

Effects of anoxia and metabolic arrest on turtle and rat cortical neurons

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DOLL, CHRISTOPHER J., PETER W. HOCHACHKA, AND PETER B. REINER. *Effects of anoxia and metabolic arrest on turtle and rat cortical neurons*. *Am. J. Physiol.* 260 (Regulatory Integrative Comp. Physiol. 29): R747–R755, 1991.—The responses of turtle and rat cortical pyramidal neurons to various pharmacological treatments were measured using intracellular recordings. Turtle neurons survived both anoxia and pharmacological anoxia for 180 min with no noticeable effect. Rat pyramidal neurons responded with a loss in membrane resistance, followed by a transient hyperpolarization, and a subsequent depolarization to a zero membrane potential (41.3 ± 6.5 min, anoxia; 25.8 ± 12.6 min, pharmacological anoxia). Metabolic arrest caused a rapid loss in membrane resistance, transient hyperpolarization, and a rapid depolarization in both turtle (4.6 ± 1.1 min) and rat (3.1 ± 0.5 min) neurons. Iodoacetate alone had a similar effect on the rat as metabolic arrest (6.5 ± 0.8 min), but the turtle exhibited more prolonged survival (53.5 ± 4.6 min). Ouabain caused a rapid depolarization in the rat cortical neuron (8.6 ± 1.1 min), but no initial loss in membrane resistance or a hyperpolarization. These results demonstrate that the turtle neuron, which survives anoxia, is no better at surviving total metabolic inhibition than the rat neuron. In addition, anoxia takes 13 times longer to depolarize a rat cortical neuron than metabolic arrest, and neither of these treatments is totally mimicked by ouabain alone.

pyramidal neurons; membrane resistance; intracellular recording

MAMMALIAN BRAINS are very sensitive to lack of oxygen. When blood PO_2 reaches critical levels, brain function ceases. Of particular interest is the mechanism by which this rapid failure occurs because of the brain's importance to survival. For the purpose of this paper, these responses are divided into two categories, anoxia and ischemia. Although in the past these insults have been considered to be similar in terms of their evoked neuronal responses, from a biochemical standpoint they are quite different. In vivo, anoxia refers to the absence of oxygen in the system; however, the tissue can still receive metabolites for glycolysis and have waste products such as lactate and protons carried away. Ischemia refers to a lack of blood flow, which means that the tissue can neither receive nor rid itself of any products and thus must rely on endogenous stores of glucose and glycogen to sustain ATP production. The high metabolic rate of the mammalian brain combined with low amounts of endogenous anaerobic fuels cause energy stores to be

depleted within a minute (19), leaving the brain without any fuel source for ATP production. Although it is impossible to precisely mimic the ischemic situation in a flow-through slice chamber, we have chosen a set of inhibitors that block both glycolysis (iodoacetate) and the electron transport system (cyanide and nitrogen) to mimic the energy deficit created by an ischemic insult.

The in vivo extracellular ion shifts observed during ischemia can be classified into several stages (13). The initial stage for hippocampus and cortex occurs within a minute and involves isoelectricity of the electroencephalogram (EEG) (13, 33), leading to a slow gradual increase in $[K^+]$ (brackets indicate concentration) (13, 33), followed by 10-fold increase in the $[Na^+]$ (2) and $[K^+]$ (13, 33) and a 10-fold decrease in the $[Ca^{2+}]$ (13, 33). In addition, there is an 80% decrease in whole brain ATP levels (19). These results imply a rapid breakdown in neuronal ion gradients leading to a nonfunctioning state.

With respect to anoxia, more is known about the changes that occur to the neuron itself in vitro. In hippocampus, synaptic activity is reduced by 90%, and there is a 50% decrease in neuronal membrane conductance usually after or concurrent with a slight hyperpolarization of the cell for the CA1 and less often for CA3 (5, 17). These initial events have been shown to occur in vivo for the cortex (11). Little is known about the long-term effects of anoxia on membranes, but hypoxia and anoxia have been shown to ultimately cause a depolarization to a zero membrane potential in the hippocampus in 15–30 min (9, 13).

Chrysemys picta provides an excellent contrasting model for the study of anoxia. This turtle species has been shown to survive low-temperature ($3^\circ C$) anoxic dives for over 6 mo (34) and up to 48 h at $25^\circ C$ (25). EEG (31), evoked field potentials (6), and ouabain-induced extracellular K^+ accumulation in the brain (7) are all reduced, whereas extracellular $[K^+]$ rises <2 mM over a 48-h period. Remarkably, ATP levels remain constant throughout this period (21) and have even been reported to rise (15). When the turtle brain is exposed to iodoacetate (IAA) as well as anoxia, a rapid rise in extracellular $[K^+]$ is observed (31), suggesting a dependence on glycolytic energy production for neuronal ion homeostasis. However, little is known about the changes that occur to the turtle neuron during these insults.

In this paper, we focus on the mechanism behind the rapid brain failure during anoxia and ischemia charac-

terizing the full response of both turtle and rat cortical neurons to several pharmacological treatments including those that mimic anoxia and ischemia.

METHODS

Young male Wistar rats, 50–100 g, were anesthetized with halothane, decapitated, and the brain was rapidly dissected free and immersed in precooled oxygenated artificial cerebrospinal fluid (aCSF). After a few minutes of precooling, the brain was bisected along the midline. A block containing frontal-parietal cortex was dissected free, glued with cyanoacrylate to a mounting block, and sliced (400 μm thickness) on a vibratome. Slices were stored at room temperature $\approx 22^\circ\text{C}$ in a holding chamber for at least 1 h until their use in the recording chamber at 25 or 35°C. The recording chamber was a modification of a previous design (12) in which slices were continuously superfused with aCSF at a flow rate of 1.5–2.0 ml/min. Turtles (*C. picta*) ranged in weight from 250 to 600 g. The dissection has been previously discussed in Ref. 8. After the cortex was removed, it was used whole or divided in half depending on the size. All other procedures were the same as those discussed above for the rat.

Intracellular recordings were carried out using 1.2-mm-OD micropipettes filled with 2 M KCl with resistances ranging from 40 to 90 M Ω connected to an Axoclamp 2A amplifier. Data were acquired using the Pclamp suite of programs and an Axolab 1100 interface, which also served to generate current commands. Data were also independently digitized at 49 kHz and stored on videotape for off-line analysis.

Pyramidal neurons in the turtle were identified by location, action potential size and duration, as well as input resistance and time constant, as previously discussed (8). Pyramidal neurons in the rat were identified by location as well as action potential duration and size, as discussed elsewhere (24). Criteria for a healthy neuron included positive-going action potentials, a minimum of 40 M Ω resistance for rat neurons and 100 M Ω resistance for turtle neurons, and stable membrane potential for 15 min. Whole cell resistances were measured in both the turtle and the rat by a 15- to 20-mV hyperpolarizing pulse, as discussed in Ref. 8.

The rat aCSF consisted of (in mM) 126 NaCl, 2.5 KCl, 1.2 MgCl₂, 2.5 CaCl₂, 1.2 NaH₂PO₄, 11 glucose, 25 NaHCO₃, and 0.03 phenol red as a pH indicator. The aCSF for the turtle was a modification of that of Ref. 8 and consisted of (in mM) 96.5 NaCl, 2.6 KCl, 2.5 CaCl₂, 2.0 MgCl₂, 2.0 NaH₂PO₄, 10 glucose, 26.5 NaHCO₃, and 0.03 phenol red as a pH indicator. Final pH of both solutions was 7.4 when saturated with 95% O₂-5% CO₂. To mimic anoxia, the solution was switched to a presaturated aCSF solution of 95% N₂-5% CO₂. For pharmacological anoxia, the aCSF solution was the same as for anoxia and contained 1 mM sodium cyanide. For metabolic arrest, the solution was the same as for pharmacological anoxia and also contained 10 mM IAA titrated to a pH of 7.4 with concentrated NaOH. For the ouabain and IAA experiments, the aCSF contained 100 μM ouabain and 10 mM IAA, respectively, equilibrated with 95% O₂-5% CO₂.

Rats were obtained from the University of British Columbia breeding facility, and turtles were purchased from Lemberger. Chemicals were purchased from Sigma and Fisher Scientific. Data were analyzed using Pclamp software (Axon Instruments), statistics were carried out using Systat (Systat), and graphics were done using Sigmaplot (Jandel).

RESULTS

Figure 1 represents the mean time for cell survivability, which for the purpose of this paper is the time it takes for a cell to depolarize from its resting membrane potential to 0 mV once a drug was applied. These measurements are corrected for the lag time for the drug to reach the slice and equilibrate in the slice chamber (≈ 30 s). All turtle cells were recorded at 25°C because of problems associated with survivability of ectothermic cells at unphysiologically high temperatures. Rat neurons were recorded at 25°C as a comparison with the turtle. In addition, some insults were repeated at 35°C in the rat

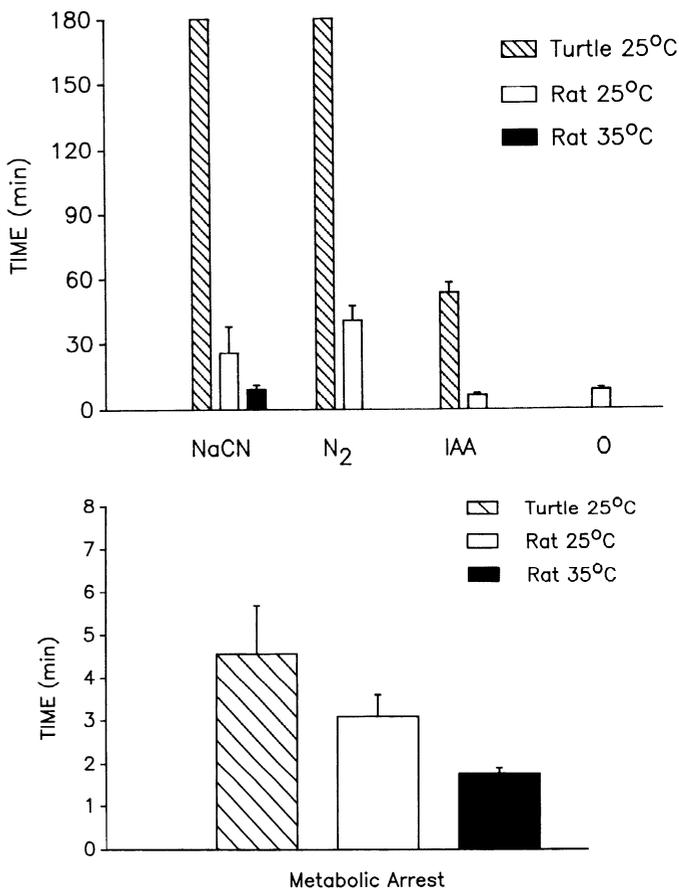


FIG. 1. Time to depolarization for turtle and rat pyramidal cortical neurons in response to various pharmacological treatments: pharmacological anoxia [sodium cyanide (NaCN)], anoxia (N₂), iodoacetate (IAA), ouabain (O), and metabolic arrest. Data that do not have SE bars do not represent survival times. All treatments are significantly different ($P \leq 0.05$; Newman-Keuls multiple comparison test) from each other, except IAA acid vs. ouabain. All values within a treatment group are significantly different ($P \leq 0.05$; independent t test), except rat pharmacological anoxia 35 vs. 25°C and rat vs. turtle metabolic arrest 25°C. All treatments had $n = 6$ except rat 35°C ($n = 3$), turtle 25°C NaCN ($n = 5$), and turtle N₂ ($n = 5$). Note different time scales on ordinate.

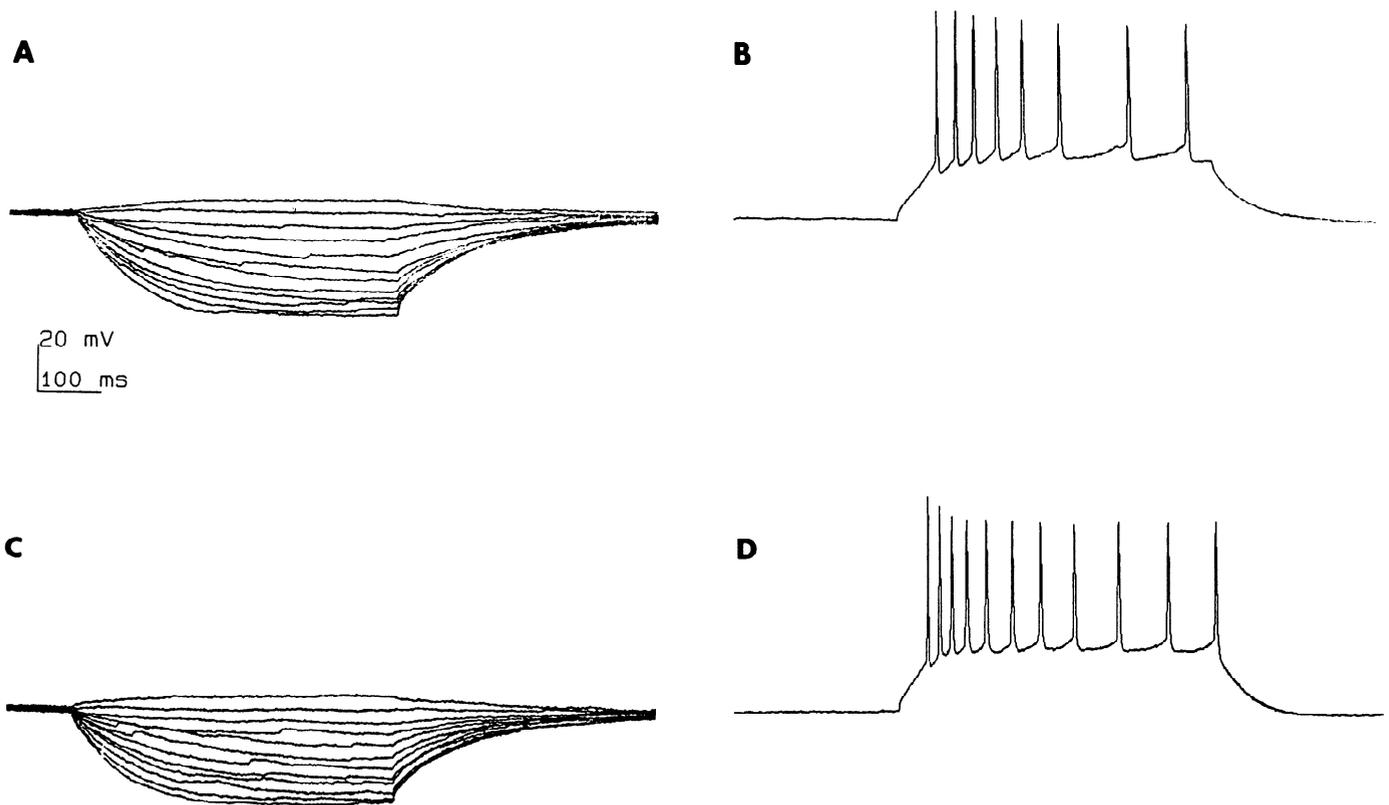


FIG. 2. Effect of 180 min of anoxia on a continuously impaled turtle cortical pyramidal cell. Cell initially was impaled and recorded from using oxygenated artificial cerebrospinal fluid (aCSF) (A and B) and then was subjected to 180 min of anoxia while measurements were repeated (C and D). Membrane potential in both cases was -65 mV. Current steps are from 0.1 to -0.5 nA by 50 pA in A and C. A stimulating pulse of 0.5 nA was used in B and D.

to demonstrate the repeatability of the low-temperature results at a more physiological temperature. The average whole cell resistances were as follows: turtle at 25°C , 151 ± 12 M Ω ($n = 23$); rat at 25°C , 86 ± 5 M Ω ($n = 30$); and rat at 35°C , 66 ± 13 M Ω ($n = 6$). These values are similar to those reported for both turtle and rat using whole cell patch clamping (3).

The turtle neurons maintained a healthy whole cell resistance throughout the anoxic and pharmacological anoxic insults and were able to fire action potentials for the duration of the experiment (180 min) (Fig. 2). Indi-

TABLE 1. Effect of anoxia and pharmacological anoxia on membrane potential and action potentials of turtle neurons

Treatment	Parameter	<i>n</i>	Control	180 min
<i>Membrane parameter</i>				
N ₂	MP, mV	4	-67.8 ± 3.8	-70.0 ± 6.4
NaCN	MP, mV	4	-74.0 ± 4.2	-74.5 ± 3.6
<i>Action potential parameters</i>				
N ₂	Spike rise, mV/mS	4	92.6 ± 4.7	91.9 ± 5.5
	Spike fall, mV/mS	4	28.4 ± 0.9	33.9 ± 3.7
	Threshold, mV	4	-36.0 ± 1.5	-43.0 ± 2.5
	Spike amplitude, mV	4	62.7 ± 1.1	63.4 ± 1.6
	Spike width, mS	4	4.8 ± 0.4	4.7 ± 0.4

Values are means \pm SE; *n*, no. of turtles. Values given are an average from a set of continuously impaled cells. There were no statistical differences between any data sets ($P > 0.05$; paired *t* test). N₂, anoxia; NaCN pharmacological anoxia; MP, membrane potential.

vidual turtle cells impaled under normoxic conditions were held up to 5 h under anoxia. In addition, slices were held in a holding chamber bathed with nitrogen-equilibrated aCSF for up to 18 h. These cells qualitatively showed no effect of the treatment. To quantitatively study the effect of anoxia on certain cellular parameters, turtle neurons were impaled under control conditions (oxygenated aCSF) and held for 180 min under anoxic conditions (Table 1). Cells were tested for changes in resting membrane potential, action potential rate of rise and fall, threshold, spike amplitude, and width after various treatments. Spike amplitudes were measured from threshold, and the rates of spike rise and fall were measured at the maximum slopes. The results indicate that there were no significant changes in any of the measured parameters.

When the turtle cells were exposed to metabolic arrest, the neurons quickly died (4.6 ± 1.1 min) (Fig. 1). This experiment was carried out under oxygenated conditions (Fig. 3C) as well as anoxic conditions (Fig. 3D). In both instances, the turtle cortical neurons exhibited a slight hyperpolarization (4.6 ± 1.8 mV) preceded by or concurrent with a loss in whole cell input resistance followed by a rapid depolarization to a zero membrane potential.

Rat cortical neurons responded to metabolic arrest similarly to the turtle, also exhibiting a slight hyperpolarization (8.0 ± 2.7 mV, 25°C ; 1.0 ± 0.6 mV, 35°C) preceded by or concurrent with a loss in whole cell input resistance leading again to a rapid depolarization (3.1 ± 0.5 min, 25°C ; 1.8 ± 0.2 min, 35°C) (Figs. 1 and 3, A and

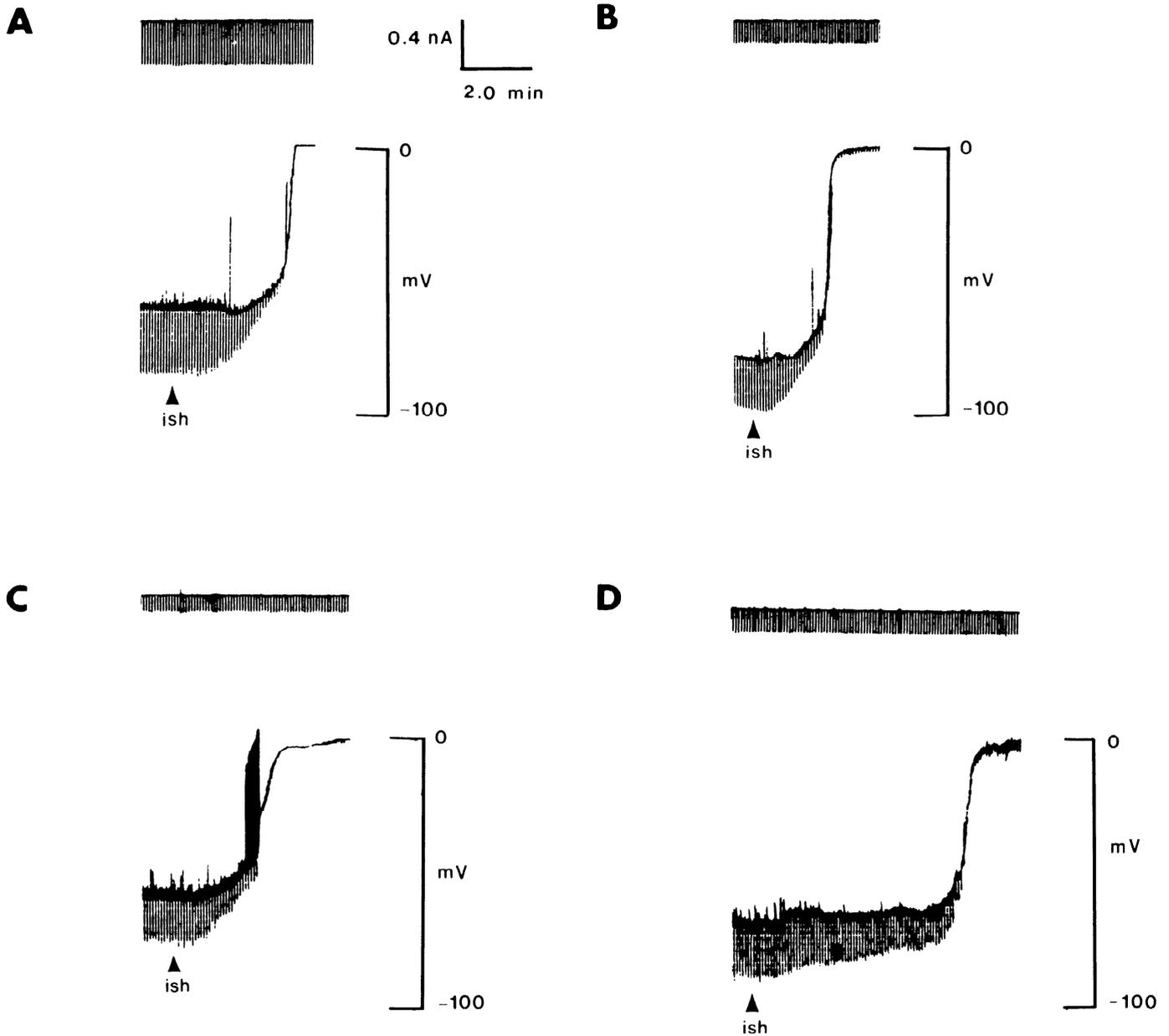


FIG. 3. Response of rat (*A* and *B*) and turtle (*C* and *D*) pyramidal cortical neurons to metabolic arrest (*ish*). Treatment was applied to a rat cortical cell at 25 (*A*) and 35°C (*B*). Treatment was applied to a turtle cortical pyramidal neuron at 25°C (*C*), and iodoacetate was applied after cell had been subjected to pharmacological anoxia for 180 min at 25°C (*D*).

B). There was a significant temperature effect for both the loss of membrane potential (Q_{10} 0.56) and hyperpolarization (Q_{10} 0.13) ($P \leq 0.05$; independent *t* test).

The results of anoxia and pharmacological anoxia show that in both instances the rat responded with slight hyperpolarization (anoxia: 3.2 ± 0.1 mV, 25°C; pharmacological anoxia: 5.5 ± 1.7 mV, 25°C; 1.3 ± 0.3 mV, 35°C) preceded by or concurrent with a loss in whole cell input resistance and a slow and gradual depolarization (anoxia: 41.8 ± 6.6 min, 25°C; pharmacological anoxia: 25.8 ± 12.6 min, 25°C; 9.3 ± 1.8 min, 35°C) (Figs. 1 and 4). Again, there was a significant effect of temperature on both the loss of the membrane potential (Q_{10} 0.36) and the hyperpolarization (Q_{10} 0.24) for pharmacological anoxia ($P \leq 0.05$; independent *t* test).

The addition of IAA by itself was applied to test the cell's ability to rely on other sources of substrate besides glucose from intracellular reserves. Because IAA blocks the glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase, the cell can no longer utilize glucose and glycogen as glycolytic substrates. Under these conditions, rat cells depolarized to 0 mV in an average of 6.5 ± 0.8 min compared with 53.5 ± 4.6 min for turtle cells (Figs. 1 and 5). This treatment also caused a hyperpolarization (3.0 ± 1.2 mV, turtle; 4.0 ± 1.7 mV, rat).

To test whether metabolic arrest or anoxia may simply reflect an inability of the $\text{Na}^+\text{-K}^+$ -adenosinetriphosphatase (ATPase) to function, ouabain (100 μM) was perfused onto the rat cortical slice at 25°C. The average time to depolarization was 8.7 ± 1.1 min (Fig. 6). The

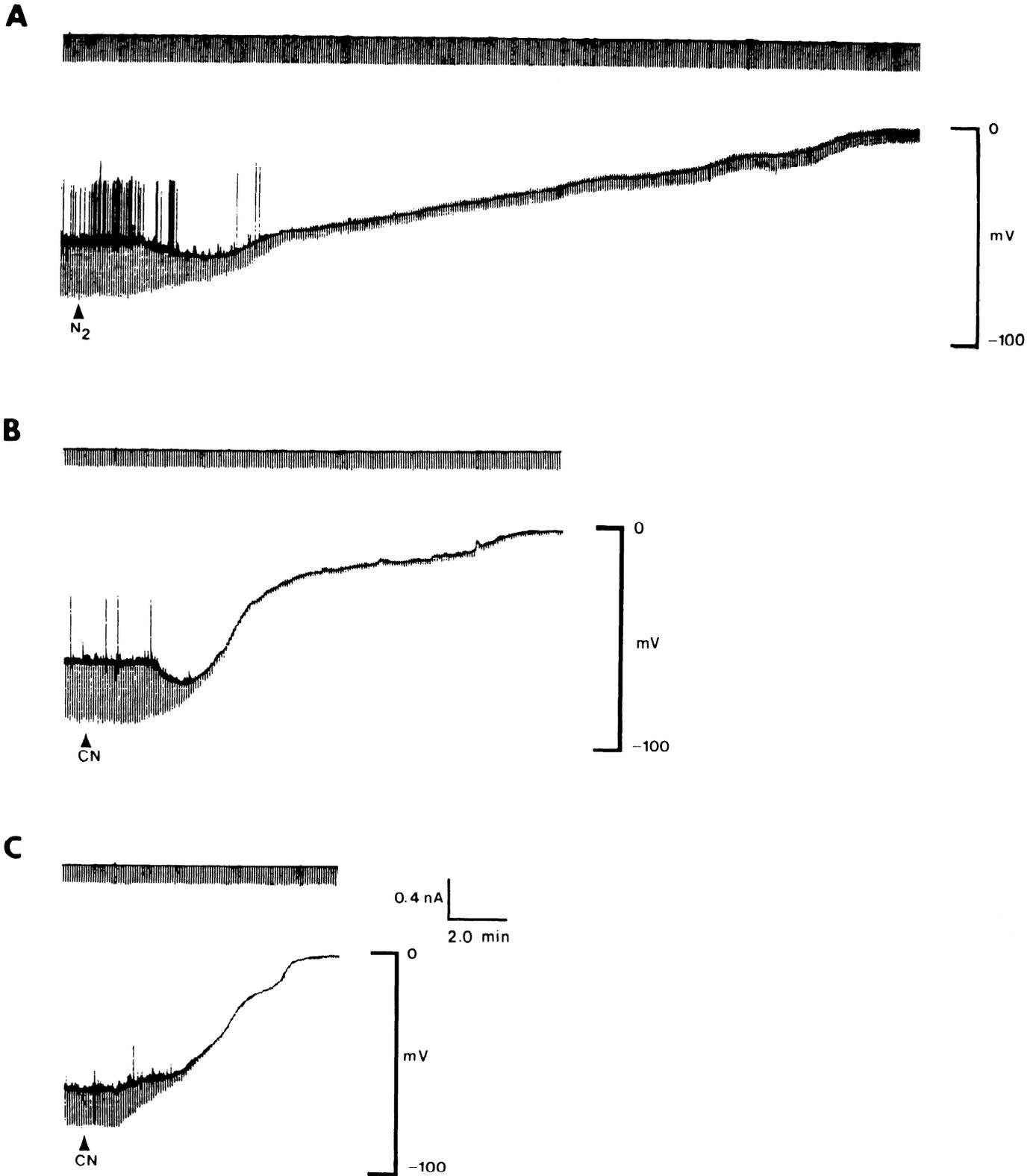


FIG. 4. Effect of perfusing solutions of anoxia at 25°C (A), pharmacological anoxia at 25°C (B), and pharmacological anoxia at 35°C (C) on a rat cortical pyramidal neuron.

cells never showed a hyperpolarization, and there was no loss in membrane resistance until there was a loss in membrane potential. The time to depolarization was faster than anoxia or pharmacological anoxia but less than total metabolic inhibition.

In addition to testing the significance of these pharmacological treatments to each other, each treatment was individually tested (Pearson Correlation Matrix) to see if there were any correlations within treatments corresponding to membrane potential, whole cell resist-

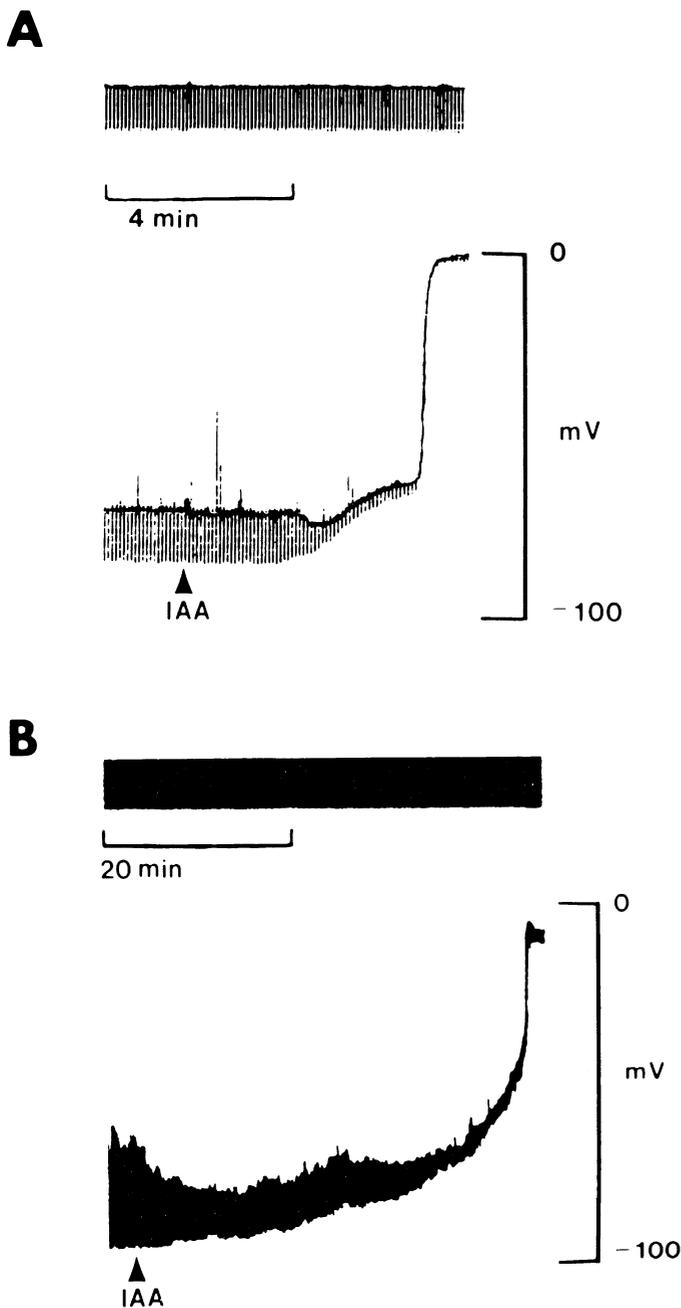


FIG. 5. Effect of perfusing IAA on rat (A) and turtle (B) cortical pyramidal neuron at 25°C.

ance, degree of hyperpolarization, and the time to depolarization. Although there were a few correlations, there were no consistent correlations throughout all of the treatments. The most consistent correlation was with the membrane potential vs. the time to depolarization with the treatments of nitrogen and pharmacological anoxia in the rat and with IAA in the turtle ($P \leq 0.05$).

DISCUSSION

The evidence presented in this paper strongly supports the supposition that the mechanism responsible for the rapid failure of the brain during anoxia and ischemia involves the breakdown of ion gradients across the neuron. This observation is not surprising in view of the

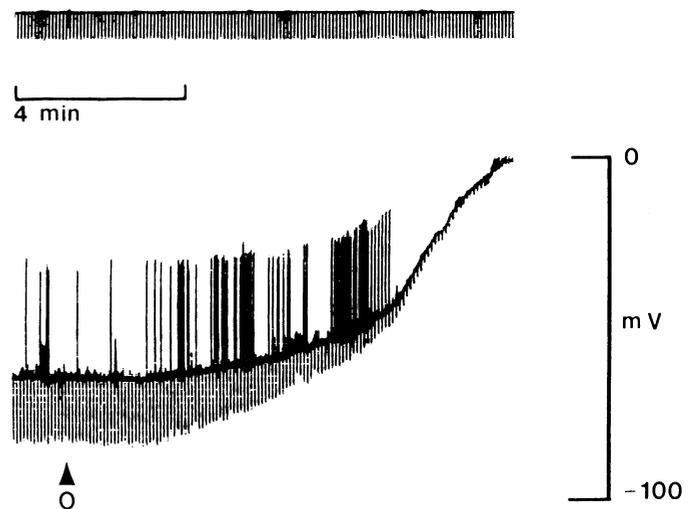


FIG. 6. Effect of perfusing 100 μ M ouabain (O) on a rat cortical pyramidal neuron at 25°C.

evidence discussed earlier. However, the question further explored here is why cortical neurons lose resistance under these treatments.

One hypothesis for the loss of membrane resistance observed here and elsewhere concerns the loss of metabolism and hence the energy state of the cell (13, 14, 18). This hypothesis centers around the idea that high-energy phosphates are directly or indirectly responsible for maintaining ion gradients through pumping and regulation of ion channels. Brain tissue is metabolically very active compared with other tissues. The ability of maintaining an adequate ATP concentration, as discussed earlier, for more than a minute during ischemia is impossible. This inability is related to the fact that during ischemia the brain must rely on the glycolytic pathway using endogenous stores of glucose and glycogen for fuel, which are rapidly consumed. The resulting low ATP concentrations would cause energy-consuming processes to cease, including the pumping action of Na^+ - K^+ -ATPase and Ca^{2+} -ATPase. The low ATP concentrations might also cause opening of ATP-sensitive K^+ and non-selective cation channels (1), as well as a rise in intracellular $[\text{Ca}^{2+}]$ from both extracellular and intracellular stores. The rise in intracellular $[\text{Ca}^{2+}]$ would in turn cause the opening of Ca^{2+} -dependent K^+ (32) as well as nonselective ion channels (31). In addition, the high $[\text{Ca}^{2+}]$ might lead to the release of neurotransmitters and activation of phospholipases. These events overall would cause a loss in ion homeostasis through the loss of pumping and the opening of channels. It is currently thought that the activation of phospholipases ultimately leads to irreversible membrane damage (14).

Support of this hypothesis is indirect but includes such observations as bathing the slice with creatine leading to prolonged anoxic and ischemic survival (17, 35), the extracellular addition of high-energy compounds to neurons after cyanide exposure leading to a partial reestablishment of the membrane ion gradient (4), hypothermia protecting the neuron (26), and low $[\text{ATP}]$ correlating with the loss of electrical activity (18).

Our data support the concept that metabolic inhibition and, hence, limiting ATP plays a major role in the loss

of neuronal membrane resistance. First, the cell's ability to maintain resistance and membrane potential is inversely related to the degree of metabolic inhibition. Thus the use of nitrogen gave the longest degree of survival followed by pharmacological anoxia, IAA, and, finally, the most inhibitory regimen used, metabolic arrest (Fig. 1). The use of nitrogen presumably allows the cell to exist longer because of the fact that the cytochrome system is not immediately inhibited; in contrast, sodium cyanide leads to an almost immediate and total inhibition of the system aided by the fact that the aCSF was equilibrated with nitrogen. The use of IAA alone is the most severe single drug treatment. The inhibition of glycolysis by IAA leaves only the low levels of endogenous fatty acids, lactate, tricarboxylic acid cycle intermediates, and the reserves of high-energy phosphates to fuel metabolism. The most severe was the use of metabolic arrest, which inhibited all energy production and allowed the cell to use only its reserve of high-energy phosphates.

Second, the observation that the turtle lasted 10 times longer during treatment of IAA compared with the rat also supports this statement. In an earlier paper (32), we showed that the turtle brain had $\sim 1/10$ to $1/20$ difference in resting metabolic rate compared with a rat in vivo. Assuming a Q_{10} of 2 for turtle metabolism (10), the expected difference between the turtle and the rat neuronal depolarization would be 5- to 10-fold, since in the turtle ATP would be consumed 5-10 times more slowly assuming usable energy stores were similar between the two tissues (23).

The third piece of evidence that supports this hypothesis is the effect of temperature on the survivability of rat cortical cells during the treatments of metabolic arrest and pharmacological anoxia. These treatments both exhibited a Q_{10} effect (0.58 for metabolic arrest and 0.37 for pharmacological anoxia). Because the observed metabolic change in rats between 25 and 35°C is 2 (S. Osborne and W. K. Milsom, personal communication), the expected Q_{10} for the rate of membrane depolarization would be 0.50 if metabolism were directly or indirectly responsible.

Although there have been some suggestions that cyanide does not provide a good mimic of anoxia (2), our data suggest the opposite. Pharmacological anoxia and anoxia provided qualitatively similar results (Figs. 1 and 4). The turtle responded well with the treatment and illustrated no difference to either treatment, suggesting that pharmacological anoxia is not alternately inhibiting other processes that are necessary for survival under these circumstances.

The pharmacological mimic of ischemia appears to reflect the observed extracellular observations well (13, 28). The initial slow gradual rise in $[K^+]$, as observed in the extracellular space, could be caused by an evoked K^+ current resulting in the initial loss of membrane resistance and concurrent neuronal hyperpolarization (16). The sequential sudden and massive loss in membrane resistance resulting in the depolarization of the neuron would correspond to the sudden drop of $[Na^+]$ and $[Ca^{2+}]$ in the extracellular compartment, since these ions would be entering the neuron flowing down their electrochemical gradient. Further confirmation that metabolic arrest

provides a plausible in vitro mimic of the in vivo response awaits simultaneous recording of changes in membrane potential and extracellular ion concentrations.

The fact that turtle brain is able to maintain ATP concentration and possibly even increase it throughout an anoxic insult is remarkable. This observation suggests that the turtle can more than adequately meet the energy demands of an anoxic insult. Two mechanisms to aid the turtle brain in maintaining ATP concentration include a low resting metabolic rate combined with a large glycolytic capacity (15, 32). In addition, there is currently evidence to suggest that evoked field potentials, EEG, and ouabain-sensitive K^+ leakage are all reduced during anoxia, as discussed in the introduction, indicating that ion fluxes, and hence energy-dependent pumping, associated with synaptic activity and action potentials may be reduced. One possible mechanism that would decrease the electrical activity of the neuron as the above evidence suggests would be by hyperpolarizing the neuron, thus causing the cell to be further from threshold. The anoxia data did not support this hypothesis (Table 1).

Another possible mechanism for the reduction of activity in the turtle brain would be through an increase in release of the inhibitory neurotransmitter γ -aminobutyric acid (GABA). It is known that GABA concentration increases 147% in the anoxic turtle brain (20), but whether this increase represents increased release or decreased catabolism is unknown. We have observed synaptic potentials increasing during anoxia but whether they are inhibitory or excitatory has not been investigated.

The idea of reducing ion fluxes to conserve ATP could also be accomplished by reducing the number of open leakage channels resulting in less ion pumping and, hence, reduced ATP expenditure (22, 14). This mechanism is currently being investigated.

The emphasis throughout this paper has been on the importance of supplying cells with an adequate amount of energy (ATP) to maintain ion homeostasis during anoxia. If this theory is correct, then the turtle cell in the absence of any energy production should rapidly lose its membrane potential. The results of metabolic arrest on the turtle neuron are illustrated in Fig. 3, C and D. Although this is the first study to observe turtle neuronal response directly to total metabolic inhibition, it has been reported that extracellular $[K^+]$ rises with both the addition of IAA plus nitrogen (31) as well as clamping the arterial supply to the turtle brain (30). The results in all cases clearly demonstrate the importance of glycolysis for maintaining ion homeostasis across the neuronal membrane. Statistically, the turtle neuron maintains a membrane potential no longer than the rat neuron, which suggests that the turtle cell is not designed to survive on low amounts of ATP but is designed to maintain ATP concentrations during anoxia. This hypothesis is further strengthened by the observations that the turtle brain has been shown to exhibit a Pasteur effect (15) and that there are comparatively large amounts of key glycolytic control enzymes in the turtle brain compared with the rat at physiological temperatures (32). The rat cell cannot adequately cope with either anoxia or ischemia because of its inability to maintain adequate

energy supply due to its high metabolic rate and comparatively low glycolytic scope (32).

Our data support metabolism playing a direct role in the maintenance of ion homeostasis, but it is unclear whether the rapid failure of the neuron that accompanies anoxia and ischemia and their pharmacological mimics is due to a lack of energy-dependent pumping, channel opening, or the release of excitatory neurotransmitters. To look at one facet of this model, ouabain was used to block Na^+ - K^+ -ATPase and observe the resulting time to depolarization. The results indicate that the ouabain treatment (Figs. 1 and 6) did not adequately mimic any of the previous times of survival. In addition, there was no initial hyperpolarization in any of the cortical cells. This observation is in contrast to hippocampus in which a slight hyperpolarization has been observed using lower concentrations of ouabain (9). In addition, there was no initial loss in whole cell resistance until there was a change in the membrane potential of the cell. The current evidence indicates that the initial hyperpolarization in the CA1 region of the hippocampus is due to a Ca^{2+} -dependent K^+ current (16). Taken together these results suggest that although a part of the anoxic depolarization may be explained by a gradual loss of the pumping system, it seems unlikely that the rapid failure observed during metabolic arrest can be totally explained by this insult.

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