Disordered Cholinergic Neurotransmission and Dysautoregulation After Acute Cerebral Infarction

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Abstract:

The possible role of displaced neurotransmitter acetylcholine (ACh) in dysautoregulation was examined after experimental regional cerebral infarction was produced by occluding the middle cerebral artery (MCA) in baboons. Regional cerebral blood flow (rCBF) was measured after intracarotid injection of 133Xenon using the gamma camera. Autoregulation was tested with metaraminol or angiotensin infusion and the autoregulation index (A.I.) was calculated. Acetylcholinesterase (AChE) was measured in brain tissue of noninfarcted and infarcted hemispheres. Cerebral arteriovenous (A-V) differences for cholinesterase (ChE) were also measured. Regional dysautoregulation was found in infarcted gray matter and correlated with increased AChE levels in the same zones of cortex and basal ganglia. The time course of onset of dysautoregulation correlated with increased ChE uptake by the brain. Intravenous infusion of the cholinergic neurotransmitter blocker, scopolamine, restored autoregulation to the ischemic zones. Autoregulation appears to be a myogenic reflex, influenced by neurogenic and metabolic mechanisms.

Additional Key Words
cerebral infarction
acetylcholine
cholinesterase
autoregulation
regional cerebral blood flow

d Apart from the well-known cholinergic neuronal neurotransmitter systems occurring at synapses throughout the central and peripheral nervous system, whereby acetylcholine (ACh) is released from presynaptic vesicles and is inactivated by cholinesterase (ChE), it has been recently confirmed by histochemical methods that there is a rich cholinergic innervation of cerebral vessels accompanying their adrenergic nerves. Nevertheless, there is meager information concerning the functional significance of the cholinergic nerves in controlling cerebral blood flow (CBF). It has been shown by direct application to pial vessels as well as by systemic administration of pharmacological agents that inhibit ChE or block ACh receptor sites that the diameter of pial vessels and cortical blood flow were altered as a result of these maneuvers. Recent data obtained in this laboratory showed correlation between the development of acute cerebral infarction in the baboon after middle cerebral artery (MCA) occlusion and increased levels of acetylcholinesterase (AChE) and ChE in the infarcted brain tissue which is generally assumed to be associated with increased ACh levels.

The intrinsic ability of cerebral vessels to maintain CBF constant despite changes in cerebral perfusion pressure (CPP) has been defined as "autoregulation." Autoregulation is frequently impaired by ischemia of the brain so that CBF passively follows changes in CPP (dysautoregulation). Some believe that dysautoregulation is caused, at least in part, by maximal vasodilatation (vasoparalysis) of cerebral vessels due to accumulation of acid metabolites such as lactic acid with brain tissue acidosis, while others feel that neurogenic influences may play some part. The present communication reports a series of experiments in baboons showing that following MCA occlusion regional cerebral infarction alters...
DISORDERED CHOLINERGIC NEUROTRANSMISSION AND DYSAUTOREGULATION

The control of regional cerebral blood flow (rCBF) associated with regional accumulation of AChE in the cortical and subcortical gray matter and that this contributes to regional dysautoregulation.

**Methods**

Twelve baboons (Papio anubis) of either sex weighing 8 to 15 kg were anesthetized with pentobarbital (30 mg per kilogram I.V.) which was supplemented as required to maintain the same level of anesthesia. Tracheostomy was performed and the animals were respirated by means of a variable speed respirator to keep arterial carbon dioxide tension (Paco₂) constant after immobilization with gallamine (15 mg per kilogram I.V.). Tracheal end-tidal CO₂ was monitored with an infrared gas analyzer and the body temperature kept at about 36.8°C by means of heating pads.

A polyethylene catheter was introduced into the ascending aorta via one femoral artery and was connected to a pressure transducer to monitor mean arterial blood pressure (MABP). Arterial samples were drawn from this catheter to measure Paco₂, hemoglobin and hematocrit intermittently throughout the experiment. Superior sagittal sinus wedge pressure was measured by means of a pressure transducer connected to an indwelling catheter inserted through a burr hole and the skull was then closed with acrylic cement. The right linguistic artery was cannulated with a polyethylene catheter and its tip placed about 0.5 cm below the bifurcation of the common carotid artery. All other branches of the right external carotid artery were ligated. Intravenous fluids and supplemental pentobarbital were administered via a catheter inserted into one femoral vein. Another polyethylene catheter was introduced into the right internal jugular vein for sampling purposes after all tributaries were ligated.

The right MCA was approached by enlarging the optic canal and occluded using a Zeiss operating microscope. Skin and temporalis muscle including the peristeum were resected to expose the entire extent of the right calvarium.

Regional CBF was measured by intracarotid injection of 133Xenon using the gamma camera. An outline of the baboon's skull was marked on the collimator and its main axis was matched with that of the baboon's head in a standard manner. A 1 ml bolus of 133Xenon (1.5 to 2 ml) was then injected via the catheter into the linguofacial artery and the clearance curve recorded over a ten-minute interval on magnetic tape. A specially designed computer permitted automatic analysis and printout for 14 contiguous square areas in the infarcted hemisphere. A standard grid for assessment of 14 regions of interest (ROI) throughout the lateral aspect of the infarcted hemisphere was used in all animals in a comparable manner (fig. 1). Flow values were calculated from the clearance curves of 133Xenon by stochastic analysis (rCBFa) as well as for the fast component, generally considered flow in gray matter (Fg), and for the slow component, generally considered flow in white matter (Fw), by biexponential (two-compartmental) analysis.

The presence of ischemic and/or hyperemic regions was arbitrarily defined from those regions showing reduction or increase of rCBF exceeding the interregional coefficient of variation (ICV) of 20%. Hence, an area was designated "ischemic" or "hyperemic" when its average rCBF was at least 20% below or above the mean rCBF and the change in rCBF was found to be consistent in at least two adjacent regions. The remaining regions showing changes of less than 20% were arbitrarily termed "nonischemic."

Arterial hypertension was produced by the intravenous infusion of metaraminol (Aramine®) in six animals and by angiotensin (Hypertensin®) in the other six; by both drugs the MABP was increased by 30% to 40% above the steady state levels (table 1). Arterial and cerebral venous samples for ChE were drawn immediately before and four hours after MCA occlusion. ChE estimation in serum was made with a Dionex Automatic Chemical Analyzer using the standard colorimetric method depending on the following chemical reaction:

\[
\text{ChE} + \text{butyrylthiocholine} + H_2O \rightarrow \text{butyric acid} + \text{thiocholine} + \text{DIP}^* \rightarrow \text{dithiocholine} + \text{DIP} - H_2 \text{O} \ (\text{colorless})
\]

The significant positive A-V difference and increased ChE measured in infarcted brain tissue were taken as evidence of increased uptake or diminished release of ChE by brain. Since measurements of ChE were done in a standard manner (before and after infarction) it was thought unlikely to be a reflection of red blood cell sludging.

**Table 1**

| arterial PaCO₂, MABP and Regional Cerebral Vascular Resistance (CVR) During Tests of Autoregulation by MABP Elevation Before and After Intravenous Infusion of Scopolamine in Acute Experimental Cerebral Infarction in the Baboon

| PaCO₂ (mm Hg) | MABP (mm Hg) | Nonischemic zones | | Ischemic zones | | Hyperemic zones |
|----------------|----------------|-------------------|-------------------------|-------------------|-------------------|
| Before scopolamine infusion | Steady state | 38.0 ± 3.2 | 90.7 ± 10.3 | 2.19 ± 0.41 | 3.28 ± 0.52 | 1.65 ± 0.67 |
| | Induced hypertension | 39.0 ± 2.1 | 129.7 ± 18.3 | 3.01 ± 0.49* | 2.87 ± 0.48* | 2.71 ± 0.63* |
| | Change | + 1.0 NS | + 39* | + 0.82* | - 0.41* | + 1.06* |
| After scopolamine infusion | Steady state | 40.0 ± 2.4 | 99.5 ± 12.9 | 2.12 ± 0.45 | 2.46 ± 0.49 | 2.11 ± 0.43 |
| | Induced hypertension | 41.8 ± 2.0 | 141.6 ± 16.3* | 3.12 ± 0.69* | 3.50 ± 0.76* | 3.01 ± 0.67* |
| | Change | + 1.8 NS | + 42.1* | + 1.00* | + 1.04* | + 0.90* |

N = 12. NS = not significant.

*Statistically significant change compared with steady state values (P < 0.01).

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Photography from the screen of the gamma camera after injection of \(^{133}\)Xenon into the right carotid artery of a baboon in this series of experiments four hours after clipping the right middle cerebral artery. The primate brain is well outlined by the isotope. The grid was placed to overlay the hemisphere from occipital (O) to frontal (F) poles in a standard manner so that the same 14 areas of interest were measured for rCBF in a comparable manner in all experiments.

Results are expressed in International Units (IU per milliliter).

CPP was calculated by subtracting sagittal sinus wedge pressure from MABP and regional cerebrovascular resistance (rCVR) was derived by dividing CPP by rCBF.

Impairment of cerebral autoregulation (dysautoregulation) was assessed using the standard A.I. derived from the formula \(\Delta \text{CBF}/\Delta \text{CPP}\), where \(\Delta \text{CBF}\) indicates the change in CBF when CPP was increased (\(\Delta \text{CPP}\)). When autoregulation is intact, A.I. should equal zero: Statistical significance was considered when differences reached the level of 5% (P < 0.05) or less using the paired t-test.

The experimental design was as follows: Four hours after MCA occlusion, the lateral aspect of the baboon's head was placed on the collimator of the gamma camera and fixed in position with adhesive tape. Once positioned, no movement was permitted until the experiment was completed. Regional CBF measurements in the steady state and during pharmacologically induced hypertension were repeated 30 minutes after the intravenous infusion of scopolamine (0.6 mg per kilogram) which is an effective blocker of the cerebral receptor sites of ACh. Scopolamine (0.6 mg per kilogram) was used for effective ACh blockade, and a dosage higher than 0.6 mg per kilogram was avoided. However, dosages as high as 1 to 5 mg per kilogram of scopolamine were used in guinea pigs by Beani et al. (1964) to significantly block ACh in the brain. Samples for arterial and cerebral venous ChE were taken immediately before and four hours after MCA occlusion. Arterial Paco\(_2\), hemoglobin and hematocrit were controlled by intermittent sampling. Since no abnormal Paco\(_2\) values occurred, no adjustment of rCBF for Paco\(_2\) was necessary.

After the measurements were completed, the animals were killed by a lethal dose of intravenous pentobarbital and the brain removed at necropsy and sectioned to determine the extent of the infarction and verify that the MCA was indeed occluded by the clip.

Results

NONISCHMIC REGIONS
Before the injection of scopolamine, rCBF in nonischemic regions did not change significantly during induced hypertension (fig. 2), although CVR increased indicating relatively well-preserved autoregulation (table 1). When hypertension was induced 30 minutes after the intravenous infusion of scopolamine, nonischemic areas continued to show intact autoregulation although Fg decreased significantly and Fw did not change (fig. 2) and CVR increased as might be expected (table 1).

ISCHEMIC REGIONS
As already defined, regions with rCBF at least 20% below mean rCBF were designated ischemic and such zones were observed in ten animals. Before intravenous scopolamine, rCBF\(_0\) increased during hypertension (dysautoregulation), which was primarily due to an increase in Fg rather than in Fw (fig. 3), and CVR decreased (table 1). Thirty minutes
following scopolamine infusion, hypertension now caused only a tendency for rCBF, and Fg to decrease which was not significant (dysautoregulation improved), while CVR increased markedly (fig. 3 and table 1). Fw showed no significant change but a tendency to increase (fig. 3).

**HYPEREMIC REGIONS**

Regions with rCBF at least 20% above the mean rCBF (hyperemic areas) occurred in all 12 experiments. In those ten cases, in which they were found together with ischemic regions, the focal hyperemia bordered the ischemic zones. Before the intravenous infusion of scopolamine, rCBF, Fg and Fw decreased (excessive autoregulation) and a tendency toward this phenomenon was also observed when hypertension was repeated 30 minutes after intravenous infusion of scopolamine but the changes were no longer significant (fig. 4). During induced hypertension CVR increased before and after scopolamine infusion (table 1).

The degree of hemispheric dysautoregulation in each animal was assessed by calculating the A.I. from the mean values for rCBF, Fg and Fw and the changes in CPP (fig. 5). Dysautoregulation was consistently found to be greater in gray than in white matter and was not dependent on the pharmacological...
EFFECT OF INDUCED HYPERTENSION ON \( \text{CBF} \) IN HYPEREMIC ZONES AFTER MCA OCCLUSION

**BEFORE SCOPOLAMINE**

**AFTER SCOPOLAMINE**

\[ \text{rCBF} - \text{ml/min} \times \text{cm}^3 \]

- Before hypertension
- During hypertension

**FIGURE 4**

EFFECT OF INDUCED HYPERTENSION ON REGIONAL CEREBRAL AUTOREGULATION (\( \Delta \text{rCBF} / \Delta \text{CPP} \)) AND OF GRAY \( (\Delta F_g) \) AND OF WHITE \( (\Delta F_w) \) MATTER AFTER MCA OCCLUSION

\[ \Delta \text{rCBF} / \Delta \text{CPP} \]

- Hypertension with Angiotensin
- Hypertension with Metaraminol

**FIGURE 5**
DISORDERED CHOLINERGIC NEUROTRANSMISSION AND DYSAUTOREGULATION

CEREBRAL A-V DIFFERENCES FOR CHOLINESTERASE BEFORE AND AFTER MCA OCCLUSION

DYSAUTOREGULATION IN GRAY MATTER CORRELATED WITH CEREBRAL A-V DIFFERENCES FOR CHOLINESTERASE (AChE) AFTER MCA OCCLUSION

agent used, metaraminol and angiotensin producing similar results. Following intravenous infusion of scopolamine, A.I. became negative indicating that the vasoconstriction response to autoregulation had now become excessive and was greater in gray than in white matter (fig. 5).

After sacrifice samples of tissue were taken from the infarcted zone and the “normal” noninfarcted hemisphere including cortical gray matter, basal ganglia gray matter and subcortical white matter. These were analyzed for AChE and ChE. Detailed results will be reported elsewhere but both AChE and ChE were elevated in ischemic gray matter with relatively little change in white matter, AChE being elevated more than ChE compared to the nonischemic hemisphere.

Cerebral A-V differences for ChE (determined from blood samples) increased significantly four hours after MCA occlusion (fig. 6), indicating a cerebral uptake of ChE presumably to inactivate the increased ACh in ischemic tissue. This uptake of ChE correlated closely with the degree of dysautoregulation in gray matter (fig. 7), particularly in ischemic gray matter (fig. 8).*

Discussion

The use of the gamma camera for measuring rCBF in baboons before and after MCA occlusion has been discussed in detail elsewhere. In normal anesthetized baboons the ICV for rCBF values did not exceed 10%. Therefore, an ICV of 20% after MCA occlusion may be assumed to indicate areas of ischemia and/or hyperemia with reliability. In that study, it was confirmed that regions of ischemia assessed by intracarotid injection of 133Xenon corresponded well with the actual territory of the infarct obtained by injection of a small bolus of 133Xenon into the MCA and by examination of the brain at autopsy. In all these experiments, including the present ones, excision of

*Although mean hemispheric rather than regional CPP was used for the calculation of regional autoregulation, the significance of the result would not be influenced by regional tissue pressure changes since the increase of local tissue pressure in the ischemic area following MCA occlusion has been measured in comparable experiments in this laboratory to be in the order of 4 to 6 mm Hg.
skin and temporalis muscle underlying the gamma camera was considered essential to avoid extracranial contamination.21

AChE is located mainly in gray matter synaptosomes while ChE is found in glial cells supporting arterioles, capillaries and venules.22, 23 It is generally agreed that ACh is hydrolyzed by both cholinesterase enzymes, although more rapidly by AChE.22 A sharp increase of ACh released from damaged brain cells and with subsequent leakage into cerebrospinal fluid (CSF) has been described by several investigators following brain injury.24, 25 Following MCA occlusion an increase of AChE and ChE was found in infarcted brain tissue and the increase of ChE in the infarcted tissue coincided with an increase in cerebral uptake for ChE.2 It may be assumed that this increased cerebral uptake as judged from cerebral A-V differences reflects increased hydrolysis of the neurotransmitter ACh in infarcted brain tissue since ACh is presumed to be liberated from synaptosomes by ischemic anoxia in the same manner as following brain injury.

Scopolamine, a powerful and specific anticholinergic agent capable of crossing the blood-brain barrier,17, 18 was therefore infused intravenously to block this putative increased ACh activity and to analyze any disorder of cholinergic neurotransmitters in the subsequent cerebral dysautoregulation following acute cerebral ischemia.

Quantitative testing of dysautoregulation by the use of the A.I. provides comparable information about changes in CBF considered in direct proportion to changes in CPP.26 In the present study two pharmacological agents were purposely used to evaluate dysautoregulation (fig. 5) because of possible differences in their pharmacological action. It has been shown that angiotensin stimulates release of ACh from the neurons of the cerebral cortex,27 from both preganglionic and postganglionic nerve endings,28 and increases total ACh brain content in the mouse and rabbit.29 However, there was no significant difference in the degree of dysautoregulation assessed by either angiotensin or metaraminol (fig. 5).

During pharmacologically induced hypertension, dysautoregulation occurred only in the focal ischemic regions (fig. 3), while CBF in hyperemic border zones decreased (fig. 4) and did not change significantly in nonischemic regions (fig. 2). Dysautoregulation occurred predominantly in ischemic gray matter (fig. 5) and correlated anatomically with regional increases of cerebral ChE and with the time course of increased ChE uptake by brain as judged by A-V differences for ChE (figs. 7 and 8).

Loss of autoregulation limited to ischemic zones of the brain as confirmed in this study (fig. 3) has been reported previously by many other investigators.9, 10, 30 Most authors believe that impaired autoregulation is caused in part by vasodilatation due to accumulation of acid metabolites and regional tissue acidosis41 resulting in rCBF in the ischemic zone passively following changes in CPP.9, 10 However, alterations in neurogenic control and cerebral neurotransmitters also have been incriminated as additional factors influencing the degree of dysautoregulation after cerebral infarction.12, 13 Following acute cerebral infarction AChE accumulation in the gray matter of cerebral cortex and basal ganglia in the infarcted zone likewise may be assumed to occur in a similar manner.8 Cholinergic innervation has been confirmed in cerebral vessels from the carotid down to arterioles less than 400 µ in diameter.9, 6, 31 It is known that these cerebral vessels (of the size innervated by cholinergic neurons) keep their structural integrity for several hours after infarction.29 Hence, as a result of release of ACh in infarcted brain, excessive vasodilatation of these vessels is likely to occur and ACh release may contribute to dysautoregulation, which should affect gray matter more than white matter (fig. 5). The two theories, namely regional acidity and neurotransmitter disorder, are mutually synergistic since it has been shown that lowering tissue pH by hypercapnia was accompanied by an increase of brain tissue ACh.33, 34

The improvement of regional cerebral dysautoregulation after the intravenous infusion of scopolamine (fig. 5) provides substantive support for the hypothesis that a cholinergic mechanism may be involved in dysautoregulation following acute cerebral infarction. After the infusion of scopolamine the passive pressure-flow relationship in ischemic regions was no longer evident during hypertension (fig. 3) since CVR increased (table 1) and the A.I. not only approached zero but actually became negative indicating excessive vasoconstriction, which might be anticipated to result if a cholinergic vasodilator tonus has been blocked (fig. 5).

The findings of decreased rCBF in hyperemic border zones during induced hypertension (fig. 4) indicate preserved responsiveness of these blood vessels to maximal stimulation by undergoing vasoconstriction. Similar results have been reported by other investigators who suggested that this vasoconstriction is mediated by vessels limited to the collateral circulation located peripheral to the ischemic area.20, 25 Furthermore, it is known that accumulation of acid metabolites and lowered tissue pH is less in bordering zones with high flow providing a collateral circulation.56 Likewise, enhanced cholinergic influence appeared to be relatively minor in this area, since after intravenous administration of scopolamine vasoconstriction was not enhanced (fig. 4).

The constancy of CBF during induced hypertension when autoregulation is intact (fig. 2) is known to be associated with vasoconstriction of the superficial cervical vessels28 which is thought to be predominantly a myogenic reflex (Bayliss effect) but influenced by the autonomic innervation and metabolic factors.15, 21, 37 The present study confirms that there is a cholinergic influence on autoregulation, and that the displaced
cholinergic neurotransmitter, ACh, plays a part in regional dysautoregulation after brain ischemia and that administration of scopolamine has a restorative effect so that now vasoconstriction during autoregulation actually becomes excessive.

In conclusion, cholinergic mechanisms appear to be involved in regional dysautoregulation in acute experimental cerebral infarction of four hours' duration. Cholinergically mediated dysautoregulation occurs predominantly in ischemic gray matter of cortex and basal ganglia correlating anatomically with increased levels of AChE in these zones and in time course with increased uptake of ChE as judged from cerebral A-V differences. Cholinergically mediated dysautoregulation following experimentally induced acute cerebral ischemia in the baboon may be reversed by cholinergic blockade with scopolamine.

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