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A pharmacological model of ischemia in the hippocampal slice

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The effects of various metabolic inhibitors on the time course of changes in membrane potential was studied using intracellular recordings from CA1 hippocampal neurons in vitro. Concurrent application of cyanide and iodoacetic acid, agents which block oxidative phosphorylation and glycolysis respectively, result in more rapid loss of membrane function than blockade of either pathway alone. This pharmacological regimen mimics the anoxia and the hypoglycemia encountered during ischemia in vivo, both in terms of the metabolic derangement as well as the time course of changes in membrane function. Thus, this treatment appears to represent a well-controlled pharmacological model of ischemia in vitro.

It is axiomatic that mammalian brain is dependent upon oxygen for its function. A prevalent hypothesis is that loss of the ability to generate high-energy phosphates such as ATP underlies the complex array of physiological derangements seen in anoxia and ischemia [6, 9]. Given this hypothesis, it seems clear that there is a fundamental difference between these two pathophysiological conditions. During anoxia, only oxidative phosphorylation is impaired leaving ATP production via anaerobic glycolysis intact. In contrast, the combined hypoxia and hypoglycemia incurred during ischemia cripples both oxidative phosphorylation and glycolysis.

Experimental studies of ischemia in intact animals provide a reasonable model for the human condition. However, the complex pathophysiology of ischemia (which include changes in the EEG, extracellular pH, temperature, cell volume, interstitial space volume, osmolarity, energy metabolism, neurotransmitter release, and ionic composition) makes precisely controlled experiments in vivo difficult. On the other hand, in vitro studies in which a variety of experimental manipulations may be carried out with rigor commonly utilize anoxic conditions to study the response of the brain to such insult. Because the metabolic consequences of anoxia are considerably different from that of ischemia, we have developed a highly reproducible pharmacological model of ischemia in vitro which may prove to be a useful tool in unravelling the early mechanisms which lead to ischemic neuronal damage.

Hippocampal slices were prepared using standard techniques. Briefly, young male Wistar rats (75–150 g) were anesthetized with halothane and decapitated. The brain was removed rapidly and immersed in cold artificial cerebrospinal fluid (ACSF) of the following composition (in mM): Na^+ 152, K^+ 2.5, Ca^{2+} 2.4, Cl^- 136, PO_4^{2-} 1.2, Mg^{2+} 1.3, CO_3^- 25, glucose 11, pH 7.4 at 30°C when saturated with 95% O_2, 5% CO_2. The hippocampus was dissected free and 400-μm-thick coronal slices were prepared on a tissue chopper. Slices were placed in a holding chamber where they were kept at room temperature for at least 1 h prior to recording.

Slices were transferred singly to a slice chamber where they were superfused (2 ml/min) with warm (30°C) ACSF equilibrated with 95% O_2, 5% CO_2. Intracellular recordings were obtained from pyramidal neurons in the CA1 subfield using microelectrodes filled with either 2 M KCl or 2 M potassium methylsulfate. The present set of data is based upon recordings from 40 neurons obtained from 29 rats. Mean resting membrane potential for the population was 59 ± 1 mV and mean input resistance was 64 ± 3 MΩ. Drugs, obtained from Sigma or Fisher, were added directly to the ACSF in concentrations noted in the text. Only one cell was used per slice. Statistical analysis was performed using an ANOVA followed by a post-hoc Neuman–Keuls test. Data are reported as mean $\pm$ S.E.M.

Four different manipulations were applied to assess the effects of various agents upon the membrane proper-

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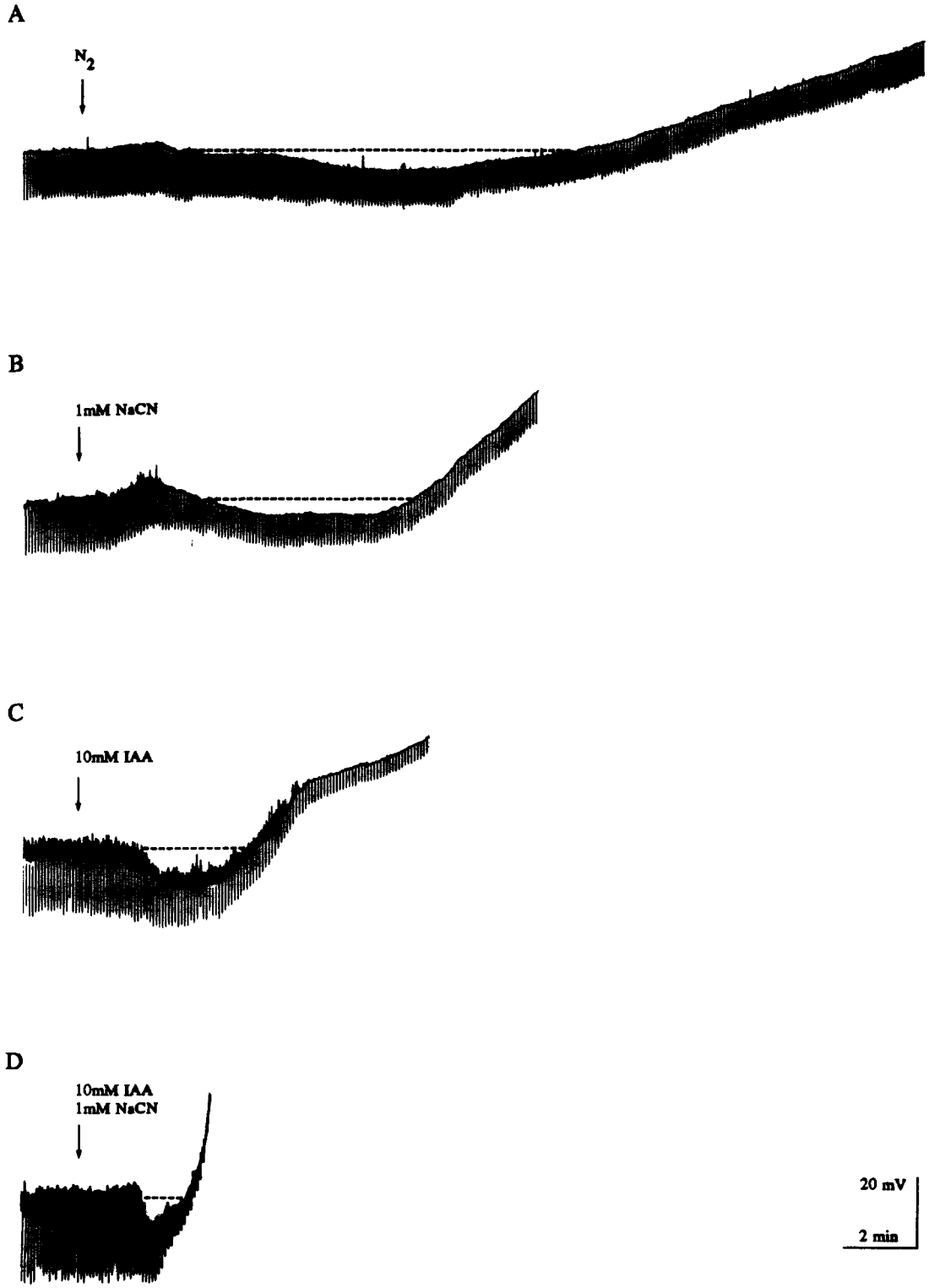

Fig. 1. Changes in membrane potential and input resistance observed with various metabolic inhibitors. In each trace, the indicated agent was applied at the arrow, the dotted line indicates the resting membrane potential, and the trace is truncated at the point where the membrane potential depolarized 30 mV from rest. The downward deflections represent voltage responses to constant current pulses of −0.3 nA amplitude, 200 ms duration applied every 5 s to monitor membrane resistance. Each record is from a different cell.

ties of hippocampal neurons in vitro. The simplest manipulation involved superfusing the slices with a solution equilibrated with 95% N_2, 5% CO_2. This treatment renders the slices anoxic and results in a characteristic se-

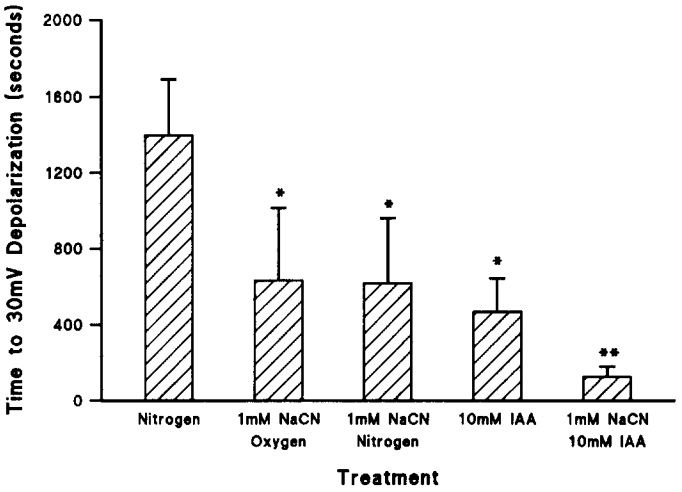

Fig. 2. Mean time to achieve a 30 mV depolarization from the resting potential for the entire populations of cells studied. *Significantly different from 95% N_2, $P < 0.05$, **Significantly different from all other treatments, $P < 0.05$.

quence of events (Fig. 1) which have been well described in the literature [4, 6, 8]. A small transient depolarization is often (but not invariably) the first observable change and is followed by a more pronounced hyperpolarization of 5–20 mV lasting 2–10 min, accompanied by a decrease in membrane resistance. The hyperpolarization gives way to a slow depolarization, ultimately leading to complete loss of membrane potential. We used as our criterion the time to a 30 mV depolarization from rest; for 8 cells tested, mean time to a 30 mV depolarization was 1397 ± 111 s.

A second paradigm involved bath application of sodium cyanide, an inhibitor of the mitochondrial cytochrome aa_3 complex. Because this treatment blocks oxidative phosphorylation, one would expect the results of cyanide treatment to be similar to those obtained with anoxia alone. Treatment of hippocampal pyramidal neurons with 1 mM sodium cyanide produced a sequence of events which were qualitatively similar to those seen with nitrogen alone, but the time course of these changes was significantly more rapid (632 ± 144 s, $n = 8$, Figs. 1 and 2). Because cyanide competes with oxygen for binding to cytochrome aa_3, the experiments were repeated with 1 mM sodium cyanide dissolved in ACSF equilibrated with 95% N_2, 5% CO_2. No differences were observed in cells treated with cyanide in the presence of oxygen versus those treated with cyanide in the presence of nitrogen (617 ± 129 s, $n = 8$, Fig. 2). The difference in the time course of responses to cyanide, which may be considered as a pharmacological version of anoxia, and anoxia produced by superfusion with ACSF saturated with N_2 is surprising. One explanation may be that cyanide has effects on cellular function other than blockade of oxidative phosphorylation. For example, it has recently been shown that cyanide impairs synaptic transmission at concentrations well below that required for toxicity [1].

A third paradigm involved the use of iodoacetic acid, an inhibitor of the glycolytic enzyme 3-phosphoglyceraldehyde dehydrogenase. Because this enzyme is key to the production of ATP via glycolysis, its blockade should mimic the effects of the severe hypoglycemia which accompanies ischemia. Bath application of 10 mM iodoacetic acid produced a sequence of events qualitatively similar to that seen with both nitrogen and cyanide (Fig. 1), and the time course of the changes in membrane potential (467 ± 67s, $n = 8$) was similar to that seen with cyanide treatment.

The fourth paradigm employed was to combine the cyanide and iodoacetic acid treatments. Once again the qualitative changes in membrane potential were similar to those seen with either drug alone, but the time course of the changes was significantly more rapid (125 ± 21 s, $n = 8$, Figs. 1 and 2). This appeared to be partly due to a shorter duration hyperpolarization as well as the invariably rapid depolarizing phase (which appeared similar to the phenomenon described as spreading depression [7]).

These data support the generally held hypothesis that the high-energy phosphate capacity of a cell determines its survival under anoxic/ischemic conditions. Treatments which block oxidative phosphorylation (anoxia, cyanide) leave intact the production of 2 ATP/mol of glucose via glycolysis. While ATP flux through this pathway does not appear to be sufficient to maintain the neuron for a prolonged period of time, it is clearly used to advantage by neurons as additional blockade of glycolysis with iodoacetate, which eliminates all metabolic production of ATP, results in much more rapid loss of membrane function.

Based upon these observations, we propose that treat-

ment of neurons with iodoacetic acid and cyanide represents a plausible pharmacological model of ischemia in vitro. This view is supported *a priori* by the fact that the metabolic derangements seen with pharmacological ischemia, namely blockade of oxidative phosphorylation and glycolysis, mimic the two major changes encountered during ischemia in vivo when insufficient blood flow and/or stasis result in both hypoxia and hypoglycemia. Significantly, the time course of changes in extracellular potassium concentrations in the cerebral cortex reported during ischemia in vivo [5] are remarkably consistent with the changes in membrane potential observed during pharmacological ischemia in vitro. In particular, the rapid depolarization phase may represent the electrical counterpart to the dramatic rise in extracellular potassium termed phase 2 by Hansen [6]. The major disadvantage of the model is the inability to reverse the effects of the metabolic impairment.

This model of pharmacological ischemia may prove useful in understanding the early events underlying the pathophysiology of ischemia. While a considerable body of evidence suggests that glutamate excitotoxicity may underly the delayed neuronal death seen subsequent to ischemia [2, 3, 10], far less is known about the immediate consequences of ischemia. Such insights may yield new strategies for the development of pharmacological agents which might ameliorate the neuronal damage accompanying ischemia in vivo.

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