
**Phystostigmine Induced Reversal of Ischemia Following Acute Middle Cerebral Artery Occlusion in the Rat**

Oscar U. Scremin, M.D., Ph.D., and A.M. Erika Scremin, M.D.

**SUMMARY** Cerebral cortical ischemia was induced in anesthetized rats by occlusion of the middle cerebral artery (MCA). Cerebral blood flow (CBF) was measured with the H2 clearance technique in the center and periphery of the ischemic territory. A decrease of CBF to about 50% of pre-occlusion values was observed in both areas. Administration of Physostigmine, a cholinesterase inhibitor, at a dose of 0.15 mg/Kg by intravenous route, induced an increase of CBF in the ischemic cortex. This change in CBF reached 120% of pre-occlusion level in the periphery and 80% of pre-occlusion value in the center of the area of distribution of the occluded artery. Although Physostigmine induced an increase in arterial blood pressure, the cerebral hyperemia observed both in normal and ischemic cortex could still be demonstrated after blockade of the pressor effect by bleeding or Phentolamine administration.

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OCCLUSION of a cerebral vessel such as the middle cerebral artery, leaves a residual blood flow in its distribution field due to the existence of interarterial anastomosis. These anastomoses are known to exist in humans and in experimental animals. Simple hemodynamic consideration, decrease in vascular resistance in ischemic areas should lead to enhancement of perfusion from arterial collaterals provided that systemic blood pressure is sustained. Some authors have failed to demonstrate an improvement of blood flow in areas of ischemia with the use of cerebral vasodilators, suggesting that blood vessels cannot dilate in this situation. This is not an unanimously accepted concept however, since both CO2 and papaverine have been shown to enhance blood flow after middle cerebral artery occlusion in animals. In view of this, it was considered of interest to explore the potential usefulness of Physostigmine, a cholinesterase inhibitor known to induce a redistribution of vascular resistance leading to cerebral vasodilatation and moderate hypertension. The increase of cerebral perfusion induced by this drug is not accompanied by metabolic activation and consequently cerebral venous oxygen content rises considerably, a fact that might help preserve the integrity of ischemic tissue. An additional feature of the cerebral vasodilatation induced by Physostigmine is that it is potentiated by hypercapnia and hypoxia. This might result in a greater sensitivity to the agent of vessels in the area of ischemia where such conditions are known to prevail. Occlusion of a cortical branch of the middle cerebral
artery supplying the sensorimotor area was selected as a model of cerebral ischemia in order to avoid damage to subcortical tissue that could complicate the interpretation of results of cholinergic interventions. A major portion of the vascular cholinergic afferents to the cerebral cortex seem to originate or be driven by brain stem, and basal forebrain centers with cortical projections traversing areas than can be rendered ischemic by a proximal occlusion of the middle cerebral artery. Thus, in the case of a massive infarction, the effect of interruption of these afferents could not be distinguished from impairment of cholinergic function at their vascular neuroeffector sites.

The hypertensive effect of Physostigmine, which is prominent in the rat, was blocked in order to simplify interpretation of results.

Methods

Animal Preparation

Sprague-Dawley male rats, 300 to 350 g body weight were anesthetized with a combination of Urethane (0.9 g/Kg i.p.) and Halothane (1.5% in air) during surgical procedures. Halothane was then lowered to 0.7% and maintained at that level during the experimental period. The use of a low dose of urethane provides adequate anesthesia at levels of Halothane below those known to impair CBF regulation. The trachea and a femoral artery and vein were cannulated. The head was rigidly held in a stereotaxic device and a craniotomy was performed over the frontoparietal and temporal cortex on the left side. The dura mater was removed avoiding damage to the underlying cortex. Blood pressure was measured from the femoral arterial catheter with a Statham P23Db pressure transducer. Body temperature was monitored with a rectal thermometer and maintained between 36–37°C with a heating pad (Aquamatic K, Hamilton).

Measurement of Cerebral Blood Flow (CBF)

Local cortical blood flow was measured with the Hydrogen clearance technique. Two platinum electrodes, 25 μm in diameter, insulated with glass except for 200 μm from the tip, were lowered to 500 μm from the piamater with micromanipulators. Two remote, sintered Ag-AgCl wires were used as reference electrodes. Current in these circuits was recorded on a Beckman multichannel recorder and used to estimate tissue H2 concentration. Hydrogen gas was added to the inhaled gas mixture at a concentration of 3% until a steady state level of current was achieved. After interruption of H2 inhalation, current decayed exponentially with time. A semi-log plot of current versus time allowed calculation of flow according to the equation:

\[ \text{Flow (ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}) = \ln(2) \cdot (T/2)^{-1} \cdot 100 \]

where \( T/2 \) = half time of decay slope in minutes.

The electrodes were positioned in what is called here, on anatomical grounds, the "center" (1 mm frontal and 4–4.5 mm lateral to Bregma) and the "periphery" (1 mm frontal and 1.5 mm lateral to Bregma) of the area of ischemia (fig. 1). The peripheral location was selected due to the fact that despite some variations in the branching pattern of the middle cerebral artery, anastomosis between terminal branches of the anterior (ACA) and middle (MCA) cerebral arteries occurred in the dorsal aspect of the brain, within 2 mm from the midline, in all animals examined. This is in agreement with previous findings by Coyle and Jokeiainen. The central location is approximately midway between the peripheral location and the site of occlusion, in an area where anastomosis between terminal branches of MCA and ACA were never found. The exposed cortex was covered with cotton pledges embedded in mineral oil.

Mechanical Ventilation

Before commencement of CBF determinations, the animals were paralyzed with Pancuronium (0.4 mg · kg\(^{-1}\) · hr\(^{-1}\)) and mechanically ventilated with air in a T system. Anaerobic samples of arterial blood were obtained and \( \text{PO}_2 \), \( \text{PCO}_2 \), and pH measured on a Radiometer BMS 3 Mk2 Blood Microsystem. Ventilation was adjusted to obtain a \( \text{PaCO}_2 \) reading between 36 and 40 mm Hg. Oxygen was added to the inspired air in order to raise \( \text{PaO}_2 \) slightly above 100 mm Hg.

Arterial Occlusion

The middle cerebral artery was exposed on its course over the temporal cortex. At the site selected for occlusion, the artery laid 3 to 4 mm below the temporal ridge and 0.5 to 1.5 mm in front of the plane of the
coronal suture (fig. 1). Occlusion was effected by touching the vessel with a needle shaped crystal of Silver Nitrate. This produced immediate coagulation of the vessel with arrest of blood flow evidenced by the presence of stable gas bubbles at both ends of the coagulated point. While performing this maneuver, the field was kept dry, taking care to minimize contact of the crystal with the adjacent cortex. Completeness of occlusion was assessed in every experiment by postmortem intravascular perfusion with colored Batson #17 corrosion compound (Polysciences, Inc., Warren, PA).

Experimental Design

After insertion of cortical electrodes and exposure of the artery to be occluded, Halothane concentration was lowered to 0.7% and 45 minutes to one hour were allowed for stabilization of local conditions and anesthetic level. After this period of time, initial determinations of CBF were performed in a number of two to four in order to establish a baseline value. Following this period, either Physostigmine was injected with or without blockade of the rise of blood pressure or the middle cerebral artery was occluded. Measurements of CBF were taken after the experimental intervention over a period of one hour in the case of Physostigmine injection or two hours for MCA occlusion.

Measurements of CBF following MCA occlusion (two to three per hour) were averaged to give first and second hour post-occlusion mean values. Since no tendency to change in CBF was observed during the second and third hour in initial experiments, Physostigmine was injected at the end of the second hour. In experiments involving cerebral ischemia, the complete sequence of baseline measurements, MCA occlusion, post-occlusion measurements, Physostigmine injection and post-Physostigmine measurements was followed in every animal.

Data Analysis

Comparison between means of CBF and BP values for the various conditions were performed by analysis of variance and the Bonferroni procedure. In the following description, group means ± standard errors are given.

Results

Effects of Physostigmine on Normal Cerebral Cortex

Blood flow in the frontoparietal cortex averaged 103 ml · 100 g⁻¹ · min⁻¹ ± (SE) 4.9 for a mean blood pressure of 89 ± 2.1 mm Hg, prior to experimental interventions. These are averages for the 46 animals in the various groups reported in this study.

After administration of a 0.15 mg/kg intravenous pulse of Physostigmine, CBF rose from 103 ± 10.1 ml · 100 g⁻¹ · min⁻¹ to 225 ± 18.7 ml · 100 g⁻¹ · min⁻¹ and BP, measured at the moment of CBF determination (six minutes after Physostigmine injection), increased to 118 ± 4.1 mm Hg from a pretreatment level of 91 ± 4.1 mm Hg, n (number of animals) = 12 (fig. 2). Both changes were statistically significant (Student’s “t” test p < 0.001). The CBF increase was proportionately greater than the BP change, leading to a 41% decrease in cerebrovascular resistance (CVR). This was calculated as the ratio of the difference between CVR after and before Physostigmine administration over CVR before the drug was given.

Two sets of experiments were performed in order to assess the cerebrovascular effects of Physostigmine in the absence of changes in BP. In the first, the BP surge associated with Physostigmine was blocked by an i.v. pulse of 0.1 mg/kg of Phentolamine mesylate given within one minute following Physostigmine administration (0.15 mg/kg i.v.). Despite blockade of the BP increase, CBF rose to 203 ± 20.3 ml · 100 g⁻¹ · min⁻¹.

Figure 2. Effects of Physostigmine (0.15 mg/kg, i.v. pulse) on CBF of normal cerebral cortex and BP. Both variables were recorded six minutes after drug injection. In three different groups of animals, BP was allowed to change spontaneously (left panel) or it was controlled by a 0.1 mg/kg i.v. pulse of Phentolamine (middle panel) or bleeding from an arterial line (right panel). The increase in CBF was statistically significant in all three groups. (p < 0.001). BP increased significantly (p < 0.001) only in the group in which this variable was not controlled. Number of animals in each group were 12 (no BP control), 10 (Phentolamine) and 22 (bleeding).
from a pretreatment level of 99 ± 9.4 ml · 100 g⁻¹ · min⁻¹ (n = 10, p < 0.001) (fig. 2). The decrease in CVR in this group amounted to 51%. In a separate group of animals, the blood pressure increase induced by Physostigmine was prevented by bleeding; this required withdrawal of 3 to 4.5 ml of blood from the arterial line over a period of three to four minutes. A significant hyperemia was observed also in this group (Control CBF = 104 ± 9.3 ml · 100 g⁻¹ · min⁻¹, post-treatment CBF = 196 ± 13.7 ml · 100 g⁻¹ · min⁻¹, n = 22 p < 0.001) (fig. 2). Cerebrovascular resistance decreased in this group by 43%.

The vasodilator effect of Physostigmine was reversible and it disappeared almost completely after 45 minutes of a single i.v. pulse of 0.15 mg/kg (fig. 3).

**Middle Cerebral Artery Occlusion**

Application of a AgNO₃ crystal induced complete occlusion of the selected vessel as evidenced by lack of filling with the Batson medium in postmortem perfusions, shown as a discontinuity in the vessel cast. The arteries distal to the occlusion did fill, however, with medium delivered by collateral branches that were the source of the residual blood flow observed after occlusion.

Two sets of experiments were performed, in groups of five animals each. As in the case of normal cortex, the blood pressure surge induced by Physostigmine was blocked by Phentolamine (group A) or bleeding (group B).

In group A, following MCA occlusion, CBF decreased from 119 ± 11.2 (control) to 39 ± 8 (post-occlusion) ml · 100 g⁻¹ · min⁻¹ in the "center" and from 100 ± 17 to 46 ± 3.0, ml · 100 g⁻¹ · min⁻¹ in the "periphery" of the area of distribution of the occluded vessel. In group B, CBF decreased from 125 ± 25 to 44 ± 3.8 in the center and from 123 ± 11.4 to 54 ± 10.0 in the periphery. In group A there was a tendency for CBF to increase over the two hour observation period that followed MCA occlusion, although the difference between the first and second hour did not reach statistical significance (fig. 4). No such tendency was observed in group B (fig. 4). Immediately after the last measurement of the second hour, a pulse of 0.15 mg/kg Physostigmine was given i.v. A significant increase of CBF was observed in the center and periphery of the area under study in both experimental groups. Although CBF in the periphery reached a mean value above that of pre-occlusion control, it was lower than that observed in the non-ischemic cortex (fig. 2). The time course of Physostigmine vasodilatation in the ischemic cortex did not differ from that of normal cortex (fig. 3).

**Discussion**

Systemic administration of Physostigmine induced a pronounced vasodilatation in the normal cortex. This is in line with previous observations in rat, rabbit, baboon. This phenomenon is not accompanied by metabolic activation. Since the cerebral hyperemia is accompanied by a significant increase in blood pressure, experiments were conducted in order to dissociate these two effects. Sympathetic blockade with Phentolamine prevented the pressor response, but the cerebral hyperemia of Physostigmine, however, was still present.

Cerebral blood vessels possess adrenergic innervation that can produce vasoconstriction in conditions of hypertension. Although the existence of a cerebral sympathetic vasoconstrictor tone is not clearly established, the possibility exists that Phentolamine, by blocking such tone, might have contributed to the cerebral hyperemia observed when this drug was used simultaneously with Physostigmine. If this were true, controlling the cholinergic blood pressure surge with bleeding rather than sympathetic blockade should produce a significantly lower cholinergic cerebral hyperemia, since this maneuver enhances sympathetic tone. When such experiments were conducted, the degree of cerebral hyperemia was similar to that observed when blood pressure was controlled with Phentolamine. These findings tend to rule out activation of the cerebral sympathetic innervation during Physostigmine administration, at least at the dose levels used in the present investigation.

Occlusion of MCA brought about a decrease in CBF of a magnitude similar to that found by other authors at comparable observation times.
The parallel decrease of CBF in the center and periphery of the area of distribution of the occluded vessels was somewhat unexpected. Previous studies in cats have shown a milder reduction of blood flow in the periphery of the ischemic field, a phenomenon also recorded in man. After Physostigmine administration, CBF in the periphery increased more than in the center, creating an asymmetry between those areas which perhaps reflects a smaller reserve capacity for vasodilatation in the center of the ischemic field. Another possible explanation for this phenomenon is that in the center of the ischemic field Acetylcholine metabolism might have been altered leading to a decreased availability of this transmitter.

Figure 4. Effects of Physostigmine on CBF in the center (upper panel) and periphery (middle panel) of the ischemic field in 10 animals in which, after Physostigmine injection, BP was controlled by Phentolamine (5 rats) or bleeding (5 rats). BP levels at the time of CBF determinations are shown in the lower panel. Control = mean CBF prior to a permanent MCA occlusion; MCAO1 = mean CBF over the first hour after MCA occlusion; MCAO II = mean CBF over the second hour after MCA occlusion; MCAO + PHYS = mean CBF recorded six minutes after i.v. administration of Physostigmine (0.15 mg/kg i.v.) while MCA remained occluded. Blood pressure was controlled by i.v. injection of Phentolamine Mesylate (0.1 mg/kg i.v.) or bleeding from an arterial line. These maneuvers were performed after the injection of Physostigmine, in the interval between the measurements labeled MCAO II and MCAO + PHYS. The complete sequence of interventions was performed in every animal. Statistical comparisons were made between means of Control and means of MCAO I, MCAO II, and MCAO + PHYS, within each group by analysis of variance and the Bonferroni procedure. An asterisk identifies the means that were statistically different (p < 0.05) from their respective controls.

It is interesting to note that the cerebral hyperemia of Physostigmine is potentiated by hypercapnia and hypoxia. Thus, the decrease in cerebrovascular resistance induced by this drug might be greater in areas of ischemia where tissue hypoxia and hypercapnia prevail than in normal areas. This will create a redistribution of vascular resistance within the brain parenchyma that will favor perfusion of the ischemic territory by collateral vessels. These considerations are merely speculative since we do not know the levels of blood pressure in the cortical pial arteries and the way in which these are modified under the influence of Physostigmine.

The pressor effect of Physostigmine is due to a central activation of the sympathetic system since it disappears with spinal transection and is not reproduced by cholinesterase inhibitors that do not cross the blood brain barrier. The fact that blood pressure increases at the same time that cerebrovascular resistance decreases during Physostigmine action, makes this drug potentially more useful than Papaverine or calcium antagonists as a means of improving circulation in ischemic areas, since administration of the latter is attended by hypotension. Whether improvement of circulation with Physostigmine following acute arterial occlusion can enhance survival of the affected tissue cannot be ascertained with the available data. Elucidation of this point will require further experimentation.

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References
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