Numerous researchers have suggested that cerebral protection from ischemia may be provided by pharmacological agents that are calcium antagonists. One such compound is flunarizine. Although its mechanisms of action are not well understood, flunarizine has demonstrated protective effects following cerebral ischemia as manifested by improved survival of hippocampal neurons, improved animal survival after prolonged global ischemia, and improved functional recovery after focal ischemic insult, even when the flunarizine is administered after termination of the ischemic event. It is also possible that flunarizine may be of prophylactic value in the population at increased risk for cerebral ischemia in enhancing the rate of cerebral recovery after an ischemic insult. We have used electroencephalogram (EEG) recovery time following bilateral forebrain ischemia in the gerbil as a method to test this potential effect.

Materials and Methods

Male gerbils weighing 65–75 g were anesthetized with halothane 2.5%, and N₂O:O₂ (2:1). Both carotid arteries were exposed under sterile technique by anterior cervical incision, and a 6-0 polypropylene loop was placed around both carotid arteries. Halothane was decreased to 0.5%, and the animals were placed in a prone position on a thermal blanket with the head and neck extending beyond the edge of the heating blanket. Monopolar platinum wire electrodes were inserted subcutaneously on either side over the frontal lobe, and a ground electrode was inserted subcutaneously in the mandible. The amplified EEG (Tektronix 5A26) was continuously monitored on an oscilloscope (Tektronix D11) and a strip recorder (General Scanning, Inc.) running at 50 mm/sec. Mean±SD body temperature was maintained at 37°±0.2°C by the heating blanket attached to a rectal probe (Harvard Instruments). Mean arterial blood pressure (MABP) was continuously monitored by a tail cuff manometer (Harvard), and O₂ saturation (O₂ Sat) was continuously monitored by a
transcutaneous oximeter (Nellcor). Temperature, MAP, and O\(_2\) Sat were stabilized during a 10- to 20-minute period before onset of ischemia and recorded every 60 seconds before, during, and after termination of ischemia. Ischemia was produced by suspending a 24-g weight from the bicaudal polypropylene loop. Adequacy of the occlusion was verified by direct inspection of the carotids, and, in the time of onset, by isoelectric EEG. Gerbils were subjected either to 4 minutes (group 1, \(n=40\)) or 6 minutes (group 2, \(n=48\)) of ischemia. For an animal to be included in the final data, an isoelectric EEG had to be achieved within 15 seconds after carotid occlusion. Following release of the occlusion, each gerbil remained anesthetized in the posts ischemic period, with rectal temperature maintained at 37°C until EEG recovery had taken place. We have divided the preischemic anesthetized gerbil EEG into three rhythm frequency categories: 4–6, 7–10, and 11–15 Hz electric activity, respectively. The EEG frequencies were determined by two methods. The EEG tracings were made on paper printed in 1-mm increments, and by knowing the speed of the paper drive (50 mm/sec), one could calculate the frequency of the EEG waveforms. The EEG frequencies were also measured using electrocardiogram calipers set at the bases of both arms of the deflection of each measured wave. The caliper was then transferred to a premeasured grid from 1 to 20 Hz to make a direct reading. If the calipers fell between two frequencies, the measurement was assigned to the higher of the frequencies. In no case was there a discrepancy between the results of the two methods of measurement. One hour before onset of ischemia, gerbils received a single intraperitoneal injection of flunarizine or cyclodextrin (Janssen Pharmaceutica, Beerse, Belgium) or the cyclodextrin vehicle alone. One investigator analyzed the EEGs without knowledge of whether animals had received flunarizine or vehicle alone.

An additional group of male gerbils, also weighing 65–75 g, were prepared in the identical manner to those above except that instead of EEG leads, a wire thermal probe (Omega Hyp-O) was inserted into the right striatum, through a hole made in the skull with a 20-gauge cutting needle, and attached to a meter (Omega 670T) capable of measuring temperature in 0.1°C increments. Both the rectal probe and the intracerebral rectal probe were calibrated against a United States Bureau of Standards-approved mercury thermometer in a water bath over a temperature range of 28–40°C. Each animal’s head was held in a small animal stereotactic apparatus (Konte) and the probe held in place by attachment to an adjustable bar mounted on the stereotactic apparatus. One hour prior to onset of ischemia, gerbils received a single intraperitoneal injection of flunarizine dissolved in 10 mg/kg hydroxypropyl β-cyclodextrin (Janssen Pharmaceutica, Beerse, Belgium) or the cyclodextrin vehicle alone. One investigator analyzed the EEGs without knowledge of whether animals had received flunarizine or vehicle alone.

The intracerebral position of the thermal probe was verified by autopsy after termination of the experiment. Ambient temperature in the laboratory was 20±1.0°C throughout these experiments and rectal temperature of the gerbils maintained at 37±0.2°C by the aforementioned heating blanket. Brain and rectal temperature were continuously monitored and recorded every 60 seconds for 20 minutes before and 60 minutes after intraperitoneal administration of either 10 mg/kg flunarizine or cyclodextrin vehicle, following which bilateral carotid occlusion was performed, as described above, for 10 minutes. Brain and rectal temperature were continuously monitored and recorded each minute during the 10 minutes of cerebral ischemia and for 60 minutes after termination of the ischemia.

Differences in the mean time necessary for return of activity at 4–5 Hz, 7–10 Hz, and 11–15 Hz in the postischemic period in flunarizine versus vehicle-treated groups were analyzed by the Levene statistic, which revealed a failure to meet parametric assumption of homogeneity of variance. Therefore, comparison of means for significant differences were achieved by the Mann-Whitney U test. Differences in mean brain temperatures in flunarizine- and cyclodextrin-treated controls were analyzed for significance by the t test because use of the Levene statistic revealed that the data met the parametric assumption of homogeneity of variance.

**Results**

The EEG failed to become isoelectric in two flunarizine-pretreated gerbils in group 1 and in one vehicle-pretreated and two flunarizine-pretreated gerbils in group 2. In two additional flunarizine-pretreated gerbils in group 2, suspension of the 24-g weight tore the carotid arteries and led to rapid exsanguination. These animals were excluded from the analysis of data. In all other gerbils, the EEG became isoelectric within 15 seconds after carotid occlusion. There were no significant differences in rectal temperatures, MABP, or O\(_2\) Sat before, during, or after cessation of ischemia between the different groups (Table 1) and no significant differences in the time of onset of isoelectric EEG in the different groups (Table 2). Pretreatment with 10 mg/kg i.p. flunarizine resulted in significantly more rapid recovery of EEG activities (\(p<0.001\)) after either 4 or 6 minutes of ischemia than that observed in vehicle-treated animals (Table 2B). Time of onset of ischemia was measured from time of carotid occlusion and not from the time of onset of isoelectric EEG. In both vehicle- and flunarizine-treated gerbils, 4–5 Hz activity was the first to reappear and 11–15 Hz activity reappeared last. During the recovery phase, paroxysmal bursts, which did not appear epileptiform, were occasionally seen on EEG in vehicle- but not flunarizine-treated animals. No clinical seizures were observed before, during, or after cessation of ischemia during the period of EEG monitoring.

In animals whose brain temperature was measured, there were no significant differences in vehi-
TABLE 1. Mean Rectal Temperature, Mean Arterial Blood Pressure, and Arterial Oxygen Saturation (±SD) Before, During, and After 4 or 6 Minutes of Bilateral Carotid Ligation in Gerbils Pretreated With 10 mg/kg Flunarizine or Its β-Cyclodextrin Vehicle

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (4 minutes of ischemia)</th>
<th>Group 2 (6 minutes of ischemia)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle (n=20)</td>
<td>Flunarizine (n=18)</td>
</tr>
<tr>
<td>Mean rectal temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preischemia</td>
<td>37.0±0.09</td>
<td>37.0±0.10</td>
</tr>
<tr>
<td>Ischemia</td>
<td>36.9±0.08</td>
<td>37.0±0.07</td>
</tr>
<tr>
<td>Postischemia</td>
<td>36.9±0.10</td>
<td>36.9±0.10</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preischemia</td>
<td>89.8±2.0</td>
<td>89.3±3.1</td>
</tr>
<tr>
<td>Ischemia</td>
<td>104.4±6.3</td>
<td>106.0±3.4</td>
</tr>
<tr>
<td>Postischemia</td>
<td>82.1±5.2</td>
<td>79.4±3.8</td>
</tr>
<tr>
<td>Oxygen saturation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preischemia</td>
<td>99.4±0.5</td>
<td>99.8±0.4</td>
</tr>
<tr>
<td>Ischemia</td>
<td>99.7±0.4</td>
<td>99.7±0.5</td>
</tr>
<tr>
<td>Postischemia</td>
<td>99.4±0.5</td>
<td>99.3±0.8</td>
</tr>
</tbody>
</table>

Statistical significance of differences in mean rectal temperatures, mean arterial blood pressure, and oxygen saturation in each group was determined by the t test. NS, not significant.

cle- and flunarizine-treated groups before (Figure 1A), or after receiving vehicle or flunarizine (Figure 1B), during forebrain ischemia (Figure 1C), or in the 60-minute postischemic period (Figure 1D).

Discussion

The results of these studies suggest that flunarizine enhances the rate of recovery of the brain from ischemia as measured by return of spontaneous electrical activity on EEG recording. We did not attempt to determine the time necessary for the EEG to return to normal in the postischemic period because we do not believe that the gerbil EEG is sufficiently characterized to accurately define such an end point by visual inspection of the tracing; instead, we measured the time required, after cessation of ischemia, for the reappearance of 4–5 Hz, 7–10 Hz, and 11–15 Hz activity. Quantitative spectrum analysis, which was not available to us, might be used to determine when the EEG returns to normal. Using quantitative analysis, Wauquier et al13 have demonstrated improved EEG recovery following global ischemia in the dog. Flunarizine administration has also produced generalized slowing in EEG background activity in human subjects with epilepsy,14 power spectrum analysis demonstrating a reduction in the alpha/theta ratio. We are unaware of similar analysis of flunarizine effects on control nonepileptic subjects. In comparing the preischemic EEG of flunarizine- and cyclodextrin-treated controls, we could detect no differences between the two groups by visual inspection. However, because our study was designed to detect the time of reappearance of EEG frequencies rather than the relative abundance of various frequencies, we believe this to be a relatively minor shortcoming of our study.

Although flunarizine treatment either before or after ischemic insult to the brain reduces neuronal damage as viewed histologically,5,8–10 prolongs animal survival,11 and enhances functional recovery,12 its

TABLE 2. Mean Recovery Time of EEG Activity After 4 or 6 Minutes of Bilateral Carotid Ligation in Gerbils Pretreated Intraperitoneally With 10 mg/kg Flunarizine or Its β-Cyclodextrin Vehicle

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (4 minutes of ischemia)</th>
<th>Group 2 (6 minutes of ischemia)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle (n=20)</td>
<td>Flunarizine (n=18)</td>
</tr>
<tr>
<td>A. Mean onset (sec) of isoelectric EEG after ischemia (mean±SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.1±1.42</td>
<td>12±2.17</td>
</tr>
<tr>
<td>B. Mean recovery time (min) of EEG activity after cessation of ischemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4–5 Hz</td>
<td>11.70</td>
<td>7.34</td>
</tr>
<tr>
<td>7–10 Hz</td>
<td>12.57</td>
<td>7.93</td>
</tr>
<tr>
<td>11–15 Hz</td>
<td>14.41</td>
<td>9.48</td>
</tr>
</tbody>
</table>

Statistical significance of differences in onset of isoelectric electroencephalogram (EEG) determined by t test; statistical significance of differences in mean EEG recovery times determined by Mann-Whitney U test. NS, not significant.

*Two animals excluded.
†One animal excluded.
‡Four animals excluded.
pharmacological mechanisms of action are not well understood. Flunarizine does inhibit calcium-stimulated vasconstriction,\textsuperscript{13} and may increase cerebral blood flow,\textsuperscript{16} the prevention of postischemic fall in cerebral blood flow being a potential protective mechanism of action. Calcium overload is an important potential mechanism of postischemic neuronal damage,\textsuperscript{1-4} and increased intraneuronal calcium has been observed ultrastructurally immediately after cessation of global cerebral ischemia.\textsuperscript{7} Flunarizine inhibits calcium conductance through T- and L-type voltage-regulated channels in cardiac muscle,\textsuperscript{17} inhibits calcium conductance through T-type channels in rat hypothalamic neurons,\textsuperscript{18} blocks presynaptic calcium uptake after sustained in vitro depolarization,\textsuperscript{19} and also delays anoxia-induced neuronal depolarization.\textsuperscript{20} Flunarizine acts as a calcium antagonist, but probably has other modes of action as well, particularly as a sodium antagonist.\textsuperscript{21} Thus, flunarizine prevents calcium-dependent sodium ionophore-induced cell death,\textsuperscript{21} probably in part due to its role as a calcium antagonist, but also possibly as a result of sodium antagonist activity. Additional direct evidence that flunarizine is a sodium antagonist is that it can block a low-threshold tetrodotoxin (a sodium-channel blocker)-sensitive channel.\textsuperscript{22} Although no specific neuronal receptor site for flunarizine has been identified, it is highly lipophilic and strongly bound to intracellular phospholipid residues, particularly phosphatidyl serine,\textsuperscript{23} and in so doing may effect membrane fluidity,\textsuperscript{24} reducing the likelihood of opening of intramembranous ion channels and thus impeding potentially damaging ionic flux following anoxia or ischemia. Flunarizine’s effects on posts ischemic ionic flux and on ischemia-induced depolarization may in part explain its use resulting in the more rapid return of EEG activity seen in our animals during the postischemic period.

We administered flunarizine intraperitoneally 1 hour before ischemia because a previous study demonstrated that we would achieve maximum levels of 2 \(\mu g\) flunarizine/g brain 45–60 minutes after intraperitoneal injection.\textsuperscript{11}

The onset of an isoelectric EEG within 15 seconds after bilateral carotid ligation is similar to the observations of others using a similar model of gerbil ischemia.\textsuperscript{25} Although clinical seizure activity is frequently seen after cerebral ischemia in the gerbil,\textsuperscript{26-28} we noted no clinical seizures or epileptiform discharges on EEG. This may in part be the result of the shorter duration of ischemia used in this study. We have observed clinical seizures within 1 hour postischemia in 100% of gerbils from the same animal colony when they were subjected to 20 minutes of bilateral carotid occlusion.\textsuperscript{11} An additional reason we may not have observed seizures is that the animals remained anesthetized in the postischemic period, in some cases up to 60 minutes.

Although we maintained body temperature via a rectal probe controlled heating blanket, the large surface area to volume ratio of a small rodent could lead to variations in head temperature which could significantly affect outcome. Not only may reduced brain temperature enhance postischemic neuronal survival,\textsuperscript{29,30} but a potential mechanism of pharmacological efficacy of some compounds in the treatment of brain ischemia may be their production of sustained hypothermia.\textsuperscript{31} Our temperature monitoring results fail to demonstrate significant variations in brain or rectal temperatures using our thermal blanket set-ups before, during, or after ischemia. Furthermore, flunarizine at the dose we used had no significant effect before, during, or after forebrain ischemia. The 10-minute duration of ischemia in the brain temperature experiments exceeded the duration of ischemia (4 or 6 minutes) in the EEG recovery studies to demonstrate that in ischemic periods inclusive of, and even exceeding, those used in the postischemic EEG recovery experiments, flunarizine had no significant effect on brain temperature during or after termination of ischemia. These results demonstrate that flunarizine’s effects on EEG recovery are not the result of drug-induced alteration in brain temperature in the intras ischemic period at least up to the first 60 minutes of the postischemic period. We chose to measure intracerebral temperature rather than scalp temperature because we wanted to determine flunarizine’s effects on brain temperature, and there may be significant variation between scalp and brain temperature.\textsuperscript{29} There was an almost 2°C difference between brain and rectal temperatures in our animals even before drug administration or onset of ischemia. Differences in accuracy of the temperature probes would not explain these differences in temperature because the accuracy of both the rectal and brain probes were verified by a US Bureau of Standards–approved thermometer. The relatively large
surface-to-volume ratio of the head, combined with the need in our set-up to extend the head and neck beyond the free edge of the thermal blanket to allow for suspension of the carotid occluding weight, may account for the differences in brain and rectal temperatures. In addition, a head-heating lamp, used in other studies to regulate brain temperature, was not used in our study because our intent was to determine whether flunarizine affected brain temperature. Unfortunately, this precluded our measuring brain temperature and EEG in the same animal because of the potential effects on EEG of the brain injury produced by insertion of the cerebral thermal probe.

In summary, flunarizine enhances the rate of recovery from cerebral ischemia in the gerbil as measured by EEG, and may be of potential prophylactic value in subjects at risk for cerebral ischemia.

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Key Words • cerebral ischemia • electroencephalography • flunarizine • gerbils
Effect of flunarizine on electroencephalogram recovery and brain temperature in gerbils after brain ischemia.

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