Pharmacological Control of Local Oxygen Regulation
Mechanisms in Brain Tissue

H. I. Bicher, M.D., Ph.D.,* and P. Marvin, B.S.

SUMMARY The effect of several agents active on autonomic nervous system functions was tested on brain oxygen autoregulation parameters. It was found that atropine, propranolol and isoproterenol had no influence in abolishing the measured parameters. Phenoxybenzamine, tolazoline and dibenamine all suppress autoregulation.

In an additional experimental series, a phenoxybenzamine infusion was given during O₂ breathing. The infusion induced, in most cases, an additional rise in TpO₂ (tissue pressure of oxygen, which refers to the partial pressure [in mm Hg] of this gas at the measuring tip of the electrode). It is concluded that an α-adrenergic mechanism is part of the autoregulation process. Also, the increase in brain TpO₂ induced by 95% O₂-5% CO₂ breathing seems to be blocked or reversed by α-adrenolytic drugs thus supporting the thinking that the effect of CO₂ on cerebral blood flow is at least in part mediated through an α-adrenergic response.

Introduction

THE TISSUES of the central nervous system are acutely sensitive to the effects of circulatory insufficiency. Interruption of the cerebral circulation is followed within seconds by a loss of consciousness and within minutes by irreversible changes in the brain. It is because the maintenance of normal cerebral function is so vitally essential and completely dependent on an adequate blood supply that the study of cerebrovascular regulatory mechanisms and the effect of drugs upon these assume a special significance. Furthermore, the physiological and pharmacological behavior of the brain circulation is sufficiently unique that drug actions exerted on most other vascular beds can only rarely be assumed to be operating similarly within the central nervous system.

In previous publications we have described a precisely controlled autoregulatory mechanism to maintain a constant brain TpO₂ (tissue pressure of oxygen, which refers to the partial pressure [in mm Hg] of this gas at the measuring tip of the electrode) level. This includes a theoretical "tissue oxygen sensor" able to simultaneously regulate cerebral blood flow (CBF) and tissue O₂ consumption through reflex inhibition-excitation of generalized neuronal activity; it is defined by four criteria in the physiological compensatory regulation. A response elicited by a short period of anoxic anaesthesia: (1) short "reoxygenation time" (RT, the time required for TpO₂ to return to the pre-anoxic level), (2) increase in CBF, (3) presence of an "overshoot" (period after reoxygenation during which TpO₂ is higher than baseline), and (4) a period of "anoxic silence" in neuronal activity.

All four responses can be suppressed by the α-adrenergic blocking agent, phenoxybenzamine. In the present experiments other autonomically active agents were tested, and a fifth autoregulation criterion, the small or non-existing rise in brain TpO₂ during normoatmospheric O₂ breathing, was added as another test. Also, the action of high respiratory CO₂ levels with these mechanisms was investigated.

Methods

The experiments were performed on 55 cats anesthetized with sodium pentobarbital and under positive-pressure breathing. Femoral artery blood pressure and carotid artery blood flow were recorded on a Brush Model 440 chart recorder using standard transducers. Blood and tissue pO₂ were determined as described below using oxygen electrodes and recorded on the Brush recorder. The different respiratory mixtures tested were administered through the artificial respiration pump (Harvard, Model 607), and all solutions were injected into the cannulated femoral vein. Results reported represent average values of five experiments for each drug or procedure.

Measurement of TpO₂ With Oxygen Ultramicroelectrode

The O₂ ultramicroelectrodes used were of the "gold in glass" type as described by Cater and colleagues. They were made by pulling a glass tube (KG-33, ID 1.5 mm, OD 2.0 mm, Garner Glass Co., Claremont, California), encasing a 20-µ gold wire (Sigmund Cohn Corp., Mt. Vernon, New York) in a David Kopf Model 700C vertical pipette puller. The exposed gold tip is about 10 µ in diameter, and is coated with Rhoplex (Rhom Haas, Philadelphia, Pennsylvania) membrane as previously described. This probe is used as an "external reference" O₂ microelectrode. The electronic circuitry to measure the polarographic current was provided by a Model 1200 Chemical Microsensor System (Transidyne General Corporation, Ann Arbor, Michigan), and the results were recorded on a Brush Model 440. The procedure for electrode calibration was the same as previously described. Cortical reoxygenation times were determined as in preceding papers. This procedure involves the determination of the time necessary to reach back baseline oxygen levels after a one-minute period of anoxic anaesthesia and seems to be a good indication of the ability of the circulation to transport oxygen to tissue.

In several experiments a platinum-iridium Teflon-coated wire, 120 µ in diameter, was used as the O₂ electrode. Although the calibration is not as reliable to determine actual TpO₂ values, it was found that in determining transients (RT and overshoot) the obtained values correlated well with those obtained using microelectrodes.

Blood pO₂

Blood pO₂ was recorded using a polarographic catheter electrode as previously described manufactured by Mediscience Technology Corporation, Cherry Hill, New Jersey, according to our specifications.

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Blood Pressure

Blood pressure was measured from a cannulated femoral artery using a Statham transducer.

Measurement of Indications of Flow and Rates of Changes in Flow

Measurement of indications of flow and rates of changes in flow were made with the use of an electromagnetic flowmeter, Model 202 (Carolina Medical Electronics, King, North Carolina). The probe or electromagnetic cuff was placed around a carotid artery and recordings were made continuously.

Operation

The animals were anesthetized with sodium pentobarbital (40 mg per kilogram), and cannulations were performed in the femoral artery and vein and in the trachea. The head of the animal was fixed in a David Kopf Model 1730 stereotaxic instrument. The surface of the brain was exposed through a small hole drilled through the periosteum. The electrode was fixed in a micromanipulator (David Kopf Model 1760) and lowered into the surface of the brain. The hole in the periosteum was resealed with Agar after electrode positioning. Micromovements of the electrode within brain tissue were achieved using a David Kopf Model 1707B hydraulic microdrive. Measurements were made only in the upper 3 mm of brain prefrontal cortex.

Materials

Those drugs used in the series were obtained from the following sources: atropine (Merck, Rahway, New Jersey), propranolol (Ayerst Laboratories, New York, New York), Isuprel (Winthrop Laboratories, New York, New York), Bemegride (Abbott, North Chicago, Illinois), tolazoline (CIBA, Summit, New Jersey), and dibenamine and phenoxybenzamine (Smith, Kline, and French, Philadelphia, Pennsylvania).

Results

O₂ Breathing

Upon respiration of pure O₂ for five to seven minutes, arterial pO₂ rises to about 600 mm Hg. TpO₂ changes are very limited. In most electrode locations there is a small increase (averaging +26% of original value) or no change. In other locations there is a small decrease.

The infusion of phenoxybenzamine, 30 mg per kilogram over a period of 30 minutes, while breathing O₂ induced a further increase in TpO₂ in seven of nine animals. The mean increase was 67% above pre-infusion levels (fig. 1).

Breathing 5% CO₂-95% O₂ caused a large increase averaging 210% in ten animals; however, after phenoxybenzamine infusion this effect was smaller (average 143% in 4 of 18 tests) or reversed (average fall of 48% in 12 of 18 tests) (fig. 2).

Drug Effects

Several agents known to act on the autonomic nervous system or to increase cerebral metabolism and blood flow were investigated. The effects on reoxygenation time and cerebral blood flow are summarized in table 1. All α-adrenergic blocking agents (tolazoline, dibenamine and phenoxybenzamine) prolonged the RT and abolished the "overshoot," and carotid flow increased as a response to N₂ breathing, as previously reported for phenoxybenzamine. The other drugs tested were devoid of such action.

Discussion

In previous papers, we have established four criteria used to identify the oxygen autoregulation mechanisms in brain tissue, following a short period of anoxic anoxia: (1) short "reoxygenation time," (2) increase in cerebral blood flow, (3) presence of an "overshoot," and (4) presence of a period of electrical silence paralleling the period of TpO₂ depression. After administration of phenoxybenzamine in increasing doses, the response to anoxic anoxia was fundamentally changed as follows: (a) on the TpO₂ record, the "reoxygenation time" is much longer, and the "overshoot" disappears, (b) the increase in carotid artery blood flow was no longer present, (c) the "anoxic silence" of cortical neurons was no longer present. Prolongation of the anoxic period for more than five minutes does not reduce the rate of neuronal firing.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Brain TpO₂ under O₂ breathing. Note small rise when changing from air to oxygen breathing (upper tracing). Marked additional TpO₂ rise when phenoxybenzamine is infused (lower tracing). Time scale: 1 square = 10 seconds.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Effect of phenoxybenzamine and CO₂ on brain TpO₂. Trace A: Small TpO₂ rise during O₂ breathing. Trace B: Large increase in TpO₂ caused by 95% O₂-5% CO₂ breathing. Trace C: After phenoxybenzamine infusion, O₂ breathing rise in TpO₂ is larger than the pre-infusion effect. Contrariwise, 95% O₂-5% CO₂ causes a fall in TpO₂. Time scale: 10 seconds between time marks (chart center).
sympathectomized monkeys promptly reduced blood flow arterioles.

Noradrenergic fibers arise from two groups of

during pure O₂ breathing, even under high blood pO₂

ten- tion was influenced by a-adrenergic blockade.

Therefore, it can be assumed that the adrenolytic agent blocks or reverses all four parameter changes used as evidence for the O₂ autoregulation mechanism.

In a recent paper, Leniger-Follert et al.⁸ described the response of brain TpO₂ to a sudden increase in arterial O₂ content induced by O₂ breathing. In most locations TpO₂ did not rise proportionally to the Pao₂ increase, or was even decreased. These authors also report that in about one-third of the sites explored, the rise in TpO₂ paralleled that of Pao₂, a finding that we could not confirm. This apparent discrepancy can probably be explained by the fact that Leniger-Follert et al. recorded surface pO₂ values, whereas we recorded TpO₂ using microelectrodes inserted into the brain matter. However, there is a good correlation between the changes that these authors describe on most of their locations and those reported in this paper.

These results suggest that a fifth autoregulation criterion should be adopted, namely, the limited rise in brain TpO₂ during pure O₂ breathing, even under high blood pO₂ tensions.⁸ Phenoxybenzamine seems to block this regulation mechanism to a limited degree when infused for a prolonged period of time.

The presence of an α-adrenergic mechanism involved in brain O₂ autoregulation seems to be confirmed by the fact that the three α-blocking agents tested (phenoxybenzamine, dibenamine, and tolazoline) did prolong the RT, abolish the overshoot, and suppress the increase in carotid blood flow induced by hypoxia. All other agents tested failed to do so.

The influence of neurotransmitters on the normal and ischemic cerebral circulation now seems to be well established.⁹⁻¹² An extensive autonomic network of catecholaminergic fibers supplies the cerebral arteries of arterioles.¹³⁻¹⁴ Noradrenergic fibers arise from two groups of neurons: postganglionic sympathetic neurons, whose cell bodies reside in the superior cervical ganglion and send axons to the extraparenchymal blood vessels, and the brainstem neurons, whose cell bodies have been shown by immunohistofluorescence to reside primarily in the locus ceruleus and to terminate near blood vessels throughout the brain. After these brainstem neurons were described by Hartman et al.,¹¹ their function was studied by Raichle et al.,¹² who found that stimulation of the locus ceruleus in sympathectomized monkeys promptly reduced blood flow and increased the permeability of the capillary endothelium to water.

Endogenous catecholamines released from noradrenergic neurons are thought to influence cerebral circulation in normal as well as pathological states. Data showing their effects on vessel constriction and blood flow have been obtained by direct application of catecholamines to blood vessels in vivo and in vitro, and by modification of endogenous catecholamine metabolism by drugs, lesions or electrical (faradic) stimulation.

In vivo or in vitro application of norepinephrine in concentrations of 10⁻⁷ to 10⁻⁴ M to the middle cerebral arteries of dogs, monkeys and goats results in constriction.¹⁰⁻²² Arteries also constrict when bathed in tyramine,²³ a sympathomimetic drug that displaces norepinephrine from presynaptic vesicles. Pretreatment with α-adrenergic blocking drugs, such as phenoxybenzamine¹⁹,²⁰ or phentolamine,²¹ or with enough reserpine to deplete noradrenergic fibers of norepinephrine prevents this constriction.²⁰ However, the nature of the mechanisms controlling cerebral blood flow is still unclear. There seems to be a dual control of cerebral blood flow, whereby (1) autoregulation maintains flow constant despite changes in perfusion pressure, and (2) chemical regulation results in changes in blood flow as Paco₂ is altered, but proof of this duality is lacking. Most authors attribute the pressure-flow autoregulatory mechanism to direct metabolic effects on the smooth muscle of the arterial wall or to myogenic reflexes due to changes in cerebral perfusion pressure (CPP). What part, if any, neurogenic innervation of the cerebral vessels plays in either or both of these regulatory mechanisms is debated. Few comparative investigations of the dual regulators have been made. In a recent paper, Meyer and associates²⁴ compared both types of vascular regulation by selective α-adrenergic blockade, and suggest that blood flow autoregulation and chemical regulation have separate, independent mechanisms. Cerebral autoregulation is strongly influenced by the autonomic nervous system since patients with brainstem lesions (the site of central neuronal control of CBF) showed loss of autoregulation but a preserved chemical regulation, this also being evidenced by the fact that autoregulation but not chemical regulation was influenced by α-adrenergic blockade.

In follow-up papers,²⁵,²⁶ these authors also suggested that phenoxybenzamine had a metabolic effect on brain, possibly by inhibition of uncoupling of oxidative phosphorylation, and that both the metabolic and vascular effects, autonominously mediated, could be of therapeutic value in stroke patients when the drug was infused directly into the carotid artery. It must be noted that other authors²⁷ have not confirmed these results, although injecting the drug intravenously.

The effect of CO₂-O₂ breathing in raising brain TpO₂ to much higher levels than O₂ breathing alone has been described by Metzger et al.²⁸ and confirmed by Leniger-Follert et al.⁸ In the present experiments we have demonstrated that α-adrenergic blockade cannot only inhibit but also reverse this phenomenon. This may seem to indicate that, at least in respect to local TpO₂ regulation, part of the response of vessels to CO₂ changes is adrenergically mediated. Similar contentions were suggested by Fraser et al.²⁹ when studying adrenergic blockade of hypoxic cerebral artery constriction.

<table>
<thead>
<tr>
<th>Drug (dosage)</th>
<th>RT (%)</th>
<th>Carotid flow (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine (2 mg/kg)</td>
<td>+21</td>
<td>-7</td>
</tr>
<tr>
<td>Propranolol (4 mg/kg)</td>
<td>+34</td>
<td>-25</td>
</tr>
<tr>
<td>Isuprel infusion (2 mg/ml, 1 hr)</td>
<td>-47</td>
<td>-15</td>
</tr>
<tr>
<td>Bemegride infusion (100 µg/ml, 0.5 hr)</td>
<td>+4</td>
<td>+66</td>
</tr>
<tr>
<td>Tolazoline (100 mg/l)</td>
<td>+95</td>
<td>-100</td>
</tr>
<tr>
<td>Dibenamine (50 mg/kg)</td>
<td>+48</td>
<td>-79</td>
</tr>
<tr>
<td>Phenoxybenzamine (50 mg/kg)</td>
<td>+98</td>
<td>-95</td>
</tr>
<tr>
<td>No drug after 2.5 hrs</td>
<td>-19</td>
<td>+92</td>
</tr>
</tbody>
</table>

Changes represent averages of five experiments, five determinations per datum point, with a 5% SD for each point. Note that all three alpha adrenergic agents (tolazoline, dibenamine, phenoxybenzamine) prolonged the reoxygenation time and depressed the carotid flow increase in response to hypoxia.

RT = reoxygenation time.
Acknowledgment

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References

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Prognosis of Occlusive Cerebrovascular Diseases in Normotensive and Hypertensive Subjects

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SUMMARY Comparison of the clinical features, especially prognosis, in cerebral infarction was made between nine normotensive subjects and 16 hypertensive patients with an 80% stenosis or occlusion of the intracranial or extracranial arteries. Our own criteria for evaluating hypertension were employed on the basis of the following items: a past history of hypertension, blood pressure levels and ischemic cerebral circulation. The authors wish to recognize and thank Ms. Gisela Davis and Mrs. Vicky Drake for their contribution to this project. Their technical abilities and assistance were necessary and valuable parts of the work.

Introduction

OUR PREVIOUS EXPERIMENTAL STUDIES3-5 have shown that bilateral carotid artery occlusion caused an extremely higher mortality and a greater increase in anaerobic glycolytic metabolites such as lactate and lactate/pyruvate ratio of the brain in spontaneously hypertensive rats than in normotensive Wistar rats, and that the ischemic lesions were diffuse and severe in the former and small and circumscibed in the latter. These observations suggest that hypertension per se, but not the vascular changes secondary to the persistent high blood pressure (BP), seems to cause the derangements of cerebral circulation, resulting in severe and diffuse cerebral infarction.
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