Introduction of cellular energy requirements

Screening for agents that may protect against CNS ischemia

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Protection of the brain and spinal cord against ischemia is a goal of vast clinical importance. One approach to this objective is to reduce the tissue's functional activity in order to preserve energy for the metabolic processes that are essential to viability. Experiments to explore ways of reducing function-related energy demands were performed on isolated rabbit retina, a well-characterized model of organized adult mammalian central nervous system (CNS) tissue. The retina was maintained in a nearly physiological state in a miniature "heart-lung" apparatus. Energy metabolism (oxygen consumption and glycolysis) and electrophysiological function (determined by electroretinogram) of the in vitro retina were monitored, and their responses to a series of agents that may reduce energy requirements were determined.

Large reversible reductions in O2 consumption, glycolysis, and electrophysiological function were seen in response to mild hypothermia (-3° to -6°C), phenytoin (Dilantin, 100 to 200 mg/kg), chlordiazepoxide (Librium, 200 μM), lithium (1 to 4 mM), Mg ++ (6 to 20 mM), strophanthidin (0.15 to 0.25 μM), CO2 (25% to 30%), 2-amino-5-phosphonovaleric acid (APV, 500 μM), amiloride (1 mM), and dantrolene (1 mM). One retina was exposed simultaneously to a combination of six of these agents, which reduced its oxidative and glycolytic metabolism to less than 50% of the control level. The retina recovered metabolic and electrophysiological function after a 2-hour exposure period. Other agents tested (diphenhydramine, midazolam, nifedipine, nimodipine, and quercetin) had effects on energy metabolism and electrophysiological function that were poorly reversible. Surprisingly little effect was seen in response to general anesthetic agents (thiopental and Althesin) and other CNS depressants (chlorpromazine, ethanol, lidocaine, paraldehyde, valproic acid, and baclofen). The presumed mechanisms through which these agents reduce cellular energy requirements, as well as their potential roles in the treatment of CNS ischemia, are discussed.

KEY WORDS · central nervous system ischemia · cellular energy requirements · rabbit retina

Approaches to preventing cell death due to ischemia generally fall into one of two categories: 1) the supply side; that is, enhancement of circulation, or 2) the demand side; that is, measures to extend cellular viability in the face of diminished circulation. One of the problems in the interpretation of research in this field has been to determine into which category a particular intervention fits: for example, in in vivo experiments, a pharmacological agent may influence cerebral circulation or metabolism, or both.

In order to study specifically the issue of cellular metabolic requirements, we have used an isolated preparation of organized adult mammalian central nervous system (CNS) tissue (rabbit retina) maintained in a miniature heart-lung apparatus. This experimental preparation has allowed us to monitor metabolic parameters (oxygen consumption and glycolysis) as well as electrophysiological function. We have found this system to be relatively simple and highly reproducible, and have used it to screen agents of potential benefit to ischemic CNS cells. Our screening to date has disclosed a number of agents which are promising in that they appear to promote reversible reductions in cellular energy requirements. Some of these data have been presented previously in preliminary form.4

Materials and Methods

Retinas were isolated from New Zealand White rabbits under darkroom conditions and incubated in the dark in a medium that simulated cerebrospinal fluid (CSF) with respect to electrolytes and 38 organic constituents. This technique has been described in detail.
A retina was secured over a mandrel in a miniature "heart-lung" apparatus and superfused with medium that had been equilibrated with a gas mixture of 40% O₂, 5% CO₂, and 55% N₂, and that was circulated by a peristaltic pump at a rate of 4.6 ml/min. The experiments were performed in a warm room maintained at 37°C. For studies of the effect of temperature itself, the apparatus was immersed in a water bath that permitted temperature to be adjusted over a 1.5°C range and maintained to within ± 0.2°C. Platinum electrodes incorporated in the apparatus on either side of the retina were used to record the electroretinogram (ERG). Pairs of electrodes sampled the circulating medium to determine O₂ and pH before and after it had passed over the retina. The electrodes' signals were conventionally amplified and displayed with a high-speed ink recorder.*

The apparatus was used in two modes for the measurement of O₂ consumption and acid production. In one, gas exchange in the "lung" was maintained, and the differences in the outputs of the paired electrodes were measured; these "arteriovenous" differences, multiplied by the flow rate of the medium, gave rates of O₂ consumption and acid production. In the other mode, the lung was bypassed so that there was a continuing fall in O₂ and pH in the relatively small volume of recirculating fluid (Fig. 1); these reductions, multiplied by the volume (2.3 ml), provided an alternative measure of O₂ consumption and acid production. The results obtained by the two methods agreed well. The first method showed the time course of the response to a change in conditions; the second method gave somewhat more reproducible results and was used for most of the studies reported here. Recordings of the electrode responses to known changes in pO₂, pCO₂, and temperature were obtained in the absence of a retina, in order to calibrate the electrodes and to correct for the effects of temperature and the relatively small movement of O₂ and CO₂ between the medium and the walls of the apparatus.

The rate of glycolysis was calculated assuming that the measured reduction in pH of the medium as it passed over the tissue was due principally to the generation of CO₂ from glucose oxidation and the generation of lactic acid from glycolysis. The generation of CO₂ from glucose oxidation was determined from the measurement of O₂ consumption. The additional fall in the pH of the bicarbonate-buffered medium was attributed to lactic acid and served as the basis for calculating its concentration. The concentration of lactic acid (HA) was determined by solving the following equation for HA:

\[
\text{pH}_v = 6.1 + \log \frac{22.6 - \text{HA}}{1.105 + \text{HA} + \text{CO}_2}
\]

In the equation, \(\text{pH_v}\) is the (lowered) pH measured in the "venous" medium containing the products of retinal metabolism; 6.1 is the pK' of carbonic acid; 22.6 is the initial concentration (mM) of HCO₃⁻ in the medium; 1.105 is the initial concentration (mM) of H₂CO₃ (and dissolved CO₂) with 5% CO₂ at 37°C; and CO₂ is the additional H₂CO₃ (and dissolved CO₂) generated by the oxidative metabolism of the tissue, as measured by its oxygen consumption.

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* Ink recorder manufactured by Gould Inc., Instruments Division, Cleveland, Ohio.
**TABLE 1**

Agents that marginally reduced energy metabolism

<table>
<thead>
<tr>
<th>Agent</th>
<th>Metabolism Change*</th>
<th>O₂ Consumption</th>
<th>Glycolysis</th>
<th>ERG b-Wave</th>
</tr>
</thead>
<tbody>
<tr>
<td>valproic acid, 5 mM (720 mg/kg)</td>
<td>-10</td>
<td>-8</td>
<td>-22</td>
<td></td>
</tr>
<tr>
<td>ethanol, 100 μM (2110 mg/kg)</td>
<td>-2</td>
<td>-14</td>
<td>-24</td>
<td></td>
</tr>
<tr>
<td>scopolamine, 20 μM (8 mg/kg)</td>
<td>-5</td>
<td>-11</td>
<td>-16</td>
<td></td>
</tr>
<tr>
<td>althesin† 0.05 ml/kg</td>
<td>+1</td>
<td>-15</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>thiopental, 340 μM (90 mg/kg)</td>
<td>+2</td>
<td>-12</td>
<td>-90</td>
<td></td>
</tr>
<tr>
<td>ether‡ 26 mM (1900 mg/kg)</td>
<td>+4</td>
<td>-12</td>
<td>+15</td>
<td></td>
</tr>
</tbody>
</table>

* Data for each drug are given in percent change from control values and represent an average of several measurements on a single retina; responses were usually quite consistent. ERG = electroretinogram.

† Althesin is a rapidly acting steroid anesthetic agent which consists of 9 mg of alphazalone and 3 mg of alphadolone acetate per milliliter. It is available from Glaxo Laboratories Ltd., Greenford, Middlesex, England.

‡ Dose may have been slightly lower due to volatilization of the gas.

Retinas incubating under control conditions appeared to be in a physiological state as judged by the stability of their electrophysiological responses and energy metabolism over many hours. Experiments lasted 6 to 12 hours. Usually each agent was introduced for 20 to 70 minutes, following which the retina was returned to control conditions in order to assess recovery; but, in some experiments, the effect of increasing the dose of the agent was observed before the retina was returned to control conditions (Fig. 2). In temperature experiments a succession of reductions (or increases) in temperature were tested before returning the retina to the starting temperature. In the one trial of a six-drug combination (see Fig. 6), the agents were introduced sequentially in the order shown so that the effects of each could be measured (each agent had been previously tested individually, and optimal doses were selected). After a 2½-hour incubation in the medium containing the drug combination, the retina was returned to control conditions, and recovery was monitored.
Protection against CNS ischemia

Results

Eight retinas perfused at 4.6 ml/min in control medium that was equilibrated with 40% O₂ consumed a mean of 0.121 ± 0.006 (standard error of the mean, SEM) μmoles of O₂ per minute and generated 0.149 ± 0.006 μM of lactic acid. The retinas' energy metabolism responded as expected to physiological stimuli. Flashing light (4 Hz), which activates on-off responses of the inner retina, increased glycolysis with little change in oxidative metabolism; and steady light, which diminishes the energy-consuming "dark current" of the photoreceptors, decreased O₂ consumption with little change in glycolysis. Moderate reductions in the O₂ content of the medium were associated with reversible reductions in O₂ consumption and increases in glycolysis (Pasteur effect).

The changes in O₂ consumption, glycolysis, and ERG B waves in response to a variety of agents are shown in Figs. 3 through 6 and Tables 1 and 2. The b-wave was used as a measure of the light-evoked electrophysiological responses of neurons postsynaptic to the photoreceptor cells (Fig. 2). Several measurements of each type were usually obtained and averaged for each retina under each condition, and these were usually quite reproducible. The numbers of experiments and error bars in the figures refer to the number of different retinas examined under each condition.

Agents Causing Reversible Reduction in O₂ Consumption and Glycolysis

The agents that were effective in reversibly reducing metabolism and electrophysiological function are shown in Fig. 3. These agents had a rapid onset of action, with a half-life (t½) for reduction of the b-wave of less than 2 minutes, and often less than 1 minute (see Fig. 2B). The time course for reduction of O₂ consumption was slower, with a t½ in the range of 2 to 5 minutes. This, along with the generally quite prompt recovery after washout of the agents, serves as evidence that it was the usage of energy, rather than its generation, that was reduced by these agents.

Hypothermia. Hypothermia caused quite parallel reductions in O₂ consumption, glycolysis, and b-wave. Regression analyses of measurements obtained between 28° and 38°C showed a mean rate change with 10°C increase (Q₁₀) of 1.9 ± 0.1 for O₂ consumption and 2.2 ± 0.3 for glycolysis. The tissue responses occurred as fast as we could change the temperature and appeared to be completely reversible over this range.

Lithium. Lithium was administered as lithium sulfate or as lithium chloride. It caused consistent reductions in all of the parameters measured, and these were independent of the nature of the anion (Figs. 1 to 3). The responses of O₂ consumption, glycolysis, and b-wave were dose-related, at least to 16 mEq/liter of lithium, and were followed by full recovery on return to control medium. The ERG monitor (Fig. 2B) showed an initial b-wave recovery to greater than control amplitude, with subsequent return to the control level.

Strophanthidin. Strophanthidin caused a dose-related reduction in O₂ consumption, to 50% at 1 μM, indicating that a major portion of the energy derived from oxidation is consumed by Na⁺K⁺-adenosine triphosphatase (ATPase). The effect on glycolysis was less consistent at lower concentrations; but, in two experiments with 0.5 μM of strophanthidin (not shown in Fig. 3), glycolysis was reduced by 31% and 33%. Winkler et al. has reported a 61% reduction in glycolysis in rat retina treated with 100 μM of ouabain. At 0.5 μM and above, strophanthidin appeared to cause irreversible damage. At 0.25 and 0.15 μM, O₂ consumption was still substantially reduced (by 28% and 24%, respectively), and this was promptly reversible. A dose of 0.1 μM was used in the drug-combination experiment (see below), and this reduced O₂ consumption by 7%.

Magnesium. Magnesium caused dose-related reductions of b-wave and glycolysis, with relatively little effect on O₂ consumption. Although the effects of Mg²⁺ were quite reversible, recovery was relatively slow (t½ =

TABLE 2

<table>
<thead>
<tr>
<th>Agent</th>
<th>O₂ Consumption</th>
<th>Glycolysis</th>
<th>ERG b-Wave</th>
</tr>
</thead>
<tbody>
<tr>
<td>gamma hydroxybutyric acid, 20 mM (2525 mg/kg)</td>
<td>+3</td>
<td>-9</td>
<td>+31</td>
</tr>
<tr>
<td>ammonium chloride, 1 mM (54 mg/kg)</td>
<td>-4</td>
<td>-1</td>
<td>-10</td>
</tr>
<tr>
<td>paraaldehyde, 8 mM (1060 mg/kg)</td>
<td>+2</td>
<td>-5</td>
<td>+15</td>
</tr>
<tr>
<td>lidocaine, 620 μM (145 mg/kg)</td>
<td>+8</td>
<td>-9</td>
<td>+26</td>
</tr>
<tr>
<td>baclofen, 100 μM (22 mg/kg)</td>
<td>+5</td>
<td>+1</td>
<td>+26</td>
</tr>
<tr>
<td>chlorpromazine, 1 μM (355 mg/kg)</td>
<td>+4</td>
<td>+9</td>
<td>+14</td>
</tr>
<tr>
<td>sodium bromide, 32 mM (3293 mg/kg)</td>
<td>-2</td>
<td>+19</td>
<td>-38</td>
</tr>
<tr>
<td>dimethylsulfoxide, 500 μM (123 mg/kg)</td>
<td>-3</td>
<td>+23</td>
<td>+10</td>
</tr>
</tbody>
</table>

* Data for each drug are given in percent change from control values and represent an average of several measurements on a single retina; responses were usually quite consistent. ERG = electroretinogram.

† Dose may have been slightly lower due to addition of drug in partial suspension in order to avoid unknown vehicle effects.

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Fig. 3 (continued →)
Protection against CNS ischemia

**Fig. 3.** Agents that reduced energy metabolism. Oxygen consumption, glycolysis, and electroretinogram (ERG) b-wave responses are expressed as percent of control values ± standard error of the mean. C = control; T1 = first test dose; T2 = second test dose; R = recovery after drug washout with return to control medium. The doses shown for phenytoin are in milligrams per kilogram; the equivalent concentrations are 365 μM (100 mg/kg) and 730 μM (200 mg/kg). The number of retinas is given in parentheses; where no number is indicated, only one retina was studied. AVP = 2-amino-5-phosphonovaleric acid.
Agents That Were Not Reversible

Phenytoin. Phenytoin (Dilantin) also caused substantial reductions in glycolysis and b-wave along with a modest reduction in O₂ consumption. There was complete recovery of metabolism and partial recovery of the ERG (at lower doses, ERG recovery improved). A dose of 1.1 mM (300 mg/kg) caused no further reduction in O₂ consumption and only minimal further decreases in glycolysis and b-wave (not shown in Fig. 3). This finding correlates with a protection study which found that phenytoin significantly prolonged survival time in hypoxic mice at an optimal dose of 200 mg/kg, but not at 300 mg/kg.

Chlordiazepoxide. Chlordiazepoxide (Librium) at 200 μM consistently reduced glycolysis, O₂ consumption, and the ERG b-wave, with full recovery. A dose of 300 μM produced no significant further reduction in metabolism or b-wave (not shown in Fig. 3).

Carbon Dioxide. Carbon dioxide caused a marked and very rapid reduction in b-wave, which recovered immediately when the CO₂ of the medium was returned to normal levels, and it reduced O₂ consumption by more than one-third. The concentration of CO₂ in these experiments reduced the pH of the medium to 6.7, preventing an accurate measurement of glycolysis on the basis of its pH effects. Higher levels of CO₂ caused irreversible changes, presumably due to the reductions in pH both intracellularly and extracellularly.

Amiloride. Amiloride produced substantial reductions in O₂ consumption and glycolysis, with a slightly lesser effect on the ERG b-wave. However, recovery of these functions was quite delayed (tᵢ = 20 minutes for b-wave recovery).

Dantrolene. Dantrolene, which posed a solubility problem in our in vitro system, caused modest reductions in metabolic and electrophysiological parameters, with good recovery.

Agents That Were Not Reversible

Some of the agents studied were not reversible. These are shown in Fig. 4.

Diphenhydramine. Diphenhydramine (Benadryl) in large doses (100 and 200 μM) caused a prompt and dramatic reduction in the b-wave, with nearly a 30% reduction in glycolysis and a lesser effect on O₂ consumption. The metabolic functions recovered quite well, but a lower dose may be needed to allow full recovery of electrophysiological function.

Midazolam. Midazolam, a newer benzodiazepine now used clinically in anesthesia, produced reductions in O₂ consumption and b-wave comparable to those seen with chlordiazepoxide, but did not reduce glycolysis or allow full recovery of the b-wave.

Nifedipine and Nimodipine. Nifedipine and nimodipine both appeared to reduce glycolysis selectively, with essentially no effect on O₂ consumption. The ERG b-wave did not return to control levels after washout of these agents.

Trial of Drugs in Combination

Based upon the above results with individual agents, a combination of six agents was selected in order to determine whether their effects were additive and still reversible after a prolonged exposure period. Figures 5 and 6 demonstrate that the agents in combination did have additive effects on O₂ consumption. Phenytoin and chlordiazepoxide appeared to have synergistic effects in reducing both O₂ consumption and glycolysis, with additional effects demonstrated by the subsequent agents. A lower dose of CO₂ (15%) was used in the drug-combination experiment. The ERG b-wave was eliminated by the addition of the second drug, and the metabolic parameters were reduced to approximately 50% of the control levels. Reversibility was quite good after a 2½-hour exposure to all six agents.

Agents With Marginal or No Effects on Energy Metabolism

Tables 1 and 2 list a variety of agents with known clinical effects on the CNS (for instance, general anesthetic, sedative, CNS depressant, and anticonvulsant drugs) or putative roles in altering cell membrane or receptor function. Each agent was administered in successive doses to a single retina. The data reported in these tables is responses to the highest doses given, with the exceptions of thiopental and ethanol; higher doses of these latter agents caused negligible further changes in metabolism.

Discussion

Protecting CNS tissue against ischemia by reducing its energy requirements depends upon inhibiting metabolic processes that are not essential for maintaining cell viability, while preserving those that are. Astrup has distinguished between "activation metabolism" (which supports signaling function) and "residual metabolism" (which supports the general "housekeeping" functions of the cells such as maintenance of membranes, ion transport, and synthesis of cellular components). An ideal protective agent would relieve the cell of its signaling workload while preserving the metabolic processes essential for viability. In addition, this hypothetical ideal agent would be quickly and easily delivered to the CNS, and would have effects that are readily
Protection against CNS ischemia

The screening system we have described here permits the identification of agents that reversibly reduce O₂ consumption and glycolysis of isolated organized CNS tissue. Our results demonstrate substantial differences in the tissue's response to various agents; there were

reversible once an adequate level of perfusion is reestablished via clot lysis or collateral enlargement. Finally, when introduced in vivo, the agent’s effects on other excitable tissues (the cardiovascular system in particular) would have to be tolerable.

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dramatic and reversible reductions in energy metabolism and electrophysiological function in the presence of a few agents (phenytoin, chlordiazepoxide, and lithium), whereas surprisingly little effect was seen with several of the general CNS depressants (thiopental, Althesin, chlorpromazine, paraldehyde, and lidocaine).

Hypothermia has been recognized as an effective means of protection for some years. Severe reductions in temperature can produce harmful effects on cells; therefore, it is important to recognize that mild hypothermia, as demonstrated here, can substantially reduce metabolic demand. Hypothermia generally reduces the rates of most enzymatic reactions. However, the "membrane-stabilizing" effect of temperature reduction may also be important in its protective role.

Lithium has been the drug of choice in the treatment of manic-depressive illness for some time, despite the fact that its mechanism of action remains unclear. Its interaction with membrane transport systems has been extensively investigated. Ehrlich and Diamond reviewed these phenomena in their paper published in 1980. More recently, it has been shown that lithium inhibits the enzyme inositol 1-phosphatase, thus preventing the regeneration of inositol. It has been proposed that lithium may exert its therapeutic effect by thus interfering with the metabolism of phosphoinositides, which may play a role in synaptic and receptor function. The reductions in energy metabolism and electrophysiological function measured in the retina, even at a therapeutic dose of 1 mM, make this an agent of considerable interest for ischemia studies.

A marked inhibition of Na⁺K⁺-ATPase by strophanthidin or other cardiac glycosides is in itself damaging to the cells. However, the data presented here suggest that partial inhibition (for instance, 20%) can be reversibly sustained and even permit continued electrophysiological function, with an appreciable saving of energy. There is previously published evidence that inhibition of this ATPase by ouabain reduces in vivo metabolism by 20% to 25% in dog brain and may be protective against ischemic injury in other actively pumping tissues such as the kidney.

Magnesium offers promise as a generalized inhibitor of synaptic transmission and of the accompanying energy expenditures. The marked and relatively selective reduction in the generation of lactic acid that was observed with Mg²⁺ may itself be protective. Magnesium crosses the blood-brain barrier slowly, but large increases in plasma levels may have central depressant effects. In recent studies of ischemia in the rabbit spinal cord, Mg²⁺ pretreatment improved neurological outcome; however, at least in the latter study, its effect was not likely due to inhibition of synaptic transmission in that it did not suppress somatosensory evoked potentials. Thus, it may have been effective via a vascular mechanism, such as enhancement of collateral circulation or prevention of delayed postischemic hypoperfusion.
Protection against CNS ischemia

Phenytoin (Dilantin) is widely used clinically for its potent anticonvulsant activity. In animal studies it has been found to substantially prolong survival time in hypoxic mice and to protect neurons in the hippocampus and dentate nucleus of rabbits subjected to 15 minutes of global cerebral ischemia. Proposed mechanisms for this protective effect have included reduction of hypoxia-induced seizure activity, an increase in cerebral blood flow, and a reduction in the cerebral metabolic rate for O2; the former study, which examined these proposals, did not find evidence to support any of them. These investigators did find that pretreatment with phenytoin resulted in a reduction in the rate of K+ accumulation in cisternal CSF of dogs after 20 minutes of anoxia. Thus, a membrane-stabilizing role was proposed for phenytoin’s mode of action. In accord with our finding of a predominant effect on glycolysis, an earlier study reported that phenytoin pretreatment (200 mg/kg) resulted in a reduction in the utilization of glucose and high-energy phosphate in mouse brain after decapitation.

Chlordiazepoxide (Librium) and midazolam are benzodiazepines that are clinically useful for treatment of alcohol withdrawal, as anxiolytic and sedative-hypnotic agents, and for induction of general anesthesia. Midazolam and another benzodiazepine, diazepam (Valium), have been found to reduce the cerebral metabolic rate for O2 in the dog, and to provide significant protection in a hypoxic mouse model. Even at the extremely high doses of chlordiazepoxide in our model, the retinas recovered promptly and completely all parameters of metabolic and electrophysiological function. This, along with this agent’s wide margin of clinical safety, make chlordiazepoxide a promising agent for further study.

The rather high levels of CO2 examined caused a surprisingly large reduction in O2 consumption; this effect was not observed with a similar CO2 concentration in rat brain in vivo. The latter study did demonstrate a significant reduction in glucose utilization, an effect probably seen in our system as well, although we were unable to measure this accurately at the higher concentrations. In a protection study, Secher and Wilhelmson found that a concentration of 5% CO2 in the inhaled-gas mixture increased survival time of anoxic mice by 137%. These findings, together with the rapidity with which electrophysiological function recovered after elevated CO2 in our system, suggest that this agent warrants further investigation, even though its effects on the cerebral vasculature will have to be taken into account.

2-Amino-5-phosphonovaleric acid (APV) is a potent representative of a new class of agents that inhibit excitotoxic amino acid receptors. There is some evidence that synaptic release of these excitatory amino acid neurotransmitters (glutamate and aspartate) may contribute to neuronal death due to hypoxia. Two of the receptor blockers (2-amino-7-phosphonoheptanoic acid and D-glutamylglycine) have been shown to protect against ischemic neuronal damage in rat brain in vivo and in vitro.

Amiloride is a K+-sparking diuretic that blocks the Na+ channel in the distal nephron. This Na+ channel is apparently characteristic of “tight” epithelia and is probably also present in the CNS. Dantrolene is used clinically as an effective antispasticity agent, and is the treatment of choice for malignant hyperthermia. It reduces contraction of skeletal muscle by a direct action on excitation-contraction coupling, perhaps by decreasing the amount of calcium released from the sarcoplasmic reticulum. An uncontrolled increase in intracellular calcium is thought to be one of the important pathophysiological events during ischemia. Thus, a logical drug combination for treatment of ischemia might include dantrolene to inhibit release of intracellularly sequestered calcium and nifedipine or nimodipine to block entry of extracellular calcium. Nimodipine administration has resulted in improved neurological outcomes after complete cerebral ischemia in dogs when given prior to ischemia and in primates when given 5 minutes after an ischemic episode. In mongolian gerbils, pretreatment with nimodipine retarded the fall in adenosine triphosphate in cortex and striatum during ischemia and facilitated the recovery of glucose in these regions during recirculation. The beneficial results found with nimodipine in ischemia studies and in patients with vasospasm after subarachnoid hemorrhage may be secondary to its metabolic as well as its vascular effects.

Several other agents depicted in Fig. 4 are of potential interest. Histamine is currently considered a likely candidate as a neurotransmitter in mammalian brain. H1 and H2 histamine receptors, as well as other subtypes, are believed to reside in the CNS, and these may mediate the well-known sedative effects of the clinically useful antihistamines. Thus, it is not altogether surprising that diphenhydramine (Benadryl) had pronounced effects upon CNS tissue metabolism and electrophysiological responses in our model. It is also of interest that, at high doses, H1 receptor antagonists like diphenhydramine have a local anesthetic action. The poor reversibility of the ERG b-wave after removal of this drug may indicate a problem with toxicity in neurons. Quercetin is a ubiquitous plant flavonoid with a wide variety of postulated physiological effects. Of particular interest is a proposal that quercetin may enhance the efficiency of Na+K+-ATPase. The reductions in energy metabolism that we observed in the retina may correlate with this hypothesis. The irreversibility of its effects is probably attributable to difficulty in eluting the drug with our protein-free medium.

Our results for the agents listed in Tables 1 and 2 indicate surprisingly little reduction in energy metabolism by these CNS depressants. This is in contrast with studies on intact brain which have shown significant reductions in metabolic rate in response to some of these agents. This may reflect a greater effect of these anesthetic agents on highly integrated systems.
containing many synapses in sequence. The literature on barbiturates and CNS protection is divided; there seem to be protective effects in focal cerebral ischemia, but not consistently in global ischemia. Lidocaine did not protect against ischemia in rabbit spinal cord.

The classification of the agents used in our study into the various categories illustrated in the figures and tables is somewhat arbitrary. For example, valproic acid was categorized as an agent that had marginal effects on energy metabolism. It did appear to reduce both oxygen consumption and glycolysis, but less dramatically than did the agents in Fig. 3. Other agents (such as Althesin, ethanol, thiopental, ether, scopolamine, lidocaine, and gamma-hydroxybutyric acid) appeared to reduce glycolysis selectively, with generally fair to good reversibility. Certain of these agents may well deserve further consideration in protection studies, in which a small but reproducible energy savings could contribute to recovery in marginally perfused tissue. In particular, the agents that reduced glycolysis selectively may be useful in combination with agents that reduced oxygen consumption primarily.

The single trial in which the retina was exposed to both Mg§ and strophanthidin represents a preliminary demonstration that agents with different mechanisms of action can be usefully combined. The experiment in which the retina was exposed simultaneously to six agents (each of which individually reduced energy metabolism) demonstrated that CNS tissue can tolerate a prolonged period of reduction of O2 consumption and glycolysis to less than 50% of control levels with essentially full recovery of both metabolic and electrophysiological function. We plan further combination experiments such as this, including combinations with mild hypothermia. Vacanti and AmesŽ reported that the rabbit spinal cord stroke model has demonstrated the efficacy of combination therapy with elevated serum Mg§ and mild hypothermia. Other animal models and combinations of agents have been tried as well.

Acknowledgments

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References

4. Ames A III, Zager EL: Reducing cellular energy requirements as protection against CNS ischemia, in Hartmann A, Kuschinsky W (eds): Cerebral Ischemia and Hemo-
25. Nugent M, Artru AA, Michenfelder JD: Cerebral meta-
Protection against CNS ischemia


44. Steen PA, Milde JH, Michenfelder JD: No barbiturate protection in a dog model of complete cerebral ischemia. Ann Neurol 5:343–349, 1979


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