and in the presence of the two K\(_{\text{ATP}}\) channel...
blocks glibenclamide and 2,3-butanedione monoxime (BDM).

MATERIALS AND METHODS

Experiments were conducted on freshwater turtles (T. scripta) weighing 300–500 g. Experimental procedures were approved by the Florida Atlantic University Institutional Animal Care and Use Committee, and all applicable National Institutes of Health guidelines were maintained.

Turtles were ventilated with 4% AE (Isoflurane, Anaquest) in ambient air. The anesthesia was administered via a small animal ventilator (Phipps and Bird, Richmond, VA) using an intratracheal cannula. Once a surgical plane was reached, turtles were maintained on 1.7% isoflurane for the duration of surgery. The animals were ventilated at 1–1.5 breaths/min with a 5- to 7-s inspiration time. This method of delivery ensured a consistent induction time of the anesthesia.

The skin and muscle above the midline of the skull were retracted, and a portion of the skull bone was carefully removed by drilling over the telencephalon. After opening the dura and leptomeninges, we chose a cortical surface for continuous monitoring of the extracellular potassium ion concentration ([K\(^+\)]. The criteria for selecting the optimal cortical area were microscopic observation of normal blood flow and absence of extravasated blood or cortical damage. Calibrated K\(^+\)-sensitive microelectrodes were inserted to a depth of ~100–200 \(\mu\)m from the brain surface (4). Turtle artificial cerebrospinal fluid (aCSF) (4) was administered continuously to the brain surface at a rate of ~1–2 ml/h.

The turtles were distributed into four sets. The first set was divided into three groups. In group A \((n = 5)\), the animals were ventilated with air for 30 min before 80 \(\mu\)mol/l glibenclamide was added to the aCSF superfusing the brain surface. Ouabain (10 \(\mu\)mol/l) was added to the aCSF after an additional 5 min. Because Na\(^+\)-K\(^+\)-ATPase activity is different in anoxic and normoxic turtle brains (10), it was necessary to use high concentrations of ouabain to ensure that all of the Na\(^+\)-K\(^+\)-ATPase was rapidly blocked (4). Group B \((n = 4)\) was treated similarly to group A except that glibenclamide was substituted with 60 \(\mu\)mol/l BDM, a nonsulfonylurea K\(_{ATP}\) channel blocker. The control group C \((n = 5)\) was treated only with ouabain, without the addition of K\(_{ATP}\) blockers to the aCSF. In the second set, after 30 min of air respiration, the animals were switched to 100% \(N_2\) ventilation for 1 h. As \(N_2\) respiration continued, they were treated similarly to the first set, viz. group A \((n = 5)\) glibenclamide administration, group B \((n = 5)\) BDM administration, and group C \((n = 5)\) control. In the third set, the experiments were repeated after 2 h of 100% \(N_2\) respiration (glibenclamide-treated group, \(n = 5\); control group, \(n = 5\)). A similar procedure was adopted for the fourth set after 4 h of \(N_2\) respiration (glibenclamide-treated group, \(n = 5\); control group, \(n = 5\)).

All experiments were conducted at room temperature (25°C).

Statistical analyses were performed with ANOVA and Dunnett’s tests, and data are expressed as means ± SE.

RESULTS

Figure 1 shows typical changes in extracellular K\(^+\) in brains after superfusion with 10 mmol/l ouabain in turtles breathing air and at 1 h \(N_2\) respiration during glibenclamide and BDM treatments. In all cases, the pattern of change in extracellular K\(^+\) was similar to that described for the anoxic mammalian brain (8), i.e., a slow increase in extracellular [K\(^+\)] (phase 1) to a threshold, followed by a rapid increase (phase 2) to a plateau associated with anoxic depolarization (phase 3).

K\(^+\) leakage rate was calculated by measuring changes in extracellular [K\(^+\)] during ouabain superfusion. The rate of K\(^+\) leakage in phase 1 best represents resting K\(^+\) leakage in the brain. This is because K\(^+\) conductance may be affected by other factors when the depolarization produced by increasing extracellular K\(^+\) is sufficiently large to open voltage-gated channels (phase 2) (4). An estimate of the K\(^+\) leakage rate during phase 1 was obtained from measuring the time it took extracellular [K\(^+\)] to increase over baseline levels by 1 mmol/l after ouabain application. The time to reach maximal extracellular [K\(^+\)], which would correspond to full depolarization, was also measured.

Compared with air-breathing animals, the time taken for extracellular [K\(^+\)] to increase by 1 mmol over resting levels had more than doubled after 1 h of anoxia.
(+236%), indicating a corresponding reduction in the rate of K\(^+\) leakage (Fig. 2). By 2 h of anoxia, the time taken for extracellular [K\(^+\)] to increase 1 mmol/l above baseline levels had increased approximately threefold (+345%) (Fig. 2), indicating that the rate of K\(^+\) leakage had reduced still further. However, after 4 h of anoxia, no further diminution in K\(^+\) efflux rates was seen (Fig. 2). The times to reach maximal extracellular [K\(^+\)] levels had also increased by the first hour of anoxia (+209%), and further at the second hour (+274%), with no subsequent increase at 4 h (Fig. 3).

Neither glibenclamide nor BDM superfusion had any measurable effect on the initial rate of K\(^+\) leakage in the normoxic brain (Fig. 2). However, superfusion of either glibenclamide or BDM at 1 h of anoxia fully suppressed the reduction in K\(^+\) efflux rates, which remained at normoxic levels (Fig. 2), and also reduced the time to full depolarization (Fig. 3). By 2 h of anoxia, glibenclamide was less effective, and at 4 h of anoxia, there were no significant differences between treated and untreated brains in K\(^+\) leakage rates (Fig. 2). Glibenclamide superfusion had no effect on the times to full depolarization in the 2-h and 4-h anoxic brains (Fig. 3).

**DISCUSSION**

We have shown that K\(^+\) leakage is reduced in the anoxic turtle telencephalon, similar to that previously described for the turtle olfactory bulb (4), indicating that channel arrest may be the general response of turtle brain to anoxia. We also show that channel arrest is progressively expressed over the first few hours of anoxia. This is illustrated in Fig. 4, in which rates of increase in extracellular [K\(^+\)] at different times in anoxia are presented. There is a reduction in K\(^+\) efflux to ~50% of normoxic values in 1 h and to ~70% at 2 h of anoxia, after which no further decrease is seen (Fig. 4).

This study also presents evidence for the involvement of the K\(_{\text{ATP}}\) channel in the initial establishment of channel arrest and indicates that, in the turtle brain, the K\(_{\text{ATP}}\) channel is not an important mediator of the rapid K\(^+\) efflux that follows Na\(^+\)-K\(^-\)-ATPase failure.

There is considerable interest in identifying the pathways for the K\(^+\) efflux that occurs during brain energy failure (23). In some mammal preparations, e.g., hypoglossal brain slice (11) and cortex (25), blocking the K\(_{\text{ATP}}\) channels with glibenclamide produces a large reduction in the rate of this K\(^+\) efflux, indicating that the K\(_{\text{ATP}}\) channels are a major route for K\(^+\) efflux in anoxia/ischemia. However, there appear to be important regional differences in K\(_{\text{ATP}}\) channel involvement in K\(^+\) efflux. For example, although K\(_{\text{ATP}}\) channels open during ischemia in the rat hippocampus, these channels make only a small contribution to the consequent K\(^+\) efflux (23). We found that in the normoxic and anoxic turtle telencephalon, K\(_{\text{ATP}}\) channel blockers did not reduce the rate of extracellular [K\(^+\)] increase that followed ouabain superfusion, suggesting little or no involvement of the K\(_{\text{ATP}}\) channel as a route for K\(^+\) efflux. Other routes for K\(^+\) efflux, such as calcium-activated K\(^+\) channel or K\(^+\) cotransport or antiport...
systems (14, 23), are probably more important, and these may be downregulated in the anoxic turtle brain. The failure of glibenclamide to reduce K\textsuperscript{+} efflux is not likely to be due simply to the lower density of K\textsubscript{ATP} channels in the turtle compared with mammals (\cite{10-30\% (12)), because similar differences are found in other energy-related processes (16). For example, turtle brain Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activities are \approx 40\% those of mammals and the density of voltage-dependent Na\textsuperscript{+} channels in turtle is \approx 30\% of that of the mammalian brain (16).

The most interesting findings in this study concern the effect of blocking K\textsubscript{ATP} channels on the expression of channel arrest in anoxia. Adding glibenclamide after 1 h of anoxia fully negated the downregulation of K\textsuperscript{+} efflux (further evidence against involvement of K\textsubscript{ATP} channel in K\textsuperscript{+} efflux). That this result was due to the blockage of the K\textsubscript{ATP} channel and was not simply a nonspecific effect of glibenclamide is indicated by the observations that BMD, a specific nonsulphonurea K\textsubscript{ATP} channel blocker, produced identical effects. At 2 h of anoxia, the effect of glibenclamide is greatly reduced, and, at 4 h, glibenclamide had no influence on the anoxic downregulation of K\textsuperscript{+} efflux.

The time course of the involvement of the K\textsubscript{ATP} channel in membrane permeability roughly corresponds to the temporal changes in brain ATP. In the turtle, there is a fall in brain ATP concentrations over the first hour of anoxia which subsequently fully recovers over the next several hours (13, 15). It is tempting to speculate that the turtle brain K\textsubscript{ATP} channels are activated during the first hour or so of anoxia, when ATP is depleted, and are closed when ATP levels are restored during later anoxia.

The mechanisms that link K\textsubscript{ATP} channel activation to the initial reduction in K\textsuperscript{+} efflux in the anoxic turtle brain are unknown. Membrane hyperpolarization is thought to be the primary protective effect of the K\textsubscript{ATP} channel activation in mammals (6), but there is much evidence that the turtle brain [telencephalon (5), cerebral hemispheres (21), brain stem (12)] maintains membrane potential during prolonged anoxia. It is, of course, possible that the activation of K\textsubscript{ATP} channels could increase K\textsuperscript{+} conductance and help stabilize membrane potential without overt hyperpolarization. However, recent evidence points to the involvement of K\textsubscript{ATP} channels in a wide variety of processes. The K\textsubscript{ATP} channel appears to play a role in long-term potentiation (3), and, in the canine heart, the K\textsubscript{ATP} channel has a synergistic interaction with the adenosine A\textsubscript{1} receptor, both being involved in ischemic preconditioning (26).

The K\textsubscript{ATP} channel may also play a role in retarding excitatory neurotransmitter (glutamate, dopamine) release during the initial energy crisis of anoxia/ischemia (14, 24). Perhaps significantly, no increase in extracellular glutamate (15) or dopamine (18) is seen in the anoxic turtle brain.

It is possible, therefore, that the K\textsubscript{ATP} channel plays an important role during the early downregulation of metabolic demand that occurs as the turtle brain becomes anoxic. When energy supply can no longer match energy demand, the fall in brain ATP acts as a signal which activates K\textsubscript{ATP} channels, which in turn initiates processes that result in a reduction in K\textsuperscript{+} efflux. This is not achieved via membrane hyperpolarization but may involve other factors such as the inhibition of excitotoxic neurotransmitter release in the energy-challenged brain and the enhancement of the adenosine A\textsubscript{1} receptor response. When a balance is achieved between ATP demand and (glycolytic) ATP supply, brain ATP levels are restored and the K\textsubscript{ATP} channel is inactivated. However, in the downregulated brain membrane, ionic permeability and other metabolic processes remain depressed, sufficient to accommodate many hours and days of anoxia.

Address for reprint requests: P. Lutz, Florida Atlantic Univ., Dept. of Biology, 777 Glades Road, Boca Raton, FL 33431

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