The Rat Hippocampal Slice Preparation as an In Vitro Model of Ischemia

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In vivo models of cerebral ischemia do not fully control for the interacting effects of many variables (e.g., anesthesia, temperature, cerebrovascular changes) and often do not clearly define the region affected. Numerous in vivo studies have indicated that hyperglycemia augments ischemic brain damage; this effect is often attributed to lactic acidosis. To separate the effects on neuronal tissue of ischemia from those due to actions on the cerebrovascular system, we used an in vitro blood-free system as an ischemic model. In our study we evaluated the effects of various combinations of oxygen and glucose levels on evoked synaptic activity in the CA1 region of the rat hippocampal slice preparation. A 50% inhibitory dose for both oxygen and glucose on neuronal synaptic function was determined. It is our intention to use this model for preliminary screening of antihypoxic/anti-ischemic drugs. (Stroke 1988;19:498–502)

The hippocampal slice preparation has been widely used in electrophysiology and basic neuroscience in recent years. Several articles have reported depressive effects of lowered oxygen concentration, glucose concentration, or both on brain slice activity. Lipton and Whittingham reported the basic mechanism of the hypoxic block of the evoked potential in the in vitro guinea pig hippocampus. Furthermore, Whittingham et al measured the metabolic and electrical alterations in the guinea pig hippocampal slice preparation as an in vitro ischemic model. Schurr et al showed that adult rat hippocampal slices are adaptable, to a certain degree, to anoxia and hypoxia. Cox and Bachelard showed that the evoked response of granule cells of guinea pig hippocampal slices under lowered glucose concentration (2 or 5 mM) or alternative substrates did not correlate with the energy state of the tissue. In none of these studies was the basic mechanism of the hypoxic block of the evoked response studied. In a recent study, we tested the combined effects of lowered oxygen concentration and increased glucose concentration on hippocampal slices. We found that hypoxic brain slices benefited from higher levels of glucose as measured by the higher rate of recovery of synaptic function upon restoration of normoxic conditions. In our study reported here, we explored the effects of different combinations of lowered oxygen and glucose levels on the synaptic function of rat hippocampal slices. Our aim was to establish an in vitro ischemic model in which the investigator controls the tissue environment and thus, can experiment with different components of that environment and their interaction. Moreover, since the CA1 region of the hippocampus is known to be one of the neuronal populations most sensitive to ischemia/hypoxia, we could expect an almost immediate response to any major change in the environment.

Materials and Methods

Tissue Preparation and Maintenance

Adult male Sprague-Dawley rats (190–340 g) were used. For each experiment one rat was decapitated and its brain removed rapidly, rinsed with cold (6–8° C) artificial cerebrospinal fluid (ACSF), and dissected. Isolated hippocampi were sectioned transversely at 400 μm with a McIlwain tissue chopper (Surrey, U.K.), and the resulting slices were rapidly transferred to a linear-flow interface chamber with a pipette. Chamber temperature was maintained at 34 ± 0.5° C. Slices, supported on a nylon mesh (105 μm²), were superfused with ACSF via a peristaltic pump at 40 ml/hr. A humidified gas mixture containing 95% O₂-5% CO₂ was circulated above the slices at a rate of 1 l/min. Hypoxia was produced by replacing oxygen in the gas mixture with nitrogen. The same gas mixture that was circulated above the slices was also passed through the ACSF before it entered the chamber.

Measurements

Extracellular recordings from stratum pyramidale of the CA1 cell body layer were made from one slice in each experiment using borosilicate micropipettes filled with ACSF (1–5 MOhms) containing fast green FCF to enhance visibility of the micropipette tip. An AC preamplifier (×100) and a field-effect transistor head-stage were used. The output was monitored on an oscilloscope, digitized, and stored on a floppy disk for later analysis. Resolution was 256 points × 8 bits. The placement of the recording electrode in the slice was controlled by a Burleigh Inchworm Micropositioning System (Burleigh Instruments, Fishers, New York).

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Experimental Design

The concentrations of glucose and oxygen are not constant within test slices; pronounced gradients exist. In addition, previous experiments in this laboratory have shown that slices incubated in 0 mM glucose-ACSF gradually lost synaptic activity within 35–45 minutes. If 10 mM glucose-ACSF was then restored to those slices, 90% (28 of 31) recovered synaptic function (evoked population spike of >3 mV). These results suggest that 35–45 minutes are required to replace 10 mM glucose-ACSF with an ACSF containing no glucose, assuming that glucose-free ACSF is not capable of supporting synaptic function. We chose 50 minutes as the time required to ensure complete replacement of a given ACSF composition with another. The effect of oxygen depletion on CA1 synaptic function in the slice preparation is much faster than that of glucose depletion. Within 1–3 minutes the population spike is usually completely abolished upon exposure of hippocampal slices to a nitrogen atmosphere. Hence, in general, slices were allowed to equilibrate for 50 minutes with any given lowered glucose-ACSF solution before applying to them a 10-minute hypoxic insult. During these 10 minutes the tissue was exposed to the desired combination of lowered oxygen/lowered glucose and the ability of synaptic function to recover from that combined insult was tested electrophysiologically.

The experimental design (Figure 1) is based on the median-effect analysis method, which has been used to evaluate the combined effects of drugs, carcinogens, physiologic stimuli, environmental pollutants, etc. Using this design, oxygen and glucose were treated as interacting drugs. Slices were allowed to stabilize in the chamber under standard conditions (95% O₂, 5% CO₂, 10 mM glucose in ACSF, pH 7.30 ± 0.10) for 90 minutes. Each slice in the chamber (10–14 slices/experiment) was tested for its responsiveness to orthodromic stimulation; nonresponsive slices were discarded. Stimulation and recording were begun at this time and continued once per minute through the duration of the experiment. Each experiment started with 15 minutes' recording of baseline activity under standard conditions followed by 60 minutes of perfusion with ACSF containing different concentrations of glucose (0, 0.5, 1, 2, 5, 8, or 10 mM). After 50 minutes of such perfusion (the time required to reach the desired concentration of glucose in the tissue), the oxygen concentration was changed for 10 minutes (0%, 10%, 21%, or 30%). Recovery followed for 30 minutes under standard conditions. At the end of this period, the automatic recording was terminated and the responsiveness of each of the remaining slices to orthodromic stimulation was checked again. If an evoked response of >3 mV could be obtained upon the application of orthodromic stimulation, the tested slice was counted as synaptically active and recovered.

Results

The quantitative effects of changing oxygen and glucose levels on the recovery of synaptic function of rat hippocampal slices are shown in Table 1. The percentage of slices recovering synaptic function after 10 minutes' hypoxia (95% N₂, 5% CO₂) increased as the glucose level in the bathing medium increased from 2 to 10 mM. In addition, the percentage recovery following perfusion with 0 mM glucose-ACSF increased as the oxygen concentration increased from 0% to 95%. Similar trends were found in other groups (10% and 21% oxygen; 1, 2, and 5 mM glucose-ACSF).

The effect of low glucose concentrations on synaptic function in the hippocampal slice preparation is shown in Table 1.
FIGURE 2. Effect of different degrees of hypoglycemia [0, 2, and 3 mM glucose in artificial cerebrospinal fluid (glucose-ACSF)] on synaptic function (population spike) of rat hippocampal slice preparation. Population spike amplitude (mV) was plotted against time. After 15 minutes of standard conditions (baseline), slice was incubated with indicated low glucose-ACSF for 60 minutes, after which 10 mM glucose-ACSF was restored.

in Figure 2. Exposure of slices to glucose concentrations of <3 mM brought about synaptic silence 35–45 minutes after the beginning of the exposure to hypoglycemia. Upon return to a normal level of glucose (10 mM) synaptic function recovered to 70–100% of its baseline level as measured by the amplitude of the evoked population spike.

Figure 3 displays a representative experiment measuring the effect of 10 minutes' hypoxia on synaptic function in a hippocampal slice perfused with 10 mM glucose. Synaptic function disappeared during the hypoxic insult but rapidly returned upon resumption of oxygenation.

The information from Table 1 was used to construct a series of dose–response curves. For example, under constant glucose concentration (1 mM), the recovery rates of synaptic function for slices exposed to 30%, 21%, or 10% oxygen were 79 of 82 (96.3%), 43 of 70 (61.4%), and 3 of 87 (3.4%), respectively. An exact binomial 95% confidence limits table was then used to construct a dose–response curve; the recovery rates of synaptic function in hippocampal slices (active/nonactive) were expressed as logarithms (Figure 4). When log active/nonactive = 0, the number of active slices = number of nonactive, and the recovery rate is 50%. Using this approach it was possible to predict the hypoxic insult that would result in 50% recovery. In Figure 4 this 50% inhibitory dose (ID₅₀) was 18.5% oxygen.

Curves representing varied concentration of oxygen (0–21%) versus calculated glucose ID₅₀ and varied concentration of glucose (0–3 mM) versus calculated oxygen ID₅₀ are shown in Figure 5. It was found that the glucose and oxygen ID₅₀ curves for synaptic function in hippocampal slices closely approximate each other. From these curves it is easy to predict the ID₅₀ for any oxygen-glucose combination.

Discussion

Several in vivo models have evaluated brain function following cerebral ischemia. Some have failed to
control adequately the effects of miscellaneous complicating factors such as anesthetics, temperature, and cerebrovascular changes, factors that could affect assessment of stroke outcome and restoration of brain function. Pulsinelli and Brierley\(^4\) set a model of bilateral hemispheric ischemia in unanesthetized rats. Although their evaluation of postischemic recovery is not confounded by lingering effects of anesthetic drugs or muscle relaxants, the density of ischemia is variable. In Pulsinelli's regional cerebral blood flow (CBF) method, some flow persists during ischemia. It remains to be clarified whether this model accurately assesses low blood flow and thus makes it difficult to interpret the results. Furthermore, Blomqvist et al.\(^9\) showed that even in animals that lost consciousness upon carotid artery clamping, the local CBF in forebrain structures varied considerably, a variability that was especially pronounced in the hippocampus and thalamus. The two-vessel occlusion model of Smith et al.\(^{20}\) was presented as an improvement of the Pulsinelli-Brierley four-vessel model. Though it has its advantages, it also fails to control for the effects of anesthesia, and it mixes tissue and cerebrovascular effects. In the global ischemic model of Siemkowicz and Hansen,\(^2\) brainstem involvement makes it difficult to maintain adequate cardiovascular and respiratory function when the period of ischemia exceeds 10 minutes.

Lipton and Whittingham\(^3\) showed that in the in vitro hippocampus, the basis for the hypoxic block of evoked potentials is depolarization of the neuronal membrane. These results are consistent with the hypothesis that depolarization is the outcome of inhibition of the Na\(^+-K^+\) pump, possibly associated with increased membrane permeability. Schurr et al.\(^4\) demonstrated the ability of adult rat hippocampal slices to adapt to transient anoxia and hypoxia. Cox and Bachelard\(^5\) reported the effect of lowered glucose concentration on evoked field potentials from dentate granule cells. These experiments evaluated the effect of one factor alone; either oxygen deficiency\(^4\) or low glucose concentration.\(^4\) Although Whittingham et al.\(^4\) perfused hippocampal slices with medium totally devoid of glucose due to nonperfusion, but also a varying and unpredictable combination of oxygen and glucose levels during recovery. Glucose is consumed by both aerobic and anaerobic metabolism\(^8\) and hence, oxygen-glucose mismatches are likely in recovering tissue.

By using different concentrations of oxygen and glucose and by measuring their effect on the rate of recovery of synaptic function, we obtained dose-response curves (ID\(_{50}\)), which allow identification of all combinations of oxygen and glucose deprivation producing a 50% recovery of synaptic function. Our present study also confirmed our previous finding\(^7\) regarding the protective effect of increasing glucose concentrations on synaptic function against hypoxia. In addition, this in vitro model allows one to screen drugs for their cytoprotective effects against ischemia/hypoxia. If a treatment produces an ID\(_{50}\) for a given combination of oxygen and glucose below that of a control, one can conclude that the treatment is protective against ischemia/hypoxia.

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