Adrenergic Control of Cerebral Blood Flow and Energy Metabolism in the Rat

KYUYA KOGURE, M.D., PERITZ SCHEINBERG, M.D., HIDEMI KISHIKAWA, M.D., YOICHI UTSUNOMIYA, M.D., AND RAUL BUSTO, B.S.

SUMMARY Studies in rats were designed to separate and define the roles of the intrinsic and extrinsic adrenergic neurons in the control of cerebral blood flow (CBF) and cerebral energy metabolism. The data suggest several conclusions:

1. Arterial sympathetic innervation plays a role in the autoregulation of cerebral circulation.
2. The central adrenergic neurons have several functions: a) They enhance cerebral vascular tone by action on alpha receptor sites, b) They play an important role in the metabolic control of CBF. The proton-sensitive receptor sites on blood vessel walls require beta-adrenergic input in order to function, c) They influence metabolic rate of brain tissue by acting on beta-receptor sites on the cell membrane.

Evidence now exists that there are at least 2 types of adrenergic innervation of cerebral vessels, one from the cervical sympathetic plexus innervating mainly extracerebral arteries, but also reaching cerebral arterioles of 15 μ diameter and the other originating in the locus ceruleus and impinging directly on the walls of intramedullary arterioles. The influences of these vascular neurogenic systems on cerebral circulatory physiology or metabolism are still not understood.

Results of recent physiologic studies of the cerebral sympathetic system have been inconsistent, probably reflecting the variety of animal models and methodology. There is evidence, however, that the sympathetic innervation of cerebral vessels influences vascular reactivity to changes in Paco2 and Pao2 and in some way functions to help maintain autoregulation. Harper recently proposed that the sympathetic nervous system is the coarse adjuster of cerebral blood flow (CBF), as contrasted to the fine control exerted by tissue metabolism.

The concept that mechanisms intrinsic to the brainstem, and probably neurogenic in character, are capable of influencing CBF and cerebral metabolic rate is supported by a variety of studies. Pial and cortical flow, as well as cerebral metabolism, may be increased by electrical stimulation of many areas in the brainstem and hypothalamus. In addition, localized brainstem lesions cause a reduction in CBF and metabolism as well as an altered responsiveness of cerebral vessels to CO2. It has been suggested that these phenomena have a neurogenic origin, mediated by intramedullary neurons whose axons exit the brainstem in the fifth or seventh cranial nerves and eventually innervate cortical arteries. The recent discovery by immunofluorescence studies of an intramedullary adrenergic system speaks for direct neuronal action on parenchymal vessels as one basis for intrinsic control of CBF and metabolism. The present experiments were designed to separate and define the roles of the intrinsic and extrinsic cerebral adrenergic neurons in the control of CBF and cerebral energy metabolism. Extrinsic adrenergic neurons are concerned with cerebral vascular autoregulation; the intrinsic (parenchymal) adrenergic system is essential to the metabolic regulation of CBF.

Methods

Studies were performed on groups of male Wistar rats weighing between 250 and 350 grams and were designed to examine the effects of unilateral cervical sympathetic denervation on homolateral CBF and vascular resistance and the effects of certain drugs which alter both central and peripheral neuronal monoamine metabolism (reserpine) or block specific actions of monoamines on alpha and beta receptors of cerebral neurons and blood vessels (phenoxybenzamine, propranolol). In order to accomplish this, it was necessary to devise a system of internal controls to minimize the variables. Two major groups of animals were studied: one received no drugs and the other received reserpine, phenoxybenzamine, or propranolol. The animals in group 1 were divided into...
For the metabolic study, the brain was frozen in situ by delivery of liquid nitrogen into a plastic cup. Care was taken to maintain cardiorespiratory function during the entire freezing processes. This method of freezing the rat brain has been evaluated by Pontén et al.28

Another group of similarly treated and prepared rats was decapitated at the time selected for the experimental endpoint; liquid nitrogen was applied 10 seconds later. A 10 second interval to calculate the change in energy reserve was chosen as the minimum necessary to obtain statistically significant changes in each of the various substances measured. The brains were then removed as previously described. The use of only the top 4 mm of brain is essential for the application of the closed system method; this gives reasonable assurance that no other changes occurred during the time required to freeze this depth of brain. The usefulness of this method in rat experiments has been discussed in a previous communication.28

The frozen samples were crushed in liquid nitrogen and subjected to quantitative analysis for glucose, glycogen, phosphocreatine and adenosine triphosphate using a spectrophotometric, enzymatic method.29

The relative rate of high energy phosphate use of the brain was calculated by a modification of the formula based on Lowry’s closed system method. Since the calculated Energy Reserve in situ is a value obtained 10 seconds prior to the actual moment of decapitation,28 there was a total time lapse of 20 seconds between the 2 measurements and a time factor

4 subgroups: 1. no denervation or ligation of carotid artery; 2. denervation but no ligation of carotid artery; 3. ligation of carotid but no denervation; 4. denervation plus ligation of the carotid.

The effects of denervation alone could thus be determined by comparing subgroup 2 with subgroup 1, the effects of ligation alone by comparing subgroup 3 with subgroup 1, and the effects of denervation plus ligation by comparing subgroup 4 with subgroup 1. Because all the drug-treated animals were studied with the right carotid artery ligated and denervated, they were compared with subgroup 4 of the untreated animals. Cerebral adrenergic innervation was modified by the intraperitoneal administration of 10 mg/kg reserpine, 5 mg/kg phenoxybenzamine (PBZ) or 10 mg/kg propranolol, 6, 1 and 1 hours, respectively, prior to CBF measurement or sampling for brain metabolites.

All the rats subjected to this experiment had free access to rat pellets and water. The basic technique for the animal preparation, sampling and the analytical methods have been reported elsewhere.26 Briefly, anesthesia was induced with ether and maintained on 70% N2O and 30% O2. The rats were immobilized with 50 mg/kg body weight of tubocurarine and ventilated to give an arterial CO2 tension (Paco2) of 40 ± 3 torr and arterial oxygen tension (Pao2) of above 100 torr. Body temperature was kept close to 37°C. The right femoral artery was used for blood samples and to monitor and/or record blood pressure.

Experimental doses and route of administration were derived from pilot experiments determining the dose dependent response of the systemic variables, EEG and evoked potential activities. The dose selected was that which produced the greatest alteration in EEG and evoked potential responses without causing changes in MAP or cardiac rate or rhythm which would have significantly altered CBF.

In the appropriate animals, the right internal carotid artery was dissected and the cervical sympathetic fibers detached and removed together with the superior cervical ganglion. The internal carotid artery was then cannulated cephalad with PE-50 polyethylene tubing for blood pressure measurement.

When CBF was measured, the left femoral vein was cannulated for infusion of 133Xe solution and a small coil of P-50 polyethylene tubing was placed between the left femoral artery and the right femoral vein as an arteriovenous shunt for the continuous recording of the concentration of radioactive tracer in the arterial blood. The hemispheric CBF was estimated as described in our previous communication,27 but with 2 modifications. First, an additional probe type gamma detector was positioned over the side of the head in order to determine and correct the arrival time difference between the external coil circuit (the femoral artery to femoral vein shunt) and the animal's head. This time difference was corrected by means of an extrapolation of the 133Xe concentration curve. Secondly, the activity of the brain tissue was determined after sealing the sample with 4 ml of Dow Corning’s 200 silicone fluid.

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of 3 was therefore used to calculate the rate of high energy phosphate use per minute.

**Results**

Pilot experiments revealed that intraperitoneal administration of the drugs produced the least effect on the systemic variables and, therefore, it was used in the following experiments. The effects of the experimental dose of drugs on EEG activity, ECG, systemic and intracranial arterial pressure, cortical sensory evoked potential responses and parameters of cerebral energy state and homeostasis, and level of cAMP and monoamines are shown in fig. 1, fig. 2 and table 1. As can be seen, reserpine provoked spontaneous seizure activity in the EEG, elongated the latency and extinguished cortical propagation of the evoked potential response. Phenoxybenzamine increased fast (20 to 50 Hz) activity in the EEG and reduced the evoked potential slightly. Propranolol slowed the EEG (5 to 3 Hz) and diminished the cortical evoked response.

None of the experimental doses of these drugs altered cerebral energy charge (EC), energy reserve (ERes) or water content of the brain (table 1). An accumulation of Na+ by phenoxybenzamine was associated with a decrease in Cl- content of the brain (from 134.8 ± 2.6 to 125.3 ± 1.8 for the right, 138.9 ± 1.3 to 126.4 ± 1.4 mEq/kg dtw for the left hemisphere). A slight decrease in the level of cyclic 3',5'-adenosinemonophosphate (cAMP) was observed in the reserpine-treated animals. The monoamine content of the reserpine-treated animals also decreased while propranolol-treated animals showed a slight increase in the level of norepinephrine (NE) and dopamine (DA).

Table 2 compares Paco2, arterial pH, hematocrit (Ht), Pao2, mean arterial pressure, intracranial arterial Pressure, and intracranial pH. The table shows the results for each group, including the means and standard errors for each parameter. The table also includes a column for significance, indicating whether the change is statistically significant compared to the control group.

### Table 1: Effects of Experimental Doses of Drugs on Cerebral Energy State, Parameters of the Homeostasis, Level of cAMP and Monoamines in the Brain

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Non-Treated</th>
<th>Reserpine</th>
<th>Phenoxybenzamine</th>
<th>Propranolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECh (mcC/kg)</td>
<td>0.94 ± 0.01</td>
<td>0.95 ± 0.00</td>
<td>0.94 ± 0.01</td>
<td>0.94 ± 0.01</td>
</tr>
<tr>
<td>Na+(mEq/Kg dtw)</td>
<td>208.9 ± 2.8</td>
<td>259.0* ± 4.3</td>
<td>225.9* ± 4.0</td>
<td>224.9* ± 2.7</td>
</tr>
<tr>
<td>K+(mEq/Kg dtw)</td>
<td>486.5 ± 5.9</td>
<td>486.5 ± 7.0</td>
<td>481.7 ± 1.5</td>
<td>474.3 ± 1.5</td>
</tr>
<tr>
<td>cAMP(pmol/kg)</td>
<td>2.20 ± 0.05</td>
<td>2.32 ± 0.08</td>
<td>2.32 ± 0.08</td>
<td>2.40 ± 0.14</td>
</tr>
<tr>
<td>NE (ug/g)</td>
<td>0.32 ± 0.02</td>
<td>0.28 ± 0.02</td>
<td>0.28 ± 0.02</td>
<td>0.38* ± 0.11</td>
</tr>
<tr>
<td>DA(ug/g)</td>
<td>0.95 ± 0.10</td>
<td>1.15 ± 0.10</td>
<td>1.15 ± 0.10</td>
<td>1.16* ± 0.03</td>
</tr>
<tr>
<td>5-HT(ug/g)</td>
<td>0.57 ± 0.04</td>
<td>0.66 ± 0.06</td>
<td>0.66 ± 0.06</td>
<td>0.71 ± 0.06</td>
</tr>
</tbody>
</table>

Number of observations in parenthesis; values are mean ± standard error; dtw = dry tissue weight. *Significantly different from the control (non-treated group) (P <0.05.

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arterial pressure and pulse rate in those animals which received drugs with the several groups that did not. There was no significant difference in these variables in treated and nontreated groups except for animals treated with reserpine. They showed a reduced mean arterial and intracranial arterial pressure but the relationship of these 2 pressures (intracranial arterial pressure is about 62% of the systemic arterial pressures) remained the same. Systemic arterial pressure did not fall below 100 mm Hg in any of the animals.

It appears that sympathetic denervation per se causes no alteration in cerebral vascular tone. Cerebral vascular resistance (CVR) decreased following ligation but denervation had no further effect. There was no change in the rate of high energy phosphate use in any of these non-treated groups (table 3).

Reserpine and PBZ produced a similar decrease in CVR in both hemispheres. Propranolol also decreased CVR in the intact hemisphere, but quantitatively less than reserpine or PBZ. Both reserpine and propranolol decreased the rate of high energy phosphate use in both hemispheres, whereas PBZ did not.

### Table 2 Systemic Variables

<table>
<thead>
<tr>
<th>Group I - Non-Treated</th>
<th>N</th>
<th>Paco₂ (torr)</th>
<th>art pH</th>
<th>H⁺ (mm Hg)</th>
<th>PaO₂ (torr)</th>
<th>MAP (mm Hg)</th>
<th>ICAP (mm Hg)</th>
<th>Pulse rate (pulse/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. No denervation or ligation</td>
<td>8</td>
<td>39.0 ± 0.5</td>
<td>7.401 ± 0.005</td>
<td>46.5 ± 0.5</td>
<td>13.0 ± 0.8</td>
<td>159 ± 3.0</td>
<td>—</td>
<td>437 ± 7.5</td>
</tr>
</tbody>
</table>

### Table 3 Effects of Drugs Used on Cerebral Blood Flow and Metabolism

<table>
<thead>
<tr>
<th>Group I - Non-Treated</th>
<th>N</th>
<th>CBF (ml/100g/min)</th>
<th>CVR (mm Hg/ml/100g/min)</th>
<th>Rate of ~ P use (mmol/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. No denervation of ligation</td>
<td>8</td>
<td>89.0 ± 4.4</td>
<td>88.7 ± 4.5</td>
<td>1.79 ± 0.05</td>
</tr>
</tbody>
</table>

### Discussion

The sympathetic fibers from the superior cervical sympathetic ganglion in the rat are distributed solely to the homolateral cerebral arteries; thus, the contralateral hemisphere can be used as a control for the denervated side in the present experimental model. Sympathetic denervation did not alter CBF or CVR, but denervation plus ligation of the carotid reduced perfusion pressure, and also hemispheric blood flow. Since CBF in the rat is ordinarily maintained at a nor-
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normal level at perfusion pressures well below 90 mm Hg, the failure of autoregulation in these animals may be secondary to sympathetic denervation of the carotid artery. This appears to be confirmed by the observation that ligation without denervation was accompanied by reduction in CVR sufficient to maintain CBF at a normal level. This argues that arterial sympathetic innervation has a role in autoregulation.

The role of the central adrenergic systems was tested by pharmacologic manipulation. Reserpine depletes neuronal monoamine content and permits evaluation of this effect upon vascular reactivity and high energy phosphate use. In this study, reserpine resulted in a decrease in cerebral metabolic rate of high energy phosphate and a decrease in perfusion pressure. The cerebral vessels dilated, apparently in response to the decrease in perfusion pressure, although the diminution in cerebral metabolism would have been expected to cause vasoconstriction. This suggests that reserpine has restored the capacity for autoregulation to the denervated cerebral arteries.

One deduction from this observation is that the metabolic control of vascular tone requires an intact central adrenergic system. Because the metabolic influence on cerebral vessels is proton mediated, the implication is that the proton-sensitive site in the vascular wall requires an adrenergic input in order to function. In the absence of this adrenergic input a reduction of cerebral energy metabolism does not cause vascular constriction, thereby resulting in flow-metabolism uncoupling.

Because reserpine is a non-specific depletor of neuronal monoamines and not NE alone, the mechanism of the central adrenergic activity was dissected further by using separate alpha and beta receptor site blockers. In this fashion the effects produced by reserpine can be attributed by comparison of the flow and metabolic data specifically to the adrenergic system without concern for the effects which might result from dopamine or serotonin depletion. The peripheral effects of the alpha and beta receptor site blockers used in this experiment (phenoxybenzamine and propranolol) had no effect upon CBF or metabolism because PaCO_2 and PaO_2 were maintained constant and MAP did not drop sufficiently to alter CBF. The cerebral vessels dilated in response to a decrease in perfusion pressure caused by PBZ, an alpha receptor blocker, implying that the role of the central adrenergic system in limiting vascular response to pressure changes is mediated by alpha receptors in the parenchymal vessels.

Beta receptor site blockade by propranolol produced a smaller decrease in vascular resistance than did reserpine or phenoxybenzamine, and caused a 50% reduction in the rate of high energy phosphate use. The reduction in ATP utilization did not cause vascular constriction, again resulting in uncoupling of the anticipated metabolism and flow relationship. This suggests that one explanation for the reserpine-induced reduction of cerebral energy metabolism is due to decreased adrenergic activity on the beta receptors of neurons.

Acknowledgment

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We are indebted to Mrs. M. Santiso, Mrs. E. Martinez and Mrs. O. Alonso for their expert technical assistance.

References

Ischemic Brain Edema Following Middle Cerebral Artery Occlusion in Baboons: Relationship Between Regional Cerebral Water Content and Blood Flow at 1 to 2 Hours

LINDSAY SYMON, F.R.C.S., NEIL M. BRANSTON, PH.D., AND OLEG CHIKOVANI, M.D.

SUMMARY The relationship between increase in water content in ischemic brain and levels of regional blood flow has been studied in 11 primates. Flows were recorded using the method of hydrogen (2-minute) clearance, from a total of 128 electrodes in cortex and white matter, and a gradation of ischemia was produced by middle cerebral occlusion transorbitally. The flows were reduced in the area of densest ischemia from control levels of 42.0 ± 12.0 ml/100g/min to 7.0 ± 5.4 ml/100g/min, with lesser decreases over the remainder of the ischemic hemisphere. Water content was measured in cortex and white matter, in regions topographically related to those of flow measurements, by densitometric assessment using precalibrated kerosene/bromobenzene columns. The average water content of cortex in regions remote from ischemia was 797.4 ± 5.8 mg/gm and in white matter 708.5 ± 8.2 mg/gm. Significant increases in water content (comparing corresponding regions of the two hemispheres) of up to 11.4 ± 7.5 mg/gm were demonstrated in the most ischemic cortical areas. A gradient of water increase was evident in the ischemic hemisphere, increases in water content being greatest in the opercular zone and least in the parasagittal area. Significant differences in white matter water content between the 2 hemispheres were demonstrated only in the most densely ischemic areas in the current experiments where ischemia was limited to 93 ± 20 mins in the 11 animals without reperfusion. The relationship between ischemic density and water content increase showed that significant increases in water content occurred in regions where terminal flows had been below 20 ml/100g/min, indicating that accumulation of water in ischemic brain begins at flow values comparable to those associated with the failure of synaptic transmission, higher than those associated with failure of the ionic pump of the cell. Possible pathophysiological mechanisms are discussed.

THE DEVELOPMENT of brain swelling secondary to focal cerebral ischemia is a common problem both in the surgery of subarachnoid hemorrhage, where a lethal outcome, particularly following aneurysm surgery, is frequently associated with extensive brain swelling, and in stroke where a common cause of death is tentorial herniation.1

In previous studies, we have established that the failure of electrical function in ischemia, assessed by monitoring the cortical evoked response, occurs at an appreciably higher level of cortical blood flow than failure of the ionic pumping mechanisms of the cell membrane as indicated by marked increases in extracellular potassium activity and tissue electrical impedance.2–4 These effects of ischemia may be reversed to some extent upon prompt restoration of the flow, either directly or with induced hypertension.5,6 It is to be expected that changes in tissue water content will accompany the above functional changes and may similarly be related quantitatively to the conditions of local flow. O'Brien and his colleagues have already shown significant water increases in areas of ischemia induced by middle cerebral artery (MCA) occlusion in cats, reaching a maximum in 24 hours,7 while Hossmann and his associates6 have indicated an earlier phase of post-ischemic brain edema, which appears to be due to intracellular swelling, and, therefore, possibly of cytotoxic type. The pattern of...
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