Side effects of spasmolytic agents in the monkey

Intracisternal phenoxybenzamine and phentolamine

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The authors report a study of the physiological and morphological responses of the rhesus monkey brain to intracisternal injections of two potentially useful spasmolytic agents, phenoxybenzamine and phentolamine. Central nervous system function remained unaffected by intracisternal injections of either drug in doses that were small but nevertheless sufficient to attain pharmacologically effective concentrations in the cerebrospinal fluid. Larger doses of phenoxybenzamine caused a chemical basilar meningitis. Phentolamine caused arterial hypotension without meningitis. It is concluded that intracisternal spasmolytic therapy deserves further experimental evaluation.

KEY WORDS - cerebral vasospasm - alpha adrenergic blockade - phenoxybenzamine - phentolamine

When spasmolytic drugs are either applied topically to affected vessels or added directly to the cerebrospinal fluid (CSF) they reverse the cerebrovascular spasm that follows experimental subarachnoid hemorrhage more effectively than when they are blood-borne.6,10,12,13 These observations imply that if spasmolytics injected intracisternally are shown to be safe, they may prove to be clinically useful to improve regional cerebral blood flow after subarachnoid hemorrhage.

Since the effects of high CSF concentrations of spasmolytic agents on brain function and structure are not known, we studied the physiological and morphological responses of the rhesus monkey brain and meninges to intracisternal injections of two potent alpha-adrenergic blocking agents: phenoxybenzamine hydrochloride (Dibenzyline)* and phentolamine mesylate (Regitine).† Both have been shown by others to be especially effective experimentally as spasmalytics when applied topically to the CSF-bathed surfaces of cerebral arteries in spasm.3,5,6,8,9,13

* Dibenzyline manufactured by Smith, Kline & French Laboratories, 1500 Spring Garden Street, Philadelphia, Pennsylvania 19101.
† Regitine manufactured by Ciba Pharmaceutical Company, Summit, New Jersey 07901.
Materials and Methods

Rhesus monkeys unselected as to age and sex, weighing 3 to 5 kg, were used for one of two experiments. To ensure repeated reliable access to the subarachnoid space, and to permit us to verify later that the drugs had been injected into the subarachnoid space, we prepared each monkey as follows: 5 to 7 days before each experiment, using sterile technique, we surgically implanted into the cisterna magna one end of a Silastic tube 1 mm in diameter and about 10 cm long. The other end was positioned under the scalp either to end blindly or to communicate with a self-sealing Silastic reservoir. The cisternostomy was usually watertight to pressures up to 40 mm Hg. In some monkeys, at the same time, we also placed EEG electrodes on the frontal and posterior parietal surfaces of the dura mater.

Experiment 1

Monkeys with cisternostomies connected to subgaleal Silastic reservoirs were placed unpremedicated and awake in a restraining box. Through the surgically prepared scalp we needle-d the reservoir and withdrew when possible 2 ml of CSF for culture and analysis. Then 2 ml of a solution of either drug were injected in less than 5 seconds into the cisterna magna via the reservoir. This was done once a day for 4 days. The daily dose of phenoxybenzamine ranged from 0.1 to 8 mg/kg, and of phentolamine ranged from 1 to 4 mg/kg (Table 1). Within minutes of each injection the monkeys were returned to their cages where we observed and examined them for abnormal behavior and motor activity. Periodically thereafter, we took them from their cages, examined them neurologically, and then tapped the reservoir to obtain CSF samples when possible. During one injection of 1 mg/kg of phentolamine we also monitored blood pressure, EEG, heart rate, and respiratory rate. At 7 to 28 days after the last injection, surviving monkeys were sacrificed as described in the necropsy protocol.

Experiment 2

Monkeys with simple cisternostomies and epidural EEG electrodes were anesthetized lightly with intravenous pentobarbital, intubated, paralyzed with tubocurarine chloride, and placed on a mechanical respirator. For one monkey that was awake during the infusion of phentolamine, we used local infiltration anesthesia. Using sterile technique, we then exposed the Silastic cisternostomy tube and connected it to a "Y"

| TABLE 1 |
| Experiment 1: effects of four daily, rapid, intracisternal injections |

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>Daily Dose (mg/kg)</th>
<th>Concentration of Solution (gm/100ml)</th>
<th>Day of Sacrifice</th>
<th>Observed Effects</th>
<th>Pathological Effects*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenoxybenzamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>1.6</td>
<td>lethal</td>
<td>vomiting, ataxia, progressive prostration, death at 14 hrs lethargy, ataxia, weakness</td>
<td>early acute meningitis meningovasculitis none</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>0.25</td>
<td>28</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>8</td>
<td>0.1</td>
<td>0.02</td>
<td>28</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>10</td>
<td>0.25</td>
<td>0.05</td>
<td>4th injection lethal</td>
<td>progressive prostration</td>
<td>acute meningitis</td>
</tr>
</tbody>
</table>

| Phentolamine |                   |                                   |                 |                  |                      |
| 12          | 1                 | 0.19                              | 7               | none             | none                 |
| 14          | 4                 | 0.9                               | lethal          | tremor, progressive prostration, death at 15 min transient lethargy | none |
| 20          | 2                 | 0.35                              | 10              | none             | none                 |
| 21          | 1                 | 0.18                              | 28              | none             | none                 |

* Multiple cultures sterile.
Intracisternal phenoxybenzamine and phentolamine

Preparation of Drugs

All solutions were prepared immediately before use, with saline solution (0.9% NaCl for intravenous use) as the diluent. During the 4-hour infusions, solutions were freshly prepared hourly. Phenoxybenzamine solution was prepared from a stock solution* that contained 50 mg/ml of phenoxybenzamine HCl dissolved in an acidified mixture of approximately equal parts of propylene glycol and ethanol. Phentolamine mesylate was prepared from the commercially available lyophilized powder that contains an equal weight of lactose. In all experiments, we regulated the dose by varying the concentration of the drug in saline, the volumes infused or injected remaining constant. For Experiment 1, the volume of each intracisternal injection was 2 ml. For Experiment 2, the solutions of the drug were infused at a constant rate of 3.3 ml/hr.

Necropsy Protocol

At the end of the observation period, the monkeys were anesthetized with intravenous pentobarbital, and the cisternostomy tubes were exposed. The CSF was withdrawn for culture and analysis. Then about 0.1 ml of sterilized India ink was injected via the

TABLE 2
Experiment 2: effects of a single 4-hour-long intracisternal infusion

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>Dose (mg/kg)</th>
<th>Concentration of Solution (gm/100 ml)</th>
<th>Day of Sacrifice</th>
<th>Observed Effects</th>
<th>Pathological Effects*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenoxybenzamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>0.06</td>
<td>3</td>
<td>weakness, lethargy</td>
<td>meningovasculitis, mild</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>0.12</td>
<td>7</td>
<td>weakness, lethargy</td>
<td>meningovasculitis, severe</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>0.27</td>
<td>28</td>
<td>ataxia, weakness</td>
<td>meningovasculitis, mild</td>
</tr>
<tr>
<td>9</td>
<td>0.4</td>
<td>0.01</td>
<td>28</td>
<td>transient weakness</td>
<td>meningovasculitis, severe</td>
</tr>
<tr>
<td>11</td>
<td>1.0</td>
<td>0.03</td>
<td>28</td>
<td>transient weakness</td>
<td>meningovasculitis, mild</td>
</tr>
<tr>
<td>Phentolamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>4</td>
<td>0.1</td>
<td>7</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>18</td>
<td>8</td>
<td>0.2</td>
<td>14</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>19</td>
<td>16</td>
<td>0.4</td>
<td>died</td>
<td>flaccid quadriplegia</td>
<td>confluent acute meningitis</td>
</tr>
<tr>
<td>22</td>
<td>4</td>
<td>0.1</td>
<td>28</td>
<td>none</td>
<td>none</td>
</tr>
</tbody>
</table>

* Multiple cultures sterile.

I. Neurosurg. / Volume 39 / November, 1973 631
cisternostomy tube into the cisterna magna. Shortly thereafter the animal was killed with an overdose of pentobarbital. Its brain and spinal cord were removed, cultured, grossly examined, and then fixed in formalin. Multiple sections of brain and cord were taken, stained with hematoxylin and eosin, and examined microscopically.

Results

India ink particles were present in the subarachnoid space of all monkeys, verifying that in each the cisternostomy had been in communication with the subarachnoid space and therefore had deposited drugs into the CSF. The results are reported only from animals that had multiple cultures of CSF and swabs of the brain that were sterile.

Experiment 1

Effects of the four daily rapid intracisternal injections are shown in Table 1.

Phenoxybenzamine. One monkey given 0.1 mg/kg/day remained free of observable abnormality following the injections up to time of sacrifice 28 days later, and histological examination of its brain showed no abnormality. Another showed increasing lethargy and anorexia after each dose of 0.25 mg/kg/day and died after the fourth injection; an acute meningitis was found on microscopic examination of the brain. A third given 1.0 mg/kg/day became increasingly ataxic and weak after each injection and remained so up to time of sacrifice 28 days later. Microscopic examination of its central nervous system showed a chronic basilar meningovasculitis. After being given 8 mg/kg, a fourth monkey began to vomit within a few minutes. Subsequently it became ataxic, progressively more flaccid, then prostrate, dying 14 hours after the injection. An acute meningitis was observed on histological examination of the central nervous system.

Phentolamine. Dosed at levels of 1 mg/kg, two monkeys showed no alteration in behavior and activity. Another given 2 mg/kg became mildly subdued temporarily after two of the injections, returning to normal in an hour. All three survived and acted normally until they were sacrificed 7 to 28 days later. Microscopic examination

![Figure 1](image-url)  
**Fig. 1.** Monkey 21. Responses to a single rapid intracisternal injection of 1 mg/kg of phentolamine. The blood pressure begins to fall soon after the injection and returns to preinjection level in 1 hour. The EEG is unchanged.
Intracisternal phenoxybenzamine and phentolamine

of their central nervous systems disclosed no abnormality. In one monkey given 1 mg/kg, vital functions were also monitored during one injection: the blood pressure began to fall within 4 minutes, reached its nadir in 30 minutes, and returned to preinjection levels at 1 hour; the heart rate transiently increased and the respiratory rate fell while the EEG remained unchanged (Fig. 1). A fourth monkey given 4 mg/kg became tremulous within 5 minutes, then progressively limp, prostrate, unresponsive, apneic, and died less than 15 minutes after the injection. Its brain appeared normal to gross and microscopic examination.

Experiment 2

The effects of a single 4-hour intracisternal infusion are shown in Table 2.

Phenoxybenzamine. All five animals survived the infusion until they were sacrificed 3 to 28 days later. After the infusion all five showed a dose-related ataxia, weakness, and lethargy, which usually improved markedly after a week or two; all five also had a dose-related basilar meningovasculitis (Fig. 2). When compared with the control (saline) infusion, the drug infusion had no consistent effect on blood pressure, central venous pressure, CSF pressure, heart rate, or EEG.

Phentolamine. The three monkeys infused with either 4 or 8 mg/kg survived the infusion and remained normal until they were sacrificed 7 to 28 days later. The infusion usually caused a dose-related fall in blood pressure. The central venous pressure, CSF pressure, electrocardiogram, and EEG remained essentially unchanged from the control period regardless of whether the monkey was anesthetized or awake (Fig. 3). Microscopic examination of their central nervous systems disclosed no abnormality.

Fig. 2. Photomicrographs of sections through the medulla and adjacent leptomeninges of monkeys given phenoxybenzamine intracisternally. Left: Monkey 9, given 0.4 mg/kg over 4 hours, sacrificed after 28 days. Hypercellular thickened arachnoid encases the vertebral artery at the lower right. H & E, X 100. Right: Monkey 5, given 4 mg/kg over 4 hours, sacrificed after 7 days. Acute inflammatory cells predominate in a vasculitis that involves all three layers of the vessel wall. H & E, X 100.
A fourth monkey infused with 16 mg/kg remained limp and immobile after the infusion, dying 30 hours later of pneumonia. Microscopic examination of its central nervous system revealed a confluent acute sterile meningitis.

**CSF Findings**

Intracisternal phenoxybenzamine causes a dose-related moderate pleocytosis and a rise in protein that returned to normal in about 2 weeks. Phentolamine caused a similar but milder response (Fig. 4).

**Discussion**

The apparent lack of toxicity of the smaller doses of these drugs in monkeys suggests that intracisternal spasmolytic therapy may prove safe and useful in reversing the vasospasm that often accompanies subarachnoid hemorrhage. By injecting the drug intracisternally, one would in essence make the CSF a relatively concentrated solution of the vasodilator, an effect impossible to achieve by systemic routes of administration because of the blood-brain barrier. In such high concentrations the vasodilator theoretically could compete more effectively with the spasmogen, presumed to be present in the bloody CSF, for the receptor sites in vessel walls.

Although encouraging, the results of these experiments must be regarded as preliminary. It would be premature to assume that either drug is safe for intracisternal use in patients with subarachnoid hemorrhage. The dose-dependent chemical meningovasculitis consistently evoked in monkeys by all but the smallest intracisternal dose of phenoxybenzamine tested tends to discourage its clinical use. Whether the meningitis is caused by the well-known irritating qualities of the drug itself, by its solvent, or by both, is unknown. These considerations are relevant to reports of phenoxybenzamine being used intracisternally experimentally and being advocated for topical use clinically at craniotomy. On the other hand, intracisternal phentolamine was consistently nontoxic to monkeys in doses as high as 2 mg/kg.

The results of these initial efforts have led us to study further the physiological effects...
Intracisternal phenoxybenzamine and phentolamine

CELLS PER PROTEIN

CISTERNAL CSF

PHENTOLAMINE

Figu. 4. Monkey 22. Study of CSF samples shows that the protein and cell count are mildly abnormal for a short period after drug infusion, returning to normal by the 28th day after infusion.

of intracisternally administered phentolamine. Investigations are presently underway in the monkey to determine what effect well-tolerated intracisternal doses of phentolamine has on regional cerebral blood flow after an induced subarachnoid hemorrhage.

References


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