INFLUENCE OF HYPOTENSION AND HYPOTENSIVE TECHNIQUE ON THE AREA OF PROFOUND REDUCTION IN CEREBRAL BLOOD FLOW DURING FOCAL CEREBRAL ISCHAEMIA IN THE RAT

D. J. COLE, J. C. DRUMMOND, H. M. SHAPIRO AND M. H. ZORNOW

SUMMARY
We have studied the effect of induced hypotension on reduction in regional cerebral blood flow (CBF) during middle cerebral artery occlusion (MCAO). Rats were anaesthetized with 2% isoflurane. MCAO was performed during four conditions: normotension (mean arterial pressure (MAP) 107 (sd 15) mm Hg); hypovolaemic hypotension (blood withdrawn to reduce MAP to 43 (2) mm Hg); nitroprusside (SNP) hypotension (MAP reduced to 45 (3) mm Hg); isoflurane hypotension (MAP reduced to 44 (3) mm Hg with 3.5 (0.48) % isoflurane). Hypotension was established before MCAO and was maintained for 10 min, at which time the dimension of the brain areas with zero CBF was determined autoradiographically. All hypotensive regimens were associated with significantly larger areas of extreme CBF reduction. In a coronal section at the centre of the MCA distribution, the area with zero CBF (expressed as a percentage of the area of the entire coronal section) was: normotension 4.7 (4.5)%; hypovolaemic hypotension 10.1 (2.8)%; nitroprusside hypotension 13.5 (2.0)%; and isoflurane hypotension 11.8 (3.9)%. There were no differences between the three hypotensive regimens. The data indicate that, when focal cerebral ischaemia occurs during hypotension (MAP 45 mm Hg) induced by any of the three regimens evaluated, extreme CBF reduction occurs over larger areas than are observed during normotension. These data confirm the importance of arterial pressure as a determinant of collateral flow during focal cerebral ischaemia.

KEY WORDS

Induced hypotension is used both electively and in urgent circumstances during the surgical management of intracranial aneurysms. Focal cerebral ischaemia may occur during these operations, for several reasons. Vasospasm of varying degree may be present, especially when clipping is performed in the acute phase following subarachnoid haemorrhage. Inadvertent occlusion of a cerebral artery (e.g., the middle cerebral artery) or of its branches (e.g., the lenticulostriates) may occur during temporary placement of aneurysm clips, and vessel occlusion may occur also as a result of clipping an aneurysm under the circumstances of reduced visibility that arise following rupture of an aneurysm. Elective "trapping" of an aneurysm may also cause temporary focal cerebral ischaemia.

Because of the risk of focal cerebral ischaemia during aneurysm surgery, it is important that the effect of anaesthetic agents and techniques on...
CBF DURING INDUCED HYPOTENSION

neuronal survival is understood. The present study has examined the dimension of the area of extreme cerebral blood flow (CBF) reduction occurring with three methods of induced hypotension following occlusion of the middle cerebral artery in the rat.

METHODS

The study was approved by the Animal Use Subcommittee of the Veterans' Administration Medical Center, La Jolla, California. Male Sprague-Dawley rats (wt 350–450 g) were fasted for 14 h before the study, but were allowed free access to water. Anaesthesia was induced with the animal in a plexiglass box, using 4% isoflurane in oxygen. The trachea was intubated and the lungs were ventilated mechanically with an inspired gas mixture of 2% isoflurane and 40% oxygen in nitrogen. Ventilation was adjusted to maintain normocapnia. Rectal temperature was maintained at 37 °C. Catheters (PE-50) were placed in both femoral arteries and veins. Mean arterial pressure (MAP), the electrocardiogram and rectal temperature were monitored continuously. Arterial blood-gas tensions and haematocrit were measured intermittently. A left subtemporal craniectomy was performed according to the method of Tamura and colleagues [1]. The subsequent middle cerebral artery occlusion was performed according to the method of Bederson and colleagues [2].

Following completion of the craniectomy, the rats were assigned randomly to one of four experimental groups: normotensive control; hypovolaemic hypotension; nitroprusside hypotension; isoflurane hypotension. In the control group, the inspired isoflurane concentration was maintained at 2.0%. In the other three groups, hypotension was induced 15 min after completion of the craniectomy. MAP was reduced slowly over a period of 15–20 min by gradual withdrawal of blood via an arterial line, a gradual increase in inspired concentration of isoflurane, or a gradual increase in rate of infusion of 0.5% sodium nitroprusside. When mean arterial pressure (MAP) had been decreased to 50–55 mm Hg, a small incision was made in the dura over the middle cerebral artery (MCA) in the vicinity of the olfactory tract. The MCA was occluded in all four groups, using micro-bipolar forceps under saline irrigation. The occlusion extended from a point just proximal to the olfactory tract, to the inferior cerebral vein [2]. During the final exposure and occlusion of the MCA, arterial pressure was decreased further, to 45 mm Hg. Arterial pressure was held stable at this value for 10 min after MCA occlusion (MCAO), at which time non-quantitative determination of CBF was performed.

$^{14}$C-Iodo-antipyrine 100 μCi kg$^{-1}$ was infused over a period of 46 s. The animal was decapitated at $t = 45$ s and the brain was removed rapidly and placed immediately in 2-methyl-isobutane in a freon bath. The brains were subsequently cut in a cryostat at −13 °C. Coronal sections (20 μm) were exposed on Kodak OM-1 film for 21 days. Five standard coronal sections (designated 1–5) spanning the anterior–posterior extent of the zone of ischaemia were analysed (fig. 1). Section 2 was located at the anterior midline extent of the corpus callosum; section 4 was located at the posterior midline extent of the corpus callosum; section 1 was 1.8 mm anterior to section 2; section 3 was midway between sections 2 and 4; section 5 was 1.8 mm posterior to section 4. Sections 1–5 correspond to Plates 5, 11, 22, 34 and 40, respectively, in Maps and Guides to Microdissection of the Rat Brain by Palkovits and Brownstein [4].

A Drexel DUMAS image processing system (Drexel University Image Processing Center, Philadelphia, PA) was used to identify the regions of each brain section with an optical density equivalent to background—that is, those areas in which cerebral blood flow was zero and in which there had been no accumulation of isotope. The area of zero flow was expressed as a percentage of...
Table I. Physiological variables (mean (SD)) at the time of CBF determination 10 min after MCAO. ***P < 0.0001 vs control; **P < 0.01 vs control and isoflurane

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hypovolaemia</th>
<th>Isoflurane</th>
<th>Nitroprusside</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>107 (15)</td>
<td>43 (2)***</td>
<td>44 (3)***</td>
<td>45 (3)***</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>(P_{aco_2}) (kPa)</td>
<td>5.0 (0.2)</td>
<td>4.9 (0.1)</td>
<td>5.0 (0.2)</td>
<td>5.2 (0.4)</td>
<td>ns</td>
</tr>
<tr>
<td>(P_{ao_2}) (kPa)</td>
<td>16.9 (1.3)</td>
<td>16.9 (2.0)</td>
<td>16.0 (2.9)</td>
<td>15.0 (2.2)</td>
<td>ns</td>
</tr>
<tr>
<td>pH</td>
<td>7.40 (0.02)</td>
<td>7.39 (0.01)</td>
<td>7.39 (0.04)</td>
<td>7.38 (0.04)</td>
<td>ns</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>46 (1)</td>
<td>41 (2)**</td>
<td>45 (1)</td>
<td>44 (2)</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

Table II. Percentage (SD) of each coronal section with zero blood flow 10 min after MCAO. *P < 0.05 vs control; †P < 0.05 vs hypovolaemia

<table>
<thead>
<tr>
<th>Section</th>
<th>Control</th>
<th>Hypovolaemia</th>
<th>Isoflurane</th>
<th>Nitroprusside</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0 (1.8)</td>
<td>3.5 (3.6)</td>
<td>7.6 (5.6)*</td>
<td>9.7 (5.4)†</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>2</td>
<td>2.9 (4.2)</td>
<td>9.0 (4.4)*</td>
<td>10.6 (6.2)*</td>
<td>14.8 (3.9)*</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>3</td>
<td>4.7 (4.5)</td>
<td>10.1 (2.8)*</td>
<td>11.8 (3.9)*</td>
<td>13.5 (2.0)*</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>4</td>
<td>1.0 (1.4)</td>
<td>3.9 (3.2)</td>
<td>6.8 (3.8)*</td>
<td>7.4 (3.7)*</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>5</td>
<td>0.2 (0.2)</td>
<td>0.7 (0.8)</td>
<td>3.2 (3.2)*</td>
<td>2.7 (2.3)</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

The cross-sectional area of the entire coronal section.

Data were analysed using one-way analysis of variance. Where differences were identified, multiple pairwise comparisons were performed using the Newman–Keuls multiple range test [5].

RESULTS

Of the 31 animals studied, three (one control and two nitroprusside) were excluded for technical reasons. The remaining 28 animals were allocated evenly to the four groups. At the time of determination of CBF (10 min after MCAO) there were no differences between the groups in terms of \(P_{aco_2}\), \(P_{ao_2}\) and pH (table I). MAP was significantly \((P < 0.0001)\) greater in the control group \((107 \text{ (SD 15) mm Hg)}\) than in the three hypotensive groups (hypovolaemia 43 (2) mm Hg; isoflurane 44 (3) mm Hg; nitroprusside 45 (3) mm Hg); differences between the three hypotensive groups were not significant. The inspired isoflurane concentration was significantly greater in the isoflurane hypotension group \((3.5 (0.48)\%)\) than in the other three groups.

MCAO resulted in an autoradiographically obvious area of ipsilateral flow reduction that included lateral and dorsolateral cortex and the corpus striatum. Each of the three hypotensive regimens was associated with a significantly larger area of ischaemia than was seen in the normotensive control group (table II). The differences between the normotensive control group and the hypotensive groups were significant in five of the five sections for isoflurane, in four of the five sections for nitroprusside, and in two of the five sections for hypovolaemia. The increases in the area of ischaemia caused by hypotension were substantial. For the middle section (section 3) the ischaemic areas (expressed as a percentage of the area of the entire coronal section) were: control 4.7 (4.5)%; hypovolaemia, 10.1 (2.8)%; isoflurane, 11.8 (3.9)%; nitroprusside, 13.5 (2.0)%. The only significant difference (Newman–Keuls multiple range test) among the three hypotensive regimens occurred in section 1, in which the ischaemic area was larger \((P < 0.05)\) in the nitroprusside group than in the hypovolaemia group.

DISCUSSION

This investigation examined the area of profound CBF reduction present 10 min after MCAO in the rat. One limitation of the results is that they provide information on the status of the cerebral circulation at only one moment following MCAO. Ten minutes was chosen because it is within the range of intervals that occur when temporary vascular occlusions take place during operation. A second limitation is that CBF at one moment is not necessarily a predictor of neuronal outcome. Therefore, it is not certain that the substantial CBF differences we observed would have resulted
in differences in neuronal survival if reperfusion had occurred. However, our method measured the area in which CBF was essentially zero, and it is established that CBF reduction of this magnitude results very quickly in neuronal death [6]. Thus our data support the view that induced hypotension with all three of the regimens studied would have resulted in greater neuronal loss than would have occurred during normotensive MCAO.

A mean arterial pressure of approximately 45 mm Hg was chosen because it is similar to the values utilized in precarious clinical situations. An MAP of 45 mm Hg is slightly less than the reported lower limit of normal autoregulation (approximately 50 mm Hg) for a variety of species ranging from rats [7] to primates [8]. However, it seems unlikely that the increase in the ischaemic area seen with hypotension was entirely the result of this small reduction of MAP beyond the autoregulatory threshold. It is probable that the vasculature within and at the periphery of the area of substantial CBF reduction is pressure passive [9] and that the development of collateral flow to the ischaemic area is dependent upon perfusion pressure. The increased extent of severe ischaemia seen with all three hypotensive regimens is consistent with the concept that the degree of arterial pressure reduction is a more important determinant of CBF distribution than the effect of the hypotensive agent on normal cerebral vessels.

By the multiple comparison method (Newman–Keuls test) used in the initial data analysis, the only significant difference among the three hypotensive groups occurred in section 1, in which the ischaemic area was larger (P < 0.05) in the nitroprusside group than in the hypovolaemia group. However, inspection of the data in table II suggested a trend toward additional differences. We therefore performed post hoc comparisons between the three hypotensive groups using Student’s t test for unpaired data (without corrections for multiple comparisons). This approach increases the power of the statistical analysis, but adds to the risk of a false positive statistical error. By this method there were no differences for the comparisons of hypovolaemia vs isoflurane and isoflurane vs nitroprusside. However, the comparison of hypovolaemia vs nitroprusside revealed significantly larger areas of ischaemia in the nitroprusside group in sections 1, 2 and 3. Before the study was performed, this apparent difference had not been anticipated. In previous investigations performed in normal animals comparing hypotension induced by nitroprusside and by haemorrhage, nitroprusside was associated with either greater or equivalent preservation of CBF [10–13]. The most likely explanation is that haemorrhagic hypotension causes cerebral vasocostriction because of high circulating concentrations of catecholamine, activation of sympathetic innervation of the cerebral vasculature, or both [4, 5]. This theory may be consistent with the present results. It is possible that, with focal ischaemia (present study) rather than global ischaemia, catecholamine-mediated vasoconstriction may result in shunting (a “Robin Hood steal”) of blood away from non-ischaemic regions to the jeopardized areas (in which there is vasoparalysis). Both isoflurane and sodium nitroprusside are known cerebral vasodilators [16–19] and it follows that, in the presence of focal ischaemia, vasodilatation of normal vessels may result in the opposite phenomenon, with shunting (“steal”) of blood away from ischaemic brain to adjacent normal tissue.

The data analysis indicates that the sizes of the areas of profound ischaemia were similar for nitroprusside and isoflurane. However, it is probable that different cerebral metabolic circumstances prevailed during the two hypotensive regimens. Isoflurane caused a substantial reduction in cerebral metabolic rate (CMR) [16]; thus CMR was probably lower in the isoflurane group than in the nitroprusside group. Accordingly, equivalent degrees of reduction in CBF observed in these two groups may represent different degrees of physiological impairment, with the isoflurane group relatively “protected” as a result of metabolic suppression [20, 21].

The methods used to compare the three hypotensive regimens measured only the areas of severe reduction in flow. Neuronal death would be likely to ensue rapidly in these areas. The study did not examine the so-called ischaemic penumbra, in which the extent of reduction in CBF is such that neurones are impaired functionally, but potentially recoverable; the relative effect of the various regimens may be different in terms of the dimension of the penumbra.

In summary, the present results suggest that, when induced hypotension occurs in the presence of focal ischaemia, there may be a substantial increase in the area of severely ischaemic brain. In terms of the area of profound flow reduction...
occurring after MCAO in the rat, there was little difference between hypotension accomplished with either deep isoflurane anaesthesia or sodium nitroprusside administered during light isoflurane anaesthesia. These data suggest that, in situations in which focal ischaemia is present or may occur, induced hypotension should be undertaken only after careful consideration of the risks and benefits.

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REFERENCES