Central cholinergic control of cerebral blood flow in the baboon

Effect of cholinesterase inhibition with neostigmine on autoregulation and CO₂ responsiveness

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Cerebral autoregulation and vasomotor responsiveness to carbon dioxide (CO₂) were measured quantitatively by the use of the autoregulation index and chemical index, respectively, in normal baboons before and after intravertebral and intracarotid infusion of the anticholinesterase agent, neostigmine methylsulfate (Prostigmin). Continuous measurements were made of cerebral blood flow (measured as bilateral internal jugular venous outflow), arterial and cerebral venous pO₂ and pCO₂, cerebral arteriovenous oxygen differences, and endotracheal CO₂.

The effect of intravertebral infusion of neostigmine (12.5 µg/kg body weight) was compared to intracarotid infusion of neostigmine (25 µg/kg body weight) for assessment of any specific action of the drug on a hypothetical cholinergic vasomotor center, presumed to be located in the territory of the vertebrobasilar supply. No significant or persistent changes in cerebral blood flow (CBF) and cerebral metabolic rate for oxygen (CMRO₂) followed either intravertebral or intracarotid infusion of neostigmine. Cerebral vascular resistance (CVR) and cerebral perfusion pressure (CPP), however, decreased significantly after intravertebral infusion. Cerebral autoregulatory vasoconstriction during increases of CPP was significantly reduced following both intravertebral and intracarotid infusion. Cerebral autoregulatory vasodilatation was not altered as CPP was lowered. Cerebral vasodilatory reactivity to CO₂ inhalation was significantly enhanced following intravertebral neostigmine but not following intracarotid neostigmine. Cerebral vasoconstrictive response to hyperventilation was not influenced by neostigmine. These results support the view that central cholinergic cerebrovascular influences exist, and are vasodilatory in nature.

KEY WORDS • central cholinergic control • anticholinesterase agent • neostigmine • cerebral blood flow • cerebral autoregulation • CO₂

The anatomical distribution of the cholinergic nerve supply to the cerebral blood vessels has recently been described in detail. Histochemical and electronmicroscopic studies have revealed cholinergic as well as adrenergic innervation of the cerebral vessels. It has also been shown that density of innervation varies from proximal to distal parts of the vascular tree, with the proximal vessels receiving greater innervation.

So far, physiological, pharmacological, and neurochemical studies have provided incomplete evidence for any cholinergic con-
control of cerebral circulation. For example, it is generally accepted that cerebral blood flow (CBF) is influenced by procedures such as electrical stimulation of the parasympathetic nerves (the seventh and tenth cranial nerves), and ablation or stimulation of brain-stem centers, as well as administration of neurotransmitters and their antagonists.

Some possible functional significance of this neurogenic control of the cerebral circulation has been adduced from clinical observations in man. Impairment of cerebral adrenergic and cholinergic reflex activity in the Shy-Drager syndrome is associated in some cases with cerebral dysautoregulation, the degree of which correlates with the severity and duration of the neurological symptoms and signs. It was also shown in a series of patients with cerebrovascular disease that impaired neurogenic cerebrovascular control and dysautoregulation followed cerebral ischemia and infarction, particularly in patients with brain-stem lesions.

These findings suggest the possibility that cholinergic as well as the well-known adrenergic autonomic pathways influence CBF from the brain-stem centers. However, a precise functional role of the cholinergic system in the influence of cerebrovascular control has yet to be established. Recent data obtained in this laboratory favor a considerable influence of cholinergic vasomotor mechanisms in cerebral autoregulation, as well as cerebrovascular responsiveness to CO₂.

The present study was designed to elucidate the possible role of central cholinergic mechanisms influencing cerebrovascular autoregulatory and chemical vasomotor responsiveness in the baboon.

Materials and Methods

General Procedures and Parameters Measured

Twelve baboons (Papio anubis) of either sex, weighing 4 to 15 kg, were anesthetized with intravenous sodium pentobarbital, 20 mg/kg body weight. Additional pentobarbital was supplemented intravenously at a rate of approximately 3 mg/kg/hr to maintain constant light anesthesia. A tracheostomy was performed, the animal was paralyzed with gallamine triethiodide (Flaxedil), and ventilated with a Harvard variable speed respirator. End-tidal CO₂ was recorded continuously with a Beckman infrared gas analyzer. Systemic blood pressure was monitored with a Statham transducer by way of a catheter inserted through one femoral artery into the descending aorta. Intracranial venous pressure (ICVP) was recorded by means of a catheter wedged rostrally into the superior sagittal sinus. One femoral vein was catheterized to permit intravenous infusion. A small balloon was inserted through the other femoral artery into the thoracic aorta in order to change blood pressure by inflating or deflating the balloon.

Cerebral arterial inflow and venous outflow were isolated at the cervical level by ligating all branches of the external carotid arteries and the external jugular veins. Special care was taken to preserve the carotid arteries, the internal jugular veins, the cervical sympathetic chains, and vagal nerves on both sides. The right lingual artery or vertebral artery was catheterized as needed to make intraarterial infusions.

Electromagnetic flowmeter probes were placed around both internal jugular veins and CBF was measured as cerebral venous outflow. Cerebral arteriovenous oxygen content difference (A-VO₂) was measured continuously with a Guyton analyzer. An extracorporeal circuit and microcuvette system was connected to the animal for continuous measurement of blood gases without any blood loss. Draining catheters were inserted into the brachial artery and into the torcular Herophili by way of the posterior portion of the superior sagittal sinus in order to pump out systemic arterial and cerebral venous blood separately to the respective cuvettes, from which blood was returned to the animal by catheterization into the femoral vein.

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*Respirator manufactured by Harvard Apparatus, Inc., 150 Dover Road, Millis, Massachusetts 02054.
†Infrared gas analyzer manufactured by Beckman Instruments, Inc., 2500 Harbor Boulevard, Fullerton, California 92634.
‡Transducer manufactured by Statham Laboratories, Inc., Hato Rey, Puerto Rico 00919.
§Guyton analyzer manufactured by Oxford Instrument Company, 234 Meadow Road, Jackson, Mississippi.
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Pumping speed was maintained at a constant rate of 10 to 15 ml/min. Blood gases (pO₂ and pCO₂) were measured continuously by means of the microcuvette electronic technique and recorded simultaneously as described by Gotoh, et al. In addition, arterial and venous blood gases (pO₂ and pCO₂) were also monitored digitally by means of a mass spectrometer. Thermostatic cuvettes for the mass spectrometer were inserted in series of the respective electronic microcuvettes.

The electroencephalogram (EEG) and electrocardiogram (EKG) were also monitored. All parameters of CBF and metabolism were recorded continuously on a Grass Model 7 polygraph.

The mean arterial blood pressure (MABP), cerebral perfusion pressure (CPP), cerebral vascular resistance (CVR), cerebral metabolic rate for oxygen (CMRO₂) and cerebral blood flow (CBF), were defined as follows:

\[
\text{MABP} = \text{Diastolic BP} + \frac{1}{3} (\text{systolic BP} - \text{diastolic BP}) \text{ (mm Hg)}
\]
\[
\text{CPP} = \text{MABP} - \text{intracranial cerebral venous pressure (ICVP)} \text{ (mm Hg)}
\]
\[
\text{CVR} = \frac{\text{CPP}}{\text{CBF}} \text{ (mm Hg/ml/100 gm brain/rain)}
\]
\[
\text{CMRO₂} = \frac{\text{CBF} (\text{A-VO₂ difference/100}}{\text{ml/100 gm brain/min)}}
\]
\[
\text{CBF} = \frac{\text{Jugular outflow/brain weight}}{\text{100 (ml/100 gm brain/min)}}
\]

Cerebral Autoregulation and Vasomotor Responsiveness to CO₂

In order to test cerebral autoregulation, acute hypertension was induced by inflating the intra-aortic balloon. Its deflation produced transient hypotension. Measurements were made for 5 minutes during hypertension and for 5 minutes following deflation of the balloon. Arterial pCO₂ (PaCO₂) was maintained within normal range of 36 mm Hg to 45 mm Hg.

To assess quantitatively the degree of impairment of cerebral autoregulation and cerebral vasomotor responsiveness to CO₂, an autoregulation index (AI)⁴⁰,⁴¹ and a chemical index (CI)³⁸,⁴¹ were calculated respectively. AI is defined as the ratio of change in CBF per unit change in CPP, and expressed as:

\[
\frac{\Delta \text{CBF (ml/100 gm brain/min)}}{\Delta \text{CPP (mm Hg)}}
\]

where \( \Delta \text{CBF} \) equals the change in CBF and \( \Delta \text{CPP} \) equals the induced change in CPP from the steady-state level. Hence, AI corresponds to the slope of the CBF-CPP correlation curve. Theoretically, when autoregulation is complete AI should equal zero. The value of AI would be directly proportional to the degree of dysautoregulation.

The chemical vasomotor responsiveness was tested by subjecting the animal to inhalation of 3% to 5% CO₂ in air or mechanical hyperventilation (room air) by changing the speed of the respirator for 5 minutes. CI is defined as the ratio of change in CBF per unit change in PaCO₂, and calculated as:

\[
\frac{\Delta \text{CBF (ml/100 gm brain/min)}}{\Delta \text{PaCO₂ (mm Hg)}}
\]

where \( \Delta \text{PaCO₂} \) equals the change in PaCO₂ from the steady-state level. As during the test for autoregulation, PaCO₂ gradually changed; the CBF values were corrected (CBFc), depending upon the individual animal's chemical vasomotor responsiveness.

In a series of pilot studies of four baboons where PaCO₂ was adjusted actually in vivo by changing the pCO₂ of the inspired air during elevation of CPP, the mean value of the correction factor was found to be 0.28 × CI (ml/100 gm/min/mm Hg) (at 5 minutes after CO₂ inhalation). The same correction factor was applied to adjust CBF to any change in PaCO₂ (CBFc), and CVR was corrected likewise (CVRc). In the test for CO₂ responsiveness, CBF values were adjusted to any change of CPP assuming the individual CVR to be dependent upon PaCO₂ (CBFc).

Intervals of 15 minutes were interposed between each procedure to reestablish a steady state with normal CPP and PaCO₂.

*Medspec MS-8 spectrometer manufactured by Scientific Research Instruments Corporation, a subsidiary of G.D. Searle and Company, 6707 Whitestone Road, Baltimore, Maryland 21207.
†Polygraph manufactured by Grass Instrument Company, 101 Old Colony Avenue, Quincy, Massachusetts 02169.
### TABLE 1

*Cholinesterase (ChE) activity in blood serum before and after infusion of neostigmine*

<table>
<thead>
<tr>
<th>Time of Measurement</th>
<th>Intravertebral Neostigmine, 12.5 µg/kg (n = 4)</th>
<th>Intracarotid Neostigmine, 25.0 µg/kg (n = 4)</th>
<th>Total (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ChE</td>
<td>Change</td>
<td>ChE</td>
</tr>
<tr>
<td>before neostigmine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>11.00 ± 0.52</td>
<td></td>
<td>13.53 ± 2.15</td>
</tr>
<tr>
<td>CV</td>
<td>10.88 ± 0.37</td>
<td></td>
<td>13.81 ± 2.43</td>
</tr>
<tr>
<td>(A-V) D</td>
<td>0.12 ± 0.42 (NS)</td>
<td></td>
<td>-0.28 ± 0.38 (NS)</td>
</tr>
<tr>
<td>5 min after neostigmine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>6.41 ± 1.00</td>
<td>-4.59 ± 0.73†</td>
<td>1.69 ± 0.23§</td>
</tr>
<tr>
<td>CV</td>
<td>6.08 ± 1.19</td>
<td>-4.80 ± 1.09‡</td>
<td>1.48 ± 0.46</td>
</tr>
<tr>
<td>(A-V) D</td>
<td>0.33 ± 0.65 (NS)</td>
<td></td>
<td>0.01 ± 0.36 (NS)§</td>
</tr>
<tr>
<td>70 min after neostigmine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>7.05 ± 1.40</td>
<td>-3.95 ± 1.37†</td>
<td>7.63 ± 1.30</td>
</tr>
<tr>
<td>CV</td>
<td>7.05 ± 1.41</td>
<td>-3.83 ± 1.12‡</td>
<td>7.66 ± 1.26</td>
</tr>
<tr>
<td>(A-V) D</td>
<td>0.005 ± 0.12 (NS)</td>
<td></td>
<td>-0.03 ± 0.12 (NS)</td>
</tr>
</tbody>
</table>

* ChE values are expressed in International Units (IU/ml). Values given are the mean, ± standard error of the mean. NS = statistically nonsignificant. Change = Change in ChE compared to the corresponding control value before neostigmine. A = arterial ChE; CV = cerebral venous ChE; (A-V) D = arteriovenous difference of ChE.

† p < 0.05.
‡ p < 0.01.
§ Mean of only three animals.
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**Pharmacological Studies of Neostigmine**

The neostigmine methylsulfate (Prostigmin), an anticholinesterase agent, was diluted in saline solution and infused into the selected artery at a constant rate of 1.03 ml/min by means of a Harvard syringe infusion pump. Due to differences in blood flow between vertebral and carotid arteries, neostigmine was diluted in such concentrations as 12.5 μg/ml for intravertebral infusion and 25 μg/ml for intracarotid infusion. The drug was infused intravertebrally in a dose of 12.5 μg/kg of body weight, or 25 μg/kg of body weight was infused into the carotid artery. The measurements of cerebral autoregulatory responsiveness and vasomotor reactivity to CO₂ as well as cerebral metabolism were carried out after repeated steady states in a serial manner before and after neostigmine administration. In order to compare the effect of intravertebral infusion to that of intracarotid infusion, the animals were divided into two groups comprising six animals for each arterial route of administration of neostigmine.

At the end of the experiment, the brain was infused with trypan blue solution in the same manner as the drug infusion, and removed. The territory infused by the artery was then identified and the total weight of the brain was measured.

In eight animals (four intravertebral and four intracarotid) blood levels of cholinesterase (ChE) activity were measured before and after neostigmine administration in order to assess the anticholinesterase effect. ChE estimation was made by means of the Dupont automatic clinical analyzer.*

Statistical significance was estimated by means of student's t-test or the paired t-test at a level of confidence above 95%.

**Results**

**Arterial and Cerebral Venous ChE Activity Before and After Neostigmine**

Changes in ChE activity in blood serum before and at 5 and 70 minutes after intravertebral or intracarotid infusion of neostigmine in four animals for each group are shown in Table 1. Both intracarotid infusions of neostigmine depressed the ChE level promptly and significantly. The fall was dose-dependent at 5 minutes after neostigmine, followed by a slow recovery. No significant arteriovenous difference was observed in either group before or after neostigmine infusion.

**Effects of Neostigmine on Cerebral Hemodynamics and CMRO₂ in Steady States**

Cerebral hemodynamics and CMRO₂ were studied in two groups of six animals each before and after intravertebral and intracarotid neostigmine. Steady states were obtained by regulating PaCO₂ constant around 40 mm Hg with controlled respiration. No significant changes in any parameter were observed in steady states during the control period for 70 minutes before neostigmine. Changes during the experimental period following neostigmine were compared to corresponding control values in steady state at 5 minutes before neostigmine, as shown in Table 2. The significant decreases in MABP, CPP, and CVR were associated with a significant decrease of CBF at 5 minutes following intravertebral injection of neostigmine (12.5 μg/kg) despite relatively constant ICVP, CMRO₂, and PvO₂ values. Thereafter, CBF recovered gradually but remained below control levels. The significant decrease in CPP persisted and a progressive reduction in CVR was observed at a time interval of 70 minutes after intravertebral infusion of neostigmine. On the contrary, no significant change in any of these parameters was observed following intracarotid neostigmine (25 μg/kg).

These changes were directly related neither to dose of neostigmine nor systemic reduction in ChE activity following neostigmine; they were related only to the intravertebral route of administration of neostigmine (Tables 1 and 2).

Visual analysis of the EEG did not reveal obvious changes after neostigmine infusion, but in some experiments during injection a reduction in amplitude and appearance of faster components were observed. This EEG change was consistently seen after intravertebral infusion.

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*Dupont Automatic Clinical Analyzer made by Dupont Corporation, Wilmington, Delaware.


**TABLE 2**

Effect of neostigmine on cerebral hemodynamics and CMRO₂

<table>
<thead>
<tr>
<th>Measurements*</th>
<th>Before Neostigmine (Mean ± SD)</th>
<th>5 min after Neostigmine (Mean ± SD)</th>
<th>70 min after Neostigmine (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBF VT</td>
<td>38.7 ± 5.8</td>
<td>30.9 ± 9.8†</td>
<td>38.0 ± 7.8</td>
</tr>
<tr>
<td>C</td>
<td>34.3 ± 4.9</td>
<td>34.0 ± 4.7</td>
<td>37.1 ± 7.3</td>
</tr>
<tr>
<td>MABP VT</td>
<td>88.7 ± 10.5</td>
<td>67.5 ± 20.8‡</td>
<td>73.0 ± 6.4</td>
</tr>
<tr>
<td>C</td>
<td>79.2 ± 6.6</td>
<td>76.7 ± 12.5</td>
<td>80.0 ± 16.7</td>
</tr>
<tr>
<td>ICVP VT</td>
<td>15.2 ± 4.2</td>
<td>13.5 ± 4.7</td>
<td>15.3 ± 4.7</td>
</tr>
<tr>
<td>C</td>
<td>15.8 ± 2.2</td>
<td>16.0 ± 3.4</td>
<td>17.2 ± 3.6</td>
</tr>
<tr>
<td>CPP VT</td>
<td>73.5 ± 10.0</td>
<td>54.0 ± 17.6‡</td>
<td>57.7 ± 7.6‡</td>
</tr>
<tr>
<td>C</td>
<td>63.3 ± 6.4</td>
<td>60.7 ± 10.7</td>
<td>63.0 ± 15.4</td>
</tr>
<tr>
<td>CVR VT</td>
<td>1.91 ± 0.10</td>
<td>1.74 ± 0.10†</td>
<td>1.55 ± 0.27‡</td>
</tr>
<tr>
<td>C</td>
<td>1.87 ± 0.22</td>
<td>1.78 ± 0.17</td>
<td>1.69 ± 0.12</td>
</tr>
<tr>
<td>PvO₂ VT</td>
<td>34.0 ± 2.7</td>
<td>30.3 ± 6.4</td>
<td>34.3 ± 2.7</td>
</tr>
<tr>
<td>C</td>
<td>33.7 ± 3.2</td>
<td>33.0 ± 2.9</td>
<td>34.8 ± 3.4</td>
</tr>
<tr>
<td>CMRO₂ VT</td>
<td>2.68 ± 0.27</td>
<td>2.42 ± 0.34</td>
<td>2.50 ± 0.41</td>
</tr>
<tr>
<td>C</td>
<td>2.49 ± 0.29</td>
<td>2.39 ± 0.32</td>
<td>2.43 ± 0.44</td>
</tr>
<tr>
<td>PaCO₂ VT</td>
<td>41.0 ± 1.0</td>
<td>39.5 ± 2.4</td>
<td>42.5 ± 2.4</td>
</tr>
<tr>
<td>C</td>
<td>40.3 ± 2.0</td>
<td>40.5 ± 1.5</td>
<td>39.2 ± 2.2</td>
</tr>
</tbody>
</table>

* VT = intravertebral infusion; C = intracarotid infusion; CBF = cerebral blood flow; MABP = mean arterial blood pressure; ICVP = intracranial cerebral venous pressure; CPP = cerebral perfusion pressure; CVR = cerebral vascular resistance; PvO₂ = oxygen tension of cerebral venous blood (mm Hg); CMRO₂ = cerebral metabolic rate for oxygen; PaCO₂ = carbon dioxide tension of arterial blood (mm Hg). Statistical significance is compared to control value before neostigmine. SD = standard deviation.

† p < 0.05.
‡ p < 0.01.

**Effects of Neostigmine on Cerebral Autoregulation During Increased CPP**

Cerebral autoregulation was studied in six animals with intravertebral neostigmine and in five animals with intracarotid neostigmine by increasing CPP before and after injection of the drug. When CPP was increased in a single step, CBFₐ showed an immediate increase during the first minute, followed by a gradual but progressive decrease which eventually reached the steady-state level despite continued elevation of CPP during the period for 5 minutes as shown in Fig. 1. This change in CBFₐ was often associated with a gradual minor reduction in PaCO₂.

Autoregulatory decreases in CBFₐ and increases in CVRₐ during the 5-minute period of induced hypertension in both control groups of experiments were no longer noted after either intracarotid or intravertebral neostigmine. CBFₐ remained at significantly elevated levels (p < 0.001) and CVRₐ did not increase significantly when compared with control values (Fig. 2).

Correlations between CPP and CBFₐ in the steady state before elevation of CPP and in the terminal state, 5 minutes after CPP had been increased, were shown to be highly significant (p < 0.001) and linear following both intravertebral and intracarotid infusion of neostigmine, indicating that infusion of the drug caused impairment of autoregulation (dysautoregulation). Such correlation in both control groups were found to be nonsignificant (p < 0.02) (Fig. 3).

The degree of dysautoregulation was also analyzed by means of the autoregulation index (AI = Δ CBFₐ/ΔCPP). Changes in AI during induced hypertension before and after neostigmine are shown in Fig. 4. Following elevation of CPP in the control experiments, AI increased stepwise immediately and reached the peak value and then decreased progressively toward zero levels. Following arterial infusion of neostigmine by either the vertebral or carotid route, marked increases in AI were observed at all time intervals. Changes were significant in the initial and terminal values (p < 0.05).

As judged from these changes in AI, intravertebral neostigmine resulted in a higher degree of dysautoregulation compared with intracarotid neostigmine due to inhibition of...
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Effects of Neostigmine on Cerebral Autoregulation During Decreased CPP

The autoregulatory vasodilator response to reduction in CPP was tested in six animals with intravertebral neostigmine and in five animals with intracarotid neostigmine. In control series when CPP was decreased the prompt decrease in CBFc was noted to occur followed by progressive gradual recovery but remaining at significantly reduced levels due to constant reduction in CPP. Changes in CVRc followed changes of CBFc in the reverse direction. When compared with controls, although both intracarotid and intravertebral neostigmine caused significant reduction in CVRc, no significant change in CBFc was seen in either group during induced hypotension (Fig. 2).

Autoregulatory vasodilatation was also assessed by means of AI (Fig. 5). The initial
increase followed by progressive recovery toward zero level during induced hypotension seen in controls was also observed after neostigmine. The effects of neostigmine on AI did not reach the level of significance compared with controls except in the initial response after intravertebral neostigmine.

**Correlations Between Hypertensive and Hypotensive Dysautoregulation Before and After Neostigmine**

Significant linear correlations were noted between AI during induced hypertension (hypertensive autoregulation) and AI during induced hypotension (hypotensive autoregulation) in control animals in both groups (Fig. 6). The results indicate that intracarotid neostigmine affected these correlations differently, causing more predominant effects on hypertensive dysautoregulation than hypotensive dysautoregulation; on the other hand, intravertebral neostigmine inhibited both vasoconstrictive and vasodilatory autoregulations, but caused significant dysautoregulation only in induced hypertension.

**Correlation of Autoregulation Index During Induced Hypertension with Cerebral Venous ChE Activity**

The effect of reduction in ChE on cerebral autoregulation is shown in Fig. 7. When the degree of dysautoregulation in induced hypertension was correlated with the percent level in cerebral venous ChE in four animals for each group, as compared with arterial
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ChE in controls, a significant negative correlation was obtained in both groups. In other words, the lower the cerebral venous ChE activity was, the higher was the degree of dysautoregulation. It is interesting to note that a lower dose of neostigmine by the intravertebral route produced greater increases in AI per unit percent change in ChE (greater degree of dysautoregulation) than a higher dose of neostigmine by the carotid artery.

**Effect of Neostigmine on Cerebral Vasomotor Responsiveness to CO₂**

Cerebral vasomotor responsiveness to CO₂ was studied in five animals with intravertebral neostigmine and in six animals with intracarotid neostigmine (one case was omitted because of technical problems during the hyperventilation study) before and after infusion of neostigmine.

Cerebral hemodynamics during induced hypercarbia and hypocarbia are shown in Table 3. Alterations in PaCO₂ were generally accompanied by significant changes in CBF, CVR, and ICVP; CPP was altered to a minor degree. The values of CI were always greater during CO₂ inhalation than during hyperventilation. During induced hypercarbia, CI increased significantly following intravertebral neostigmine, but did not show significant increase after intracarotid neostigmine. During induced hypocarbia, no significant change in CI was observed following both intravertebral and intracarotid neostigmine (Fig. 8).

As judged from these changes in CI, intravertebral neostigmine enhanced the cerebral vasomotor responsiveness to increase in PaCO₂, while intracarotid neostigmine did not significantly affect it. Chemical vasomotor reactivity to hypocarbia was not influenced by neostigmine.

**Discussion**

**Validity of the Experimental Model**

The technique of measuring cerebral blood flow as internal jugular venous outflow with the use of the electromagnetic flowmeter has been described and validated previously.697 It is, however, possible that in certain extreme conditions part of the cerebral venous outflow may be drained by channels other than the two internal jugular veins.6 The extracorporeal microcuvette system, as described by Gotoh, et al., was modified for the monkey, with a reduction in capacity of the circuit and number of electrodes used. Since sodium pentobarbital in large doses is known to affect CBF and CMRO₂, a light and steady level of anesthesia was maintained with only small supplemental doses by monitoring EEG and systemic blood pressure.

The use of an intraaortic balloon for changing CPP is a relatively atraumatic and reproducible technique, although sometimes transient cardiac dysrhythmia was observed. If abnormal changes in heart rate were prolonged, the record was discarded.
The anatomy of the cerebral and cervical vessels encountered in the present study was similar to standard descriptions in the literature.6,44 The territory supplied by the neostigmine infusion was similarly identified by injecting trypan blue immediately before killing the animal. The pattern of distribution of dye clearly differentiated internal carotid artery territory from that of the vertebrobasilar artery. The subcortical border zone between the carotid and the vertebrobasilar territories was noted to be located in the thalamus, lateral geniculate nucleus, and nucleus pulvinaris ipsilateral to the injection. A wedge-shaped parasagittal area of the contralateral hemisphere in the territory of the anterior cerebral artery was also stained by the intracarotid dye. The ipsilateral hypothalamus and amygdala were found to be in the carotid territory. These findings are in good agreement with previous reports.6,44

The technique also showed differential degrees of staining due to direct regional perfusion and perfusion resulting from recirculation. An important observation in the present study was that within the medulla the ipsilateral half was always stained more than the contralateral side by the intravertebral dye. The remaining part of the brain stem up to the midbrain was stained almost equally on both sides. Thus, it may be assumed that intracarotid or intravertebral neostigmine is initially distributed in high concentration to the primary perfusing area, where it passes through the blood-brain barrier (BBB), and, markedly diluted, to the systemic circulation, and from there to other parts of the brain during recirculation. When neostigmine recirculates after systemic dilution, its concentration is lowered enough to have little or no effect on other parts of the brain.

Neostigmine ((3-hydroxyphenyl) trimethylammonium dimethylcarbamate), or prostigmine is a quaternary ammonium derivative, a compound of greater stability and of equal or greater potency as compared to physostigmine (eserine) which exists naturally as a tertiary ammonium alkaloid. One of the essential pharmacological actions of neostigmine is its potency to inhibit competitively the action of cholinesterase (ChE) enzymes which hydrolyze not only acetylcholine (Ach), the most important cholinergic neurotransmitter, but other biological choline esters as well.16,23

It has been shown that compounds consisting of the quaternary ammonium groups penetrate cell membranes and biological barriers less rapidly than do the tertiary ammonium compounds.15,23,24 Regarding the functional interplay of acetylcholinesterase (AChE) and Ach, a third barrier system
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within the nerve cell, namely the membrane of the endoplasmic reticulum, has been proposed which may protect the so-called "reserve AChE" from the highly diffusible compound anti-ChE.23 Hence, in the present experimental design, a quaternary ammonium compound, neostigmine, was selected for the purposes of 1) "differential administration" to brain stem versus hemisphere by way of intraarterial infusion into the vertebral and carotid arteries; and 2) "selective inhibition" of so-called intrinsic "functional AChE," and avoiding physiological alterations of the presynaptic neuron as much as possible.

Effects of Neostigmine on Cerebral Hemodynamics and CMRO₂

As judged from changes of the blood ChE activity, the systemic anti-ChE effects of intraarterial neostigmine appeared promptly and persisted until the end of the experiments. This would indicate induced activation of the cholinergic system by effective inhibition of ChE.

The significant decrease in MABP, CPP, and CVR following intravertebral neostigmine (12.5 μg/kg) but not intracarotid neostigmine (25 μg/kg) seems to indicate alteration of the cardiovascular regulatory function of the brain stem, including vasodepressor centers and probably the reticular formation itself. No obvious changes were observed due to the anticholinesterase effects of neostigmine on the autonomic centers such as hypothalamus, amygdala, and epithalamus, located within the carotid artery territory.4,19,28,30

Apart from the general distribution of enzymes, including cholinesterase, in the central nervous system (CNS),32 it has been shown that high AChE activity is present in specific structures of the brain, especially the nuclei of the brain stem and the cholinergic vasomotor nerve terminals of the cerebral vessels.23,28,31,45 It is suggested from present results of intravertebral injection of neostigmine that the site of activation of the cholinergic system influencing CBF is located in the brain stem.

It has been reported that intracarotid injection of eserine (a more diffusible anti-ChE) produced an increase in the frequency of firing of single reticular units in the mesencephalic tegmentum of the cat,10,23 and that the threshold for electrical stimulation of the reticular formation for EEG arousal was lowered by eserine.5,23 There is general agreement that ChE inhibitors elicit EEG patterns of arousal.10,23,62 It is assumed that this is caused by activation of the cholinergic mechanism at some level of the reticulocor-
tical pathways, which include both the "muscarinic" and atropine-resistant or "nicotinic" synapses. However, it is also suggested that EEG activation may be due, at least in part, to anti-ChE effects of the drug at the cortical level. In the present study, the EEG showed lowering of amplitude and an increase of faster frequencies (partial desynchronization) especially after intravertebral infusion of neostigmine.

Central Cholinergic Influence on Cerebral Autoregulation and Chemical Vasomotor Reactivity

In the present study, analysis of the AI yielded quantitative assessment of the degree of dysautoregulation, and the CI made it possible to analyze quantitatively the cerebralvascular response to CO₂. There is some evidence to suggest that the neurogenic influences may not only elicit obvious cerebral vasoconstriction or vasodilatation but also modulate cerebral vascular response to changes in cerebral perfusion pressure (AI) and carbon dioxide (CI).

The term "cerebral autoregulation" has been defined as the inherent property of the brain to maintain a constant blood flow despite changes in perfusion pressure. The role of neurogenic mechanisms in cerebral autoregulation is still debated. The rapidity of the initial phase of autoregulatory responses observed in the present study is in favor of a neurogenic or myogenic component of the underlying mechanism (Figs. 4 and 5). This latent interval is compatible with the observations of other authors. Metabolic or chemical mechanisms have been suggested as participants in the autoregulatory response of CBF in both late and slow phases, which begin and progress to complete autoregulation over the next 4 minutes.

The present observations showed differences in the regional effects of intravertebral versus intracarotid injection of neostigmine on AI and CI. Both intravertebral and intracarotid neostigmine produced significant cerebral dysautoregulation during induced hypertension (p < 0.01). In contrast, cerebral vasodilatory reactivity to hypercarbia was significantly enhanced only by intravertebral neostigmine (p < 0.001), but not by intracarotid neostigmine. Neither hypotensive autoregulation nor vasoconstrictor reactivity to hypocarbia was influenced significantly by neostigmine. Scremin, et al., have shown evidence that cholinergic excitation at the cerebral cortex participates in the increase in cortical blood flow of the rat. Mcderlishvili, et al., were the first to report that impairment of autoregulatory vasodilatation of pial arteries in the rabbit ensued from intravenous or topically applied postganglionic cholinergic inhibitors, suggesting the possible role of cholinergic vasodilator mechanisms in cerebral autoregulation.
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It was recently reported that intraperitoneal atropine in the rat was not effective in altering cerebral autoregulation, but that it blocked cerebral vasodilator response to CO₂, which was enhanced by eserine.4 It has also been shown in this laboratory that intravertebral atropine in baboons inhibited cerebral vasodilatory response to hypercarbia and enhanced cerebral vasoconstrictive response to hypocarbia, but neither intravertebral nor intravenous atropine changed cerebral autoregulatory vasodilatation or vasoconstriction.22 The possibility of variable responses of cholinergic cerebrovascular receptors has been shown in particular conditions, such as isolated cerebral artery46 or topical application of specific cholinomimetic drugs.25 These findings suggest different locations for cholinergically mediated cerebral resistance vessels which require normal neuronal integrity for these physiological responses.

Shapiro, et al.,58 have reported that a substantial part of arterial resistance (39%) is located proximal to the larger arteries (above 200 μ in diameter) and a further 10% resistance is produced across the arterial bed to the smallest penetrating arterioles (25 μ in diameter) of the cat pial circulation. Transposition of resistance of the cerebral vascular bed might thus occur in instances of proximal vasodilatation accompanied by marked alteration in peripheral vascular resistance.49 Deshmukh, et al.,9 have also proposed that the sympathetic vasoconstric-

Fig. 7. Graph showing the effect of reduction in cholinesterase (ChE) on cerebral autoregulation. Note difference in slopes of regression lines of intravertebral and intracarotid infusions of neostigmine.

Neostigmine caused an upward shift of the AI response curve, maintaining the kinetic pattern. This could be expressed as "tonic cerebral dysautoregulation" (Fig. 4). It is of interest to note that neostigmine did not extend the latent interval of 20 seconds before initiation of autoregulatory vasoconstriction as judged from the measured responses of AI and CVRc (Figs. 1 and 4).

A reasonable interpretation for these findings is that differential inhibition of intrinsic functional ChE enzymes and consequent activation of the cholinergic system might overcome the other monoaminergic vasoconstrictor tonus at their effector sites in the cerebral vascular resistance bed. Under the present experimental conditions, two combined factors are offered for the preserved kinetic potency of the vasoconstrictor tonus in cerebral autoregulation after neostigmine infusion: 1) preserved kinetic vasomotor responses might be carried out by non-neurogenic components of cerebral vascular bed; 2) inhibition of ChE enzymes in the cholinergic system does not completely overcome the increased vasoconstrictor tonus mediated by the intact sympathetic innervation and provoked by elevation in CPP.

Neostigmine increased AI but did not produce dysautoregulation during induced
hypotension (Figs. 2 and 5). This might be similarly explained by finite capacity of vasodilatory response of cerebral resistance vascular bed which is regulated by both vasodilator tonus and non-neurogenic components. Presumably, the limit of vasodilator capacity is partly reached by the response to hypotension itself and, therefore, neostigmine cannot dilate the vascular bed any further (Fig. 5).

In the present study, a significant negative correlation between the degree of dysautoregulation during induced hypertension and decrease in cerebral venous ChE was observed. The effect of intravertebral neostigmine (12.5 μg/kg) resulted in a higher degree of hypertensive dysautoregulation per unit percent decrease of cerebral venous ChE when compared with the effect of intracarotid neostigmine (25.0 μg/kg).

The difference in the effect of neostigmine on hypertensive dysautoregulation between intravertebral and intracarotid infusions must, therefore, be accounted for by the differences in distribution (brain stem versus hemisphere) rather than different effects on cholinesterase activity.

In conclusion, the present study shows evidence for a role of central cholinergic mechanisms located in the brain stem which appears to be cerebral vasodilator in nature in the baboon. The cholinergic vasodilator mechanism appears to play an important role in tonic modulation of autoregulatory as well as chemical vasodilatory responsiveness of the cerebral resistance vessels. The results suggest a dual autoregulatory and chemical vasomotor control of CBF which is influenced, in part, by the central cholinergic innervation in the brain stem and is therefore sensitive to neostigmine. This mechanism probably counterbalances the well-established monoaminergic vasoconstrictor tonus.

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