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ROLE OF ATP-SENSITIVE K^+ CHANNELS DURING ANOXIA: MAJOR DIFFERENCES BETWEEN RAT (NEWBORN AND ADULT) AND TURTLE NEURONS

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SUMMARY

1. It is well known that anoxia induces an increase in extracellular K^+. The underlying mechanisms for the increase, however, are not well understood. In the present study, we performed electrophysiological, pharmacological and receptor autoradiographic experiments in an attempt to examine K^+ ionic homeostasis during anoxia. Ion-selective microelectrodes were employed to measure intracellular and extracellular K^+ activity from hypoglossal neurons in brain slices.

2. During 3–4 min anoxia, adult hypoglossal neurons lose a large amount of their intracellular K^+ and this contributes in a major way to the 8-fold increase in extracellular K^+.

3. Loss of intracellular K^+ from hypoglossal neurons is, to a great extent, due to activation of certain specific K^+ channels. Glibenclamide, a potential sulphonylurea ligand and a specific blocker of ATP-sensitive K^+ (K_{ATP}) channels, has no effect on K^+ homeostasis during oxygenated states, but almost halves the anoxia-induced increase in extracellular K^+ in the adult rat.

4. [^{3}H]glibenclamide autoradiography shows that the hypoglossal nucleus in the adult rat has high sulphonylurea receptor density, a finding that is consistent with our electrophysiological observation.

5. Since we have previously shown that newborn mammals and reptiles are more resistant to O_2 deprivation than adult mammals, we performed comparative studies among adult rat, newborn rat and adult turtle. In sharp contrast to the adult rat, extracellular K^+ activity in newborn rat and adult turtle brain increases little (10 to 100 times less than the adult rat) and glibenclamide has a small and insignificant effect on K^+ efflux in the newborn rat and none in the turtle. Glibenclamide receptor binding sites are much lower in the newborn rat than in the adult rat central nervous system (CNS) and barely detectable in the turtle brain.

6. These results support the hypothesis that in the adult rat, K^+ is lost during anoxia from neurons through sulphonylurea receptor or K_{ATP} channels in a major way. Generally, however, K_{ATP} channels are poorly expressed in the newborn rat and adult turtle CNS and have little role to play during O_2 deprivation.

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INTRODUCTION

Unlike mammalian skeletal and cardiac muscle cells which store large quantities of glycogen and other energy fuels, central neurons do not. Therefore it is not surprising that the adult mammalian brain is exquisitely sensitive to O_2 deprivation. Depending on the degree of acute brain hypoxia, major ionic alterations, which underlie the changes in the electroencephalogram and behaviour, may occur (Hansen, 1985; Choi, 1990).

One important ionic species for neuronal activity is K^+. Previous studies have established that extracellular K^+ (K_o^+) increases in the adult brain *in vivo* or *in vitro* during hypoxia or anoxia (Vyskocil, Kriz & Bures, 1972; Mares, Kriz, Brozek & Bures, 1976; Hansen, 1977, 1978; Astrup, Skovsted, Gjerris & Rahbek-Sorensen, 1981; Haddad & Donnelly, 1990; Kawasaki, Traynelis & Dingledine, 1990). The source of this extracellular K^+ is, however, unknown. For example, it is not known whether K^+ is lost from neurons or from glia. In addition, it is not clear how K^+ leaks out from the intracellular to the extracellular compartment. It has been assumed thus far that the anoxia-induced increase in K_o^+ in adult neural tissue is secondary to the reduction of intracellular ATP and the inhibition of Na^+–K^+-ATPase.

We and others have previously demonstrated that the newborn brain is less prone than that of the adult to anoxia-induced alterations in extracellular ionic activities (Mares *et al.* 1976; Haddad & Donnelly, 1990). We have also demonstrated that newborn brain stem neurons depolarize by one third the magnitude in adult neurons during the same period of hypoxia (Haddad & Donnelly, 1990). An even more extreme case of anoxia resistance is illustrated by reptiles, e.g. turtle (Sick, Rosenthal, LaManna & Lutz, 1982; Chih, Rosenthal & Sick, 1989). Recent intracellular studies from our laboratory have also shown that brain stem neurons in turtles continue to fire for hours during anoxia with little change in membrane potential (Haddad & Donnelly, 1989). The mechanisms for these major differences between newborn and adult rats and between rats and turtles in terms of ionic homeostasis during O_2 deprivation are not well understood.

The purpose of this work was to try to understand some of these mechanisms. We were particularly interested in those K^+ channels that are ATP-sensitive (K_{ATP}) (Ashford, Sturgess, Trout, Gardner & Hales, 1988; Ashford, Boden & Treherne, 1990; Politi, Jones & Rogawski, 1990; Röper, Hainsworth & Ashcroft, 1990) since, as it has been previously shown, the likelihood for ATP reduction during anoxia is high, especially in adult mammalian neural tissue (Hansen, 1985; Kass & Lipton, 1989). Experiments were performed on brain slices using intra- or extracellular simultaneous measurements of K^+ activity and potentials in brain stem. Because of our experience with brain stem hypoglossal (XII) neurons and their relatively large size (Haddad & Getting, 1989; Haddad & Donnelly, 1990; Haddad, Donnelly & Getting, 1990), we focused on these particular cells. In addition, in order to substantiate the electrophysiological data about the existence (or lack thereof) of K_{ATP} channels, we took advantage of previous observations demonstrating that K_{ATP} channels are the target of sulphonylurea ligands (cf. Ashcroft, 1988; de Weille & Lazdunski, 1990). We mapped the sulphonylurea receptor binding sites in the CNS, especially the brain stem, of the adult rat using autoradiographic techniques

and [^{3}H]glibenclamide, a potent sulphonylurea receptor ligand. We adopted a comparative strategy (rat *versus* turtle, and newborn rat *versus* adult rat) using both electrophysiological and autoradiographic approaches to gain more insight into mechanisms of response and tolerance or susceptibility of central neurons to limited O_2 supply.

METHODS

Materials. Glibenclamide and tolbutamide were purchased from Sigma. Hexamethyldisilazan and the valinomycin-based K^+ ionophore (60398 ionophore, cocktail B) were obtained from Fluka Chemie AG, Switzerland. [^{3}H]glibenclamide (50·9 Ci/mmol or 1883·3 GBq/mmol) was purchased from New England Nuclear, Boston, USA.

Preparation of brain slices. Rat brain stem (adult and newborn) slices were prepared and maintained as described previously (Haddad & Getting, 1989; Haddad *et al.* 1990). Briefly, adult and neonatal rats were anaesthetized with inhalation of methoxyflurane (saturated) and decapitated. Three slices (400 μm) were obtained from a level around the obex. The slices were promptly transferred to a chamber and superfused with a medium containing (in mM): 125·0 NaCl, 3·1 KCl, 1·25 NaH_2PO_4, 1·3 $MgSO_4$, 2·4 $CaCl_2$, 26·0 $NaHCO_3$ and 10·0 D-glucose, and oxygenated with 95% O_2–5% CO_2 at a pH of 7·4. The flow rate was 1–2 ml/min with the temperature maintained at 35–36 °C. Warmed and humidified 95% O_2–5% CO_2 also flowed over the surface of the slices. Total anoxia (measured tissue $P_{O_2} = 0$ Torr) was induced in about 20 s by switching to the perfusate equilibrated with 95% N_2–5% CO_2 and by this same gas flowing over the slices. Turtle brain slices were prepared using the same technique except for the composition of the perfusate which was (in mM): 96·5 NaCl, 2·6 KCl, 4·0 $CaCl_2$, 2·0 $MgCl_2$, 31·5 $NaHCO_3$ and 10 dextrose. The temperature used for these recordings was 22 to 24 °C, the natural temperature range for these animals.

Measurements of intracellular and extracellular K^+ and membrane potential. Extracellular and intracellular recordings in the rat and turtle were made in the brain stem, in the area of the XII nucleus. Experiments were performed with double-barrelled (capillaries produced by WPI, USA) microelectrodes, with one barrel for measurement of intracellular or extracellular K^+ activity (K_i^+, K_o^+) as previously described (Ammann, 1986; Ammann, Chao & Simon, 1987) and the other for electrical activity. The tip of the whole microelectrode was less than 1 μm for intracellular recording and 3–5 μm for extracellular measurements. The ion-selective barrel was exposed to hexamethyldisilazan vapour for 1 h and then baked at 120 °C for 2–3 h. Its tip was filled with a highly selective, valinomycin-based K^+ ionophore (Haddad & Donnelly, 1990; Jiang & Haddad, 1991), and the remainder of the barrel back-filled with 0·1 M-KCl. The other barrel was filled with 3 M-KCl or 3 M-NaCl depending on whether the measurements were intracellular or extracellular respectively. Electrodes were used only if they showed an excellent sensitivity to K^+ (a voltage change more than 70 mV when K^+ activity increased from 5 to 100 mM). The potential recorded from the KCl or NaCl barrel was subtracted from the potential obtained form the ion-selective barrel. Their difference (representing K^+ potential) was amplified and filtered (0–100 Hz). Membrane potential or V_m was calculated by subtracting the measured intracellular potential from the extracellular direct current (DC) shift. K_o^+ was calculated after DC shift subtraction.

Serial calibrations of ion-selective microelectrodes were made with solutions containing 1, 3·1, 5, 10, 20, 50, 100 and 150 mM-KCl. For extracellular measurements, the ion-selective microelectrodes were calibrated before and after experiments with various concentrations of KCl (from 1 to 100 mM) in Ringer solution in which NaCl was correspondingly reduced to keep the same osmolarity. For intracellular measurements calibrations were performed with different concentrations of KCl (5–150 mM) immediately after recordings. K_i^+ and K_o^+ were calculated by comparing the voltage changes recorded from neurons with those obtained from calibration solutions. When pharmacological blockers were used in the perfusate, the calibration was performed using the working concentration of the blocker in normal Ringer solution. These pharmacological agents showed no effect on the calibration of the electrodes.

Extracellular space (ECS) was measured as described previously (Ransom, Yamate & Connors, 1985). In brief, the bathing medium was replaced with a perfusate containing (in mM): 120·0 NaCl, 3·1 KCl, 2·4 $CaCl_2$, 1·3 $MgSO_4$, 1·25 NaH_2PO_4, 26·0 $NaHCO_3$, 10·0 D-glucose and 5·0 tetra-

methylammonium bromide (TMA). Double-barrelled microelectrodes with tip diameter of 3–4 μm were filled with a K^+ ion-exchanger (Corning 477317) which is far more sensitive to TMA than to K^+ (Phillips & Nicholson, 1979; Hansen & Olsen, 1980; Ransom *et al.* 1985; Carlini & Ransom, 1987; Haddad & Donnelly, 1990). Because of molecular size, TMA is mostly restricted to the ECS (Hansen & Olsen, 1980; Ransom *et al.* 1985) and does not have any apparent effect on K^+ currents (unpublished observation from our laboratory). Therefore, changes in TMA activity (TMA_o) represent changes in ECS (Dietzel, Heinemann, Hofmeier & Lux, 1980).

Receptor quantitative autoradiography. Autoradiography was performed in adult (120-day-old) and newborn (3-day-old) rats and adult turtles. Brain sections (10 μm) were prepared at −20 °C from both rat and turtle by using a Hacker–Bright Cryostat. Before binding, the slide-mounted sections were gradually brought up to room temperature and dried in cool air. The sections were then pre-incubated for 10 min at 20 °C to deplete the tissue of endogenous ligand. In order to study saturation profiles, consecutive sections were incubated at 4 °C in 20 mM-HEPES buffer (pH 7·4) with increasing concentrations (0·03–4·6 nM) or [^{3}H]glibenclamide for 50 min (Mourre, Ben Ari, Bernard, Fosset & Lazdunski, 1989). Incubated sections were transferred to seven different washes of cold HEPES buffer (30 s each) and finally dipped in cold distilled water. The incubated sections were totally removed from slides and radioactivity counted. Sections for non-specific binding were incubated in parallel with 1 μM-glibenclamide. A concentration of 2 nM-[^{3}H]glibenclamide, corresponding to about 60 to 70% receptor occupancy for rat and turtle tissues, was then used for autoradiographic experiments. In these experiments, sections were dried after incubation with [^{3}H]glibenclamide and, along with tritiated scales (Amersham) and tissue standards, were exposed to ^{3}H-Ultrafilms (LKB) for 6 weeks. Non-specific binding, determined from adjacent sections in parallel incubation with 1 μM-glibenclamide, was close to film background throughout the CNS. The films were developed in Kodak D-19 solution and fixed.

Tissue standard. The methods were previously detailed (Xia & Haddad, 1991). In brief, the brain paste of turtles and rats (newborn and adult) was made separately and seven to eight aliquots of each paste were mixed with varying amounts of the hot ligand. The aliquots were then frozen, sectioned in 10 μm slices and mounted on slides. The adjacent sections were used to count radioactivity and measure protein concentration. The values were then normalized to fmol/mg protein.

Autoradiographic image analysis. After film exposure, all brain sections were stained with Cresyl Violet. By comparison of stained sections with images on the film, brain areas were identified according to the rat brain atlas (Paxinos & Watson, 1982; Palkovits & Brownstein, 1988) and turtle brain atlas (Cruce & Nieuwenbuys, 1974; Powers & Reiner, 1980). Autoradiographic images were analysed by using a computer image processing system (Xia & Haddad, 1991). Following subtraction of the density from non-specific binding and film background, the optical density values from an image were converted to binding density by comparison with the standards along with the image on the same film. Quantification was expressed as the mean ± S.E.M. from nine corresponding sections from three animals in each group.

RESULTS

Electrophysiologic evidence for presence and role of K_{ATP} channels

We measured K_o^+ in the brain stem XII nucleus of adult and newborn rats and in turtles. Baseline K_o^+ averaged 3·8 ± 0·5 mM (mean ± S.D.) in the adult rat, 3·2 ± 1·6 mM in the neonatal rat and 3·0 ± 0·3 mM in the turtle. Anoxia (4 min) induced a remarkable enhancement of K^+ activity in the interstitial fluid of the adult rat with an average net increase in K_o^+ of 26·1 ± 9·0 mM above baseline (n = 10). Anoxia-induced increase in K_o^+ showed an age dependence. In the neonatal rat (2–6 days), K_o^+ increased much less (1·9 ± 1·1 mM, n = 24) than in the adult. A modest increase in K_o^+ during anoxia was found in the neonate at 8–12 days (about 5 mM) and at 14–18 days (about 12 mM) (Fig. 1). Adult turtle tissue accumulated very little K_o^+ during anoxia, much less even than in the newborn rat. Over a period of 7–10 min (twice the exposure period of adult and neonatal mammalian tissue to anoxia), the increase in K_o^+ was 0·2–0·3 mM (n = 6) (Fig. 1).

This increase in K_o^+ during anoxia in adult rat XII tissue could be due to efflux of K^+ from neurons or from glial cells. To address the hypothesis that neurons lose K_i^+ during anoxia, we measured K_i^+ in twenty XII neurons. Because the increase in K_o^+ was comparatively very small in neonatal rat and adult turtle neural tissue, K_i^+

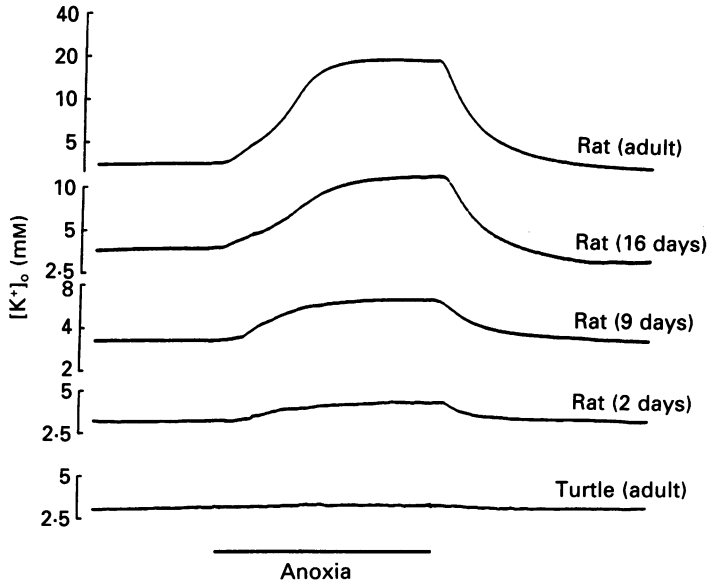

Fig. 1. Anoxia-induced increase as K_o^+ in rats (adult and neonate) and turtles. Anoxia as indicated by a straight line was maintained for 4 min in the rat and 6 min in the turtle. Note the non-linear scale for K_o^+.

measurements were made only in the adult rat. All neurons in the adult rat showed a substantial decrease in their K_i^+ during anoxia, supporting our hypothesis. Ten neurons were studied in detail in which K_i^+ was $99{\cdot}9 \pm 12{\cdot}5$ mM during baseline recordings. During anoxia, K_i^+ decreased markedly and this reduction averaged $66{\cdot}7 \pm 12{\cdot}6$ mM (Jiang & Haddad, 1991). All adult rat neurons depolarized in response to anoxia and the changes in V_m averaged $37{\cdot}2 \pm 8{\cdot}0$ mV.

These results showing an accumulation of K_o^+ and a decrease in K_i^+ strongly suggest that K_i^+ is lost from adult rat XII neurons during anoxia. However, the possibility exists that the changes in K_o^+ and K_i^+ are due to shrinkage of the extracellular space (ECS) (Dietzel *et al.* 1980; Ransom *et al.* 1985) and expansion of the intracellular compartment. For this reason, we measured the change in ECS volume as detailed previously (Dietzel *et al.* 1980; Ransom *et al.* 1985) during anoxia in four adult and four neonatal rats (6–7 days of age). ECS shrinkage during anoxia averaged $53{\cdot}0 \pm 3{\cdot}6$% in the adult and about ten times less in the newborn (< 5%).

Since the changes in K_o^+ and K_i^+ during anoxia were too large to be attributed to changes in ECS, we concluded that K^+ was actually being lost from the intraneuronal space. We then hypothesized that this K^+ efflux from adult neurons was mediated in a major way by ATP-sensitive K^+ (K_{ATP}) channels such as has been demonstrated in cardiac myocytes (Weiss & Lamp, 1987; Escande, Thuringer, LeGuern, Courteix, Laville & Cavero, 1989; Bekheit, Restivo, Boutjdir, Henkin, Gooyandeh, Assadi, Khatib, Goupg & El-Sherif, 1990; Kantor, Coetzee, Carmeliet, Dennis & Opie, 1990).

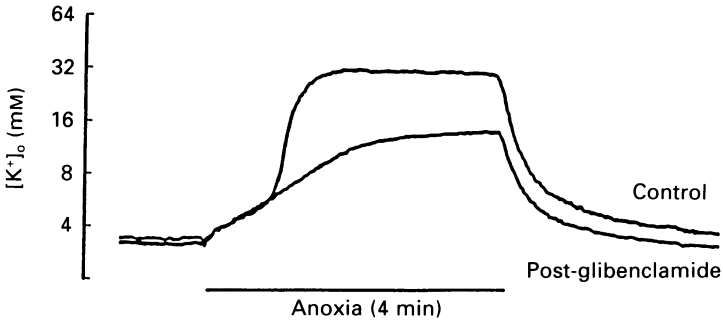

Fig. 2. Glibenclamide (40 μM) decreased anoxia-induced K_o^+ accumulation in the hypoglossal nucleus in adult rats. Effect of anoxia was tested before and after 60 min equilibration with glibenclamide in the perfusate. Both the amplitude and slope of increase in K_o^+ were reduced indicating an inhibitory effect.

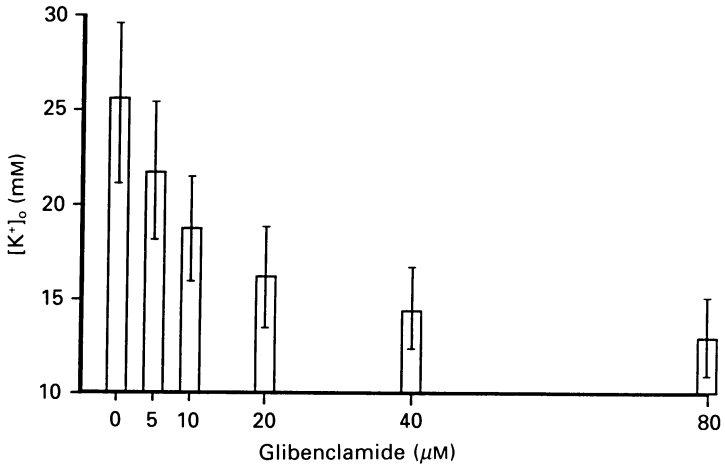

Fig. 3. Dose-dependent effect of glibenclamide (5–80 μM) on anoxia-induced increase in K_o^+. Each bar (mean $\pm$ S.D., $n = 4$) is the result of recordings obtained at least 30 min after equilibration with a glibenclamide-containing perfusate. A saturable level was reached with 40 μM of glibenclamide.

Glibenclamide (40 μM) (see below for dose-dependence), which selectively blocks K_{ATP} channels (Ashcroft, 1988; Miller, 1990; de Weille & Lazdunski, 1990), significantly decreased K^+ loss during anoxia by about 15 mM, amounting to 40–45% of total efflux (Fig. 2). A dose-dependent and saturable response of glibenclamide on K^+ efflux was observed during anoxia ($n = 4$) (Fig. 3). Although these doses of glibenclamide seem high compared to the dissociation constant of glibenclamide from receptor, diffusion distances and access to receptors may play an important role in the slice preparation. A similar response was also obtained with tolbutamide (0·25–4·0 mM), another sulphonylurea ligand.

The effect of glibenclamide on K^+ homeostasis was also studied in the neonatal rat and adult turtle, although the increase in K_o^+ was very small in the neonatal rat and almost non-existence in the turtle during anoxia. Glibenclamide (40 μM) reduced K^+

loss by a statistically insignificant amount ($\sim \frac{1}{4}$ of adult effect) in the neonate ($n = 3$), and did not produce any detectable change in the turtle ($n = 3$).

If K_{ATP} channels are activated during anoxia in adult XII neurons, then the anoxia-induced depolarization in these neurons should be enhanced when K_{ATP}

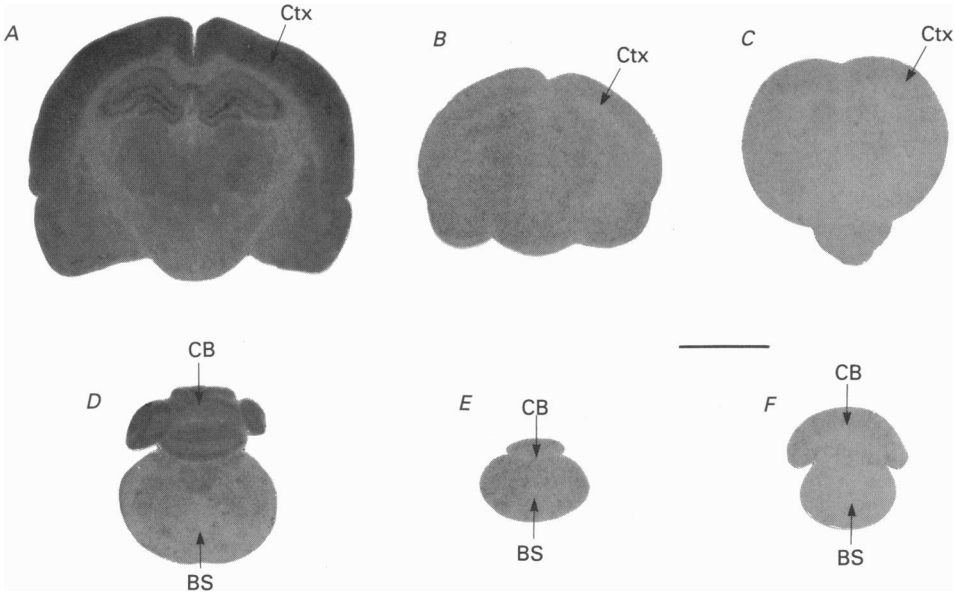

Fig. 4. Autoradiographic images of [^{3}H]glibenclamide binding sites from an adult rat (*A* and *D*), a newborn rat (*B* and *E*) and a turtle brain (*C* and *F*) at two different levels; cortex (*A*, *B*, *C*) and brain stem (*D*, *E*, *F*). BS, brain stem; CB, cerebellum; Ctx, cortex. Scale bar, 4 mm. Note that glibenclamide binding is more homogeneous and less dense in the newborn rat and the adult turtle than in the adult rat.

channels are blocked by glibenclamide. To test this hypothesis, intracellular recordings using conventional microelectrodes were performed. At rest, V_m was $-80{\cdot}3 \pm 3{\cdot}4$ mV ($n = 4$). Before the use of glibenclamide, anoxia (3 min) depolarized these neurons to $-29{\cdot}5 \pm 7{\cdot}6$ mV. After bathing with glibenclamide (40 μM) for 25–30 min but before anoxia exposure, baseline V_m did not change. With anoxia, neurons showed a significantly greater depolarization. The mean depolarization was about 26% larger and V_m reached an average level of $-12{\cdot}0 \pm 5$ mV. Three additional neurons were further tested with a longer period of anoxia (5–6 min) after glibenclamide. These neurons depolarized to almost 0 mV at the end of anoxia and, interestingly, none of them recovered when O_2 was re-instituted. All ten control XII neurons tested without glibenclamide recovered after such a period of anoxia.

Autoradiographic evidence for glibenclamide binding

To map the sulphonylurea receptor binding sites in the rat and turtle, several brain levels from rostral brain area to spinal cord were labelled with 2 nM-[^{3}H]glibenclamide. Autoradiographic images in the adult rat showed that glibenclamide binding was very heterogeneous with much higher density in rostral (Fig. 4*A*) than in caudal regions (Fig. 4*D*). The highest binding density was found in the neocortex,

cerebellum, globus pallidum and hippocampus (CA3 region). There was, in general, low density in the brain stem and spinal cord. However, binding density was much higher in some nuclei like the substantia nigra and the XII nucleus (90% higher) than in their surrounding areas. For example, Fig. 5 shows that XII nucleus has a much higher density than other regions in the medulla.

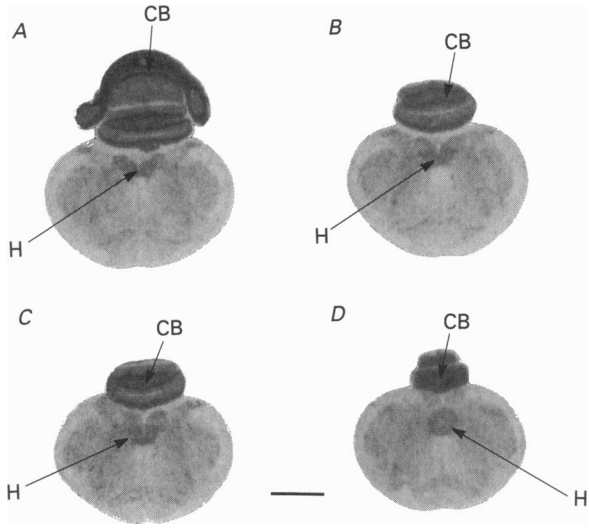

Fig. 5. Distribution of [^{3}H]glibenclamide binding sites in adult rat medulla. Images *A*, *B*, *C* and *D* show four levels from rostral to caudal medulla. Note that the glibenclamide binding density in the medulla is generally lower than in the cerebellum. However, in the hypoglossal nucleus, the density is significantly higher than in the other medullary areas. The nucleus of the solitary tract (dorsolateral to hypoglossal) also shows a higher density than most of the medulla.

In sharp contrast to the adult rat, glibenclamide binding density was low and more homogenous in the newborn CNS (Fig. 4*B* and *E*). This was especially true in phylogenetically newer structures such as the neocortex. The XII nucleus in the newborn did not show a higher density than the rest of the brain stem. In the turtle CNS, glibenclamide binding density was even lower than in the newborn rat (Fig. 4*C* and *F*). It is noteworthy that the rostral brain areas as well as the cerebellum had a higher binding density, albeit still very low in absolute terms, than the caudal regions. For example, Fig. 4*F* shows that the density was much less in the brain stem than in the cerebellum. In the spinal cord, the labelling was hardly detectable.

Figure 6 shows the quantitative differences in glibenclamide-binding densities of several major CNS structures for rat (newborn and adult) and turtle. Note that the largest difference between the newborn and the adult rat CNS is in the increase in the density of sulphonylurea receptors in rostral (e.g. cortical) areas. Also note that the receptor levels in the turtle's brain are comparatively very low throughout all levels.

DISCUSSION

Brain stem hypoglossal neurons depolarize and increase their excitability during hypoxia (Haddad & Donnelly, 1990) and anoxia (Jiang & Haddad, 1991). Cortical neurons from layer III have also been studied recently (Rosen & Morris, 1990) and

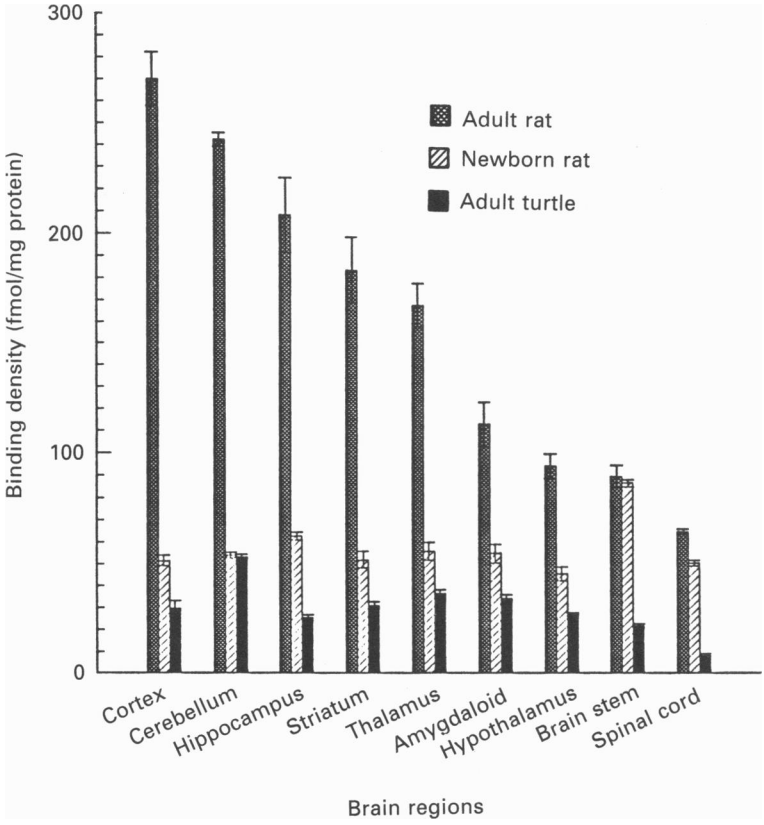

Fig. 6. Major differences in glibenclamide binding density in the CNS of the adult and newborn rat and the adult turtle. Values presented are expressed as means ± S.E.M. from nine corresponding images from three subjects.

these too, as for brain stem neurons, show depolarization, albeit of smaller magnitude than that exhibited by brain stem neurons. This response seems to be different from that of hippocampal CA1 and CA3 neurons which show first a hyperpolarization followed by a depolarization during similar conditions (Leblond & Krnjević, 1989; Mourre *et al.* 1989; Ben Ari, Krnjević & Crepel, 1990). In spite of the fact that the initial anoxic response may be different in various regions of the brain and for different cell types, a severe and continuous anoxic period can lead to a large depolarization and ultimately cellular damage, irreversible injury and cell death (Siesjó, 1988; Meyer, 1989). Mechanisms that limit this depolarization during O_2 deprivation can be particularly important but have not been appreciated or studied.

There are potentially several mechanisms that can limit such a depolarization during hypoxia and recent results have attested to some of these mechanisms (Krnjević & Leblond, 1988, 1989; Cummins, Donnelly & Haddad, 1990; Jiang & Haddad, 1991). For example, Krnjević & Leblond (1989) have shown that Ca^{2+}-activated K^+ currents are increased in CA1 neurons. Another mechanism that we focus on in this work is the activation of ATP-sensitive K^+ channels. Although we do not have data on ATP levels in brain slices, previous studies have shown that a major reduction in ATP takes place after a few minutes of hypoxia in adult brains and that the rate of reduction is higher in mature than in immature neural tissue (Hansen, 1985; Kass & Lipton, 1989), especially at the anoxic level that we have used. In previous experiments from our laboratory (Jiang, Agulian & Haddad, 1992), we have shown that P_{O_2} in these slices drops to 0 Torr within 15–20 s of inducing the stimulus. It is also important to note that there may be other regulators of these channels such as a reduction in intracellular pH, as has been recently described in skeletal muscle (Davies, 1990). When all facts are considered together – these previous observations, the fact that glibenclamide has been used as a specific probe for K_{ATP} channels (Ben Ari, 1989; Mourre *et al.* 1989; Mourre, Widmann & Lazdunski, 1990*a*; Amoroso, Schmid-Antomarchi, Fosset & Lazdunski, 1990) and our current data regarding glibenclamide effect on K^+ efflux – they indicate that sulphonylurea receptor–K_{ATP} channels are intimately involved in mediating K^+ efflux during anoxic exposure in our adult preparation but to a much lesser extent, if at all, in the newborn rat or turtle brain.

That K_{ATP} channels dampen the anoxia-induced neuronal depolarization is consistent with the hypothesis that these channels can limit excitability and O_2 consumption and possibly prolong survival by limiting the activation of voltage-sensitive Ca^{2+} channels and Ca^{2+} entry (Amoroso *et al.* 1990; Mourre, Smith, Siesjö & Lazdunski, 1990*b*). Indeed, brain stem neurons in our studies fail to recover from depolarization after prolonged anoxia (5–6 min) when bathed with glibenclamide but repolarize and recover if not exposed to this agent. We believe therefore that the activation of these channels during anoxia can be beneficial in the adult when the microenvironment of neurons is depleted of O_2.

One of the important aspects of this work is that we combine electrophysiological evidence for the activation of these channels during anoxia with studies related to their structural presence. Our study provides direct evidence that K^+ decreases intracellularly in neurons because K^+ is lost during anoxia and that this is, in large part, mediated by the activation of channels of receptors, the presence of which is ascertained autoradiographically. In correlating structure to function, it is important to note that the dosages of glibenclamide used in this study are high compared to those used in dissociated pancreatic β-cell (Niki, Kelly, Ashcroft & Ashcroft, 1989), insulinoma cells (de Weille, Schmid-Antomarchi, Fosset & Lazdunski, 1989), cardiac myocytes (Escande *et al.* 1989) and dissociated brain stem neurons (C. Jiang, T. R. Cummins & G. G. Haddad, unpublished observations from our laboratory). We believe that this discrepancy is, by and large, due to receptor access and availability of ligand across diffusion distances in the brain slice preparation.

The autoradiographic localization of [^{3}H]glibenclamide binding sites lends support to our electrophysiological data and demonstrates some interesting aspects. First, our

data show that, unlike the homogeneous binding density in the newborn CNS, there is marked heterogeneity in the density of sulphonylurea receptors in the CNS of the adult rat (Mourre *et al.* 1990*a*). It is interesting to note that the XII nucleus and the medial region of the nucleus tractus solitarii in the brain stem (Fig. 4) have a much higher level of these receptors than any other region in the brain stem and a comparable level to that in rostral areas. Second, it is clear from our results that these receptors are expressed very little in the adult turtle CNS as compared to the adult rat. Similarly, newborn rat brain sections showed only slightly higher densities than those in the adult turtle as in Figs 4 and 5. Third, the electrophysiological data on K^+ efflux presented here coincide well with the autoradiographic evidence of [^{3}H]glibenclamide binding sites. For example, the high binding sites in the brain stem XII nucleus is associated with marked loss of K^+ from the intracellular compartment, an increase in K_o^+ and a major effect of glibenclamide on K^+ efflux during anoxia. In contrast, the presence of low binding levels in the newborn rat or adult turtle CNS or in areas of the adult rat CNS such as the CA1 correspond to low levels of K^+ flux and little or no effect of tolbutamide or glibenclamide as shown in our current data and those of others (Krnjević & Leblond, 1989; Leblond & Krnjević, 1989).

It appears from recent publications that nerve cells in the newborn mammal and the adult turtle activate totally different strategies for survival than neurons in the adult mammal during O_2 deprivation (Haddad & Mellins, 1984; Hansen, 1985; Hochachka, 1986; Leblond & Krnjević, 1989; Haddad & Donnelly, 1990; Cummins *et al.* 1990; Jiang & Haddad, 1991). The newborn rat and the adult turtle brain tissues rely seemingly on intermediary metabolic strategies to survive anoxia while the adult mammal, such as the rat, activates membrane mechanisms. Both newborn mammals and adult reptiles can maintain an energy level commensurate with adequate cell function by having a remarkable capacity to shift to strategies such as glycolytic metabolism (Hochachka, 1986), usage of lactate (Hellmann, Vannucci & Nardis, 1982; Medina, 1985) and, interestingly, reduction of metabolic needs (Haddad & Mellins, 1984; Hochachka, 1986). Our observations demonstrating poor expression of glibenclamide receptors structurally (autoradiographically) and functionally (K^+ efflux from neurons) in the newborn rat or adult turtle CNS is consistent with the idea that these receptor–K_{ATP} channel complexes are present or utilized to a much lesser degree in the newborn rat or adult turtle than in the adult rat.

The activation of K_{ATP} channels during O_2 deprivation is only one membrane mechanism that adult neurons – those that are endowed with this particular channel – can utilize (Ben Ari, 1989; Mourre *et al.* 1989; Amoroso *et al.* 1990; Ben Ari *et al.* 1990; Jiang & Haddad, 1991). It is becoming clear now that membrane mechanisms other than that of K_{ATP} are utilized by adult nerve cells during O_2 deprivation (Krnjević & Leblond, 1989; Leblond & Krnjević, 1989; Cummins *et al.* 1990). Different types of channels can be involved including Ca^{2+} channels (Krnjević & Leblond, 1988, 1989; Leblond & Krnjević, 1989). Recently, we have also discovered that Na^+ channel gating is influenced by anoxia (Cummins *et al.* 1990). Using whole-cell patch techniques, we have shown that the voltage-dependent Na^+ current is decreased during O_2 deprivation in adult CA1 neurons but not in newborn ones

(Cummins *et al.* 1990). This is accompanied by a major shift to the left of the steady state inactivation curve of the Na^+ current as a function of holding potential. We argued from these results and others obtained from our laboratory that anoxia increases the probability that the Na^+ channel is in the inactive state thus reducing excitability and limiting the mismatch between O_2 supply and O_2 needs.

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