Heterogeneities of Regional Cerebral Blood Flow During Hypoxia-Ischemia in the Rat

MYRON D. GINSBERG, M.D., ROBERT MEDOFF, AND MARTIN REIVICH, M.D.

SUMMARY The distribution of regional cerebral blood flow (rCBF) within the cerebral hemispheres of rats was investigated following an hypoxic-ischemic insult consisting of a 30-minute exposure to 6.5% to 7% inspired oxygen coupled with unilateral ligation of the common carotid artery and maintenance of normal blood pressure (modified Levine preparation). rCBF was estimated by means of an autoradiographic method employing $^{14}$C-antipyrine. Mean arterial Po$_2$ values of 26.8 to 27.5 mm Hg were attained during the insult period. rCBF rose above control values in all structures of the hemisphere contralateral to carotid artery ligation.

LEVINE'S MODEL of "anoxic-ischemic encephalopathy" was developed as a means of inducing histological brain lesions in the rat. Whereas prolonged nitrogen anoxia led only to acute cardiac death, shorter periods of nitrogen exposure combined with unilateral carotid artery occlusion were found to result in pathological changes in the hemisphere ipsilateral to the occlusion.1 (Unilateral carotid artery ligation by itself produced no lesions.) Although the early work was performed in physiologically unmonitored animals, the model has recently been refined to ensure controlled hypoxia and maintenance of the normotensive state in the monitored animal. Biochemical and neuropathological studies of this preparation have been reported.2-9 Inasmuch as this model permits different degrees of cerebral perfusion to exist within the two cerebral hemispheres, a knowledge of the regional cerebral blood flow (rCBF) distribution in this preparation would be of importance in assessing the manner in which regionally selective vulnerability within the brain to hypoxia-ischemia might depend upon factors related to cerebral perfusion. Salford et al.4 have recently reported a study of rCBF in this model but did not consider the rCBF distribution in detail. Indeed, the frozen tissue-counting method employed in that study renders fine regional differentiation impossible.4 The present study was undertaken to investigate the patterns of regional perfusion during hypoxia-ischemia.

Methods

Male Wistar rats (250 to 300 gm) were anesthetized with diethyl ether and tracheostomized. D-tubocurarine (0.2 to 0.3 mg) and atropine sulfate (0.05 mg) were administered intraperitoneally. The animals subsequently were ventilated mechanically on a mixture of 30% oxygen and 70% nitrous oxide. Polyethylene catheters were inserted into both femoral veins and one femoral artery, and a Teflon No. 30 catheter was placed in the other femoral artery. The right common carotid artery was isolated and loose ligatures placed around it. End-tidal CO$_2$ concentration was measured by means of an infrared analyzer (Beckman). Rectal temperature was maintained at 37°C with a heating lamp. Arterial blood pressure and an electrocardiographic lead were recorded on a Grass polygraph.

Following a stable 20-minute control period, five experimental animals were subjected to a 30-minute period of hypoxia-ischememia produced by ligation and sectioning of the right common carotid artery and simultaneous reduction of the inspired oxygen concentration to 6.5% to 7% by the addition of nitrogen to the inspired gas mixture. Arterial blood gases were assayed in the control state and at five minutes and 25 minutes following onset of the insult. Metabolic acidosis was prevented by a slow intravenous infusion of sodium bicarbonate (mean total dose: 2.9 ± 0.5 cc containing 2.6 ± 0.4 mEq). To prevent hypotension below 90 to 100 mm Hg during the insult, methoxamine hydrochloride was slowly infused intravenously (mean total dose: 1.5 ± 0.3 mg). Three control animals were subjected to the same operative procedures, but inspired oxygen was maintained at 30% and the carotid artery remained unligated. They were given doses of methoxamine similar to those administered to the hypoxic-ischemic animals, and were infused with normal saline in volumes equivalent to those of the bicarbonate solution employed in the experimental group.

During the last minute of the insult period (or the comparable point for the control animals), rCBF was estimated by an autoradiographic technique employing $^{14}$C-antipyrine. The isotope (25 μc) was infused intravenously over one minute. The arterial blood was sampled at five-second intervals from the tip of the freely flowing Teflon catheter. The flow per volume characteristics of this catheter are such that the need for a smearing correction is obviated. The drops of blood were collected on tared paper squares, which were dried and weighed. Quantitatively pipetted volumes of arterial blood were also dried and weighed, and the volume per dry weight ratios so obtained were used to compute the volume of each arterial drop. In other respects, the method used resembled that previously reported.8 Animals were killed with an intravenous injection of saturated potassium chloride solution at the end of the antipyrine infusion period.

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TABLE 1  Arterial Blood Gas Values

<table>
<thead>
<tr>
<th></th>
<th>Po2 (mm Hg)</th>
<th>Pco2 (mm Hg)</th>
<th>pH (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxic-ischemic animals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control period</td>
<td>94.5 ± 10.5*</td>
<td>37.1 ± 3.2</td>
<td>7.376 ± 0.042</td>
</tr>
<tr>
<td>Hypoxia-ischemia, 5 minutes</td>
<td>26.8 ± 1.2</td>
<td>35.6 ± 3.0</td>
<td>7.391 ± 0.033</td>
</tr>
<tr>
<td>Hypoxia-ischemia, 25 minutes</td>
<td>27.5 ± 1.4</td>
<td>30.6 ± 3.7</td>
<td>7.356 ± 0.040</td>
</tr>
<tr>
<td>Control animals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham insult, 25 minutes</td>
<td>138.4 ± 15.9</td>
<td>32.6 ± 2.5</td>
<td>7.238 ± 0.089</td>
</tr>
</tbody>
</table>

*Mean ± SEM.

The brains were rapidly removed, frozen in a Dry Ice-acetone mixture, and sectioned at 20 μ in a cryotome at −15°C. The brain sections were allowed to expose x-ray film for ten days. Optical density measurements were performed from five representative regions of each cerebral hemisphere and the values of rCBF were computed therefrom.5

Results

Table 1 summarizes the arterial blood gas data. The hypoxic-ischemic animals sustained mean Po2 values of 26.8 to 27.5 mm Hg during the insult. Mean blood pressure was generally more than 100 mm Hg during the insult; it fell to 90 to 100 for 1.5 ± 1.3 minutes and below 90 mm Hg for at most 0.9 minute. Mean blood pressure during the antipyrine study averaged 128 ± 11 mm Hg for the hypoxic-ischemic animals and 114 ± 10 mm Hg for the control animals.

rCBF data are presented in table 2. In the hypoxic-ischemic animals, all rCBF values for structures of the unligated hemisphere were 1.4 to 2 times more than the corresponding control values. In individual structures of the ligated hemisphere, rCBF values following hypoxia-ischemia were in all cases less than in the corresponding regions of the opposite, unligated hemisphere. However, the degree to which carotid artery ligation lowered rCBF varied from region to region. The greatest rCBF decrement produced by carotid artery ligation during hypoxia occurred in the lateral cerebral cortex and the caudoputamen (fig. 1, table 2). In the dorsal cortex and hippocampus, carotid artery ligation led to a smaller reduction in rCBF, and in the central thalamus the flow decrement produced by ligation was not significant. The extent to which decrements in rCBF resulting from carotid artery ligation differed from region to region is shown in table 3: the rCBF decrement for the lateral cortex, for example, proved to be significantly greater than for the dorsal cortex, central thalamus, or hippocampus.

Discussion

In this model of hypoxia-ischemia, the generalized increase in CBF consequent upon arterial hypoxemia is modified by unilateral carotid artery ligation to create strikingly discrepant reductions of rCBF within the various structures of the hemisphere ipsilateral to the vascular occlusion. The lateral cerebral cortex and caudoputamen, which lie within the middle cerebral artery (MCA) territory, undergo the most pronounced decrement in rCBF secondary to ligation, whereas in the thalamus, carotid occlusion produces only an insignificant rCBF reduction compared to the unligated side.

A criticism of the present work relates to recent observations that antipyrine tends to underestimate rCBF, particularly at higher flow rates, owing to diffusion limitation.8 Other tracers such as 14C-ethanol, however, which are less diffusion-limited,8 are not suitable for autoradiography because of their volatility. Although the rCBF values in table 2 thus may tend systematically to underestimate true
TABLE 3. Significance* of Regional Differences of \( r\)CBF (unit:
\( \mu l\) blood/g tissue/min).

<table>
<thead>
<tr>
<th>Region</th>
<th>Lateral cortex</th>
<th>Caudoputamen</th>
<th>Dorsal cortex</th>
<th>Central thalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudoputamen</td>
<td>NS</td>
<td>P &lt; 0.02</td>
<td>P &lt; 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Dorsal cortex</td>
<td>P &lt; 0.005</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Central thalamus</td>
<td>P &lt; 0.005</td>
<td>NS</td>
<td>NS</td>
<td>P &lt; 0.02</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>P &lt; 0.005</td>
<td>NS</td>
<td>NS</td>
<td>P &lt; 0.02</td>
</tr>
</tbody>
</table>

*Paired t-test.

rCBF, they provide a basis for analyzing patterns of heterogeneous flow within hemispheric structures of the rat in a manner not possible using the frozen tissue-counting method. At present, no ideally diffusible yet non-volatile and inert radioactive tracer has been found to permit more exact quantitative autoradiography.

It is possible that the regional heterogeneity of rCBF decrements observed within structures of the ligated hemisphere in the present study might be reflected in a correspondingly varied regional susceptibility to the effects of hypoxia-ischemia. In the earlier biochemical study of Salford et al.\(^2\) using this model, a comparable insult resulted in changes characteristic of hypoxia (tissue lactic acidosis, decline in high energy phosphate compounds, normal or elevated glucose and glucose-6-phosphate, and decreased Krebs cycle intermediates) which were more pronounced on the side of carotid ligation; however, regional differentiation within a single hemisphere was not attempted. Subsequently, Salford and Siesjö\(^3\) performed separate biochemical analyses from the anterior cerebral-middle cerebral arterial border-zone region and from the MCA territory itself, and found significantly greater changes in the latter than the former.

Neuropathological study of this preparation\(^4\) has shown an absence of histological lesions in the area corresponding to our "dorsal cortex," generally mild affection of the anterior cerebral-MCA boundary zone, and involvement of many neurons in the MCA territory proper (our "lateral cortex"). The thalamus was normal in most animals, the striatum was somewhat more often affected, and the hippocampus showed frequent and prominent neuronal alterations. All such changes occurred only on the side ipsilateral to carotid ligation.\(^3\) The gradient of pathological damage in that series (MCA territory > hippocampus = striatum > boundary zone > thalamus) corresponds generally to the order of rCBF decrements produced by carotid ligation in our series. These data thus suggest that regionally selective damage may be caused primarily by heterogeneities of rCBF (as originally suggested by Spielmeyer\(^7\)), rather than by intrinsic regional differences in the metabolic properties of nervous tissue, as proposed by Vogt.\(^8\)

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References

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