The Distribution of Ischaemic Damage and Cerebral Blood Flow After Unilateral Carotid Occlusion and Hypotension in the Rat

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SUMMARY We have developed a model of haemodynamic cerebral ischaemia by inducing haemorrhagic hypotension (40-50 mmHg mean blood pressure) following unilateral common carotid occlusion, with external carotid ligation, in anaesthetised rats. The neuropathological pattern of ischaemic brain damage was correlated with the distribution of change in cerebral blood flow using the I²C-iodoantypyrine autoradiographic technique. Whereas hypotension alone (40-50 mmHg) resulted in neither ischaemic brain damage nor significant alterations in cerebral blood flow, the combination of this degree of hypotension with unilateral carotid occlusion produced predominantly unilateral ischaemic brain damage which correlated with regions of reduced cerebral blood flow. With this type of haemodynamically induced oligoemia, the most vulnerable areas were the lateral neocortex, the caudate nucleus, the hippocampus and the thalamus. Within the cortex, the greatest reductions in blood flow occurred in the deeper cortical layers, and this was the most frequent site of ischaemic cell change. These data support the concept of a haemodynamic mechanism in the pathogenesis of some transient cerebral ischaemic attacks in man.

Materials and Methods

Thirty male Sprague-Dawley rats (weighing 300-500 g) were used. Anaesthesia was induced in a perspex box with halothane (5%) and maintained with (0.5-1.0%) halothane and a nitrous oxide/oxygen mixture (70%:30%). A tracheostomy was performed and the animals' ventilation controlled by a small animal respirator. Both femoral arteries were catheterised, the one for continuous blood pressure measurements and the other for direct intra-arterial withdrawal and re-infusion of blood to maintain an exactly uniform blood pressure. A femoral vein was catheterised for infusion of radio-isotope. Arterial blood samples were withdrawn for measurement of blood gases, haematocrit and blood glucose (Corning blood gas analyser and Beckman glucose meter). Rectal temperature was maintained at 37°C with an automated heating box. After a 30 minute stabilisation period the right carotid bifurcation was exposed and the external carotid artery and all its branches ligated. The common carotid artery was isolated between ligatures, and at the start of the experiment it was ligated after an 0.8 mm outside diameter catheter had been inserted distally to monitor internal carotid artery stump pressure, and another inserted proximally to permit the sampling of arterial blood during the measurement of local CBF. Systemic

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arterial pressure was recorded on a Gould 2 channel recorder, calibrated and zeroed for the low pressure range (0–50 mmHg). In control animals (hypotension alone), where both carotids remained patent, the left brachial artery was catheterised instead of the carotid artery for withdrawal of arterial samples during autoradiographic measurement of CBF.

Measurement of CBF
The $^{14}$C-iodoantipyrine autoradiographic method to measure local cerebral blood flow (CBF) was employed in the manner described previously in this laboratory. In summary, $^{14}$C-iodoantipyrine (50 µCi) in 1.5 ml of saline was given as a ramped infusion over 30 seconds whilst simultaneously collecting arterial blood from the carotid (or brachial arterial) catheter on filter paper for subsequent weighing and for determination of radioactivity by liquid scintillation counting. At the end of the 30 second period the animal was decapitated and the brain was removed rapidly for freezing by immersion in 2-methyl butane at −45°C. Brain sections were cut (20 µm thick) in a cryostat at −22°C; three in every ten sections were mounted on glass coverslips and immediately dried on a hot plate. Autoradiographs were prepared by exposing these sections, with a set of plastic standards of known $^{14}$C concentrations (44–1175 nCi/g), to single coated X-ray film (Kodak SB5) for approximately 6 days. Tissue $^{14}$C concentrations from autoradiographs were measured on a computer based densitometer (Quantimet 500, Cambridge Instruments) with reference to the pre-calibrated standards.

Tissue optical density (OD) measurements were taken from at least 6 sections in which the anatomical regions of interest could be accurately defined. We evolved a method to minimise, as far as possible, observer bias towards high or low flow on one or the other side. In the parietal cortex, corresponding columns of high and low flow (‘dark’ and ‘light’ columns respectively) were identified on each side. While these columns may represent the architectural arrangement of cortical arteries, their precise nature is uncertain. They are, however, a well recognised phenomenon in autoradiographic sections, and cannot be ignored when making side to side comparisons. The columns selected were each equidistant from the midline and were aligned so that the counting pattern of the densitometer was accurately positioned at 90 degrees to the cortical surface. The units of size on the densitometer were measured on each radiograph from standard units in millimetres, so that measurements were made at exact depths from the cortical surface on each side. Three readings were taken from each consecutive level (1 level corresponded to two densitometer units measuring 105 µm) within the column and the mean value was calculated. Six such values from contiguous areas within the same column were taken, and the centre of these six areas was designated the cortical depth for that measurement. An average of only three areas was taken as the most superficial depth to provide a more accurate determinant of the most superficial cortical layer. The average values for CBF within the columns were thus determined at 0.16, 0.48, 0.80, 1.12, 1.44 and 1.76 mm from the cortical surface within the ‘light’ columns of the parietal cortex. This permitted standardisation from side to side and from animal to animal.

Experimental Design
The purpose of this study was to correlate CBF reductions with areas of ischaemic brain damage in animals with unilateral carotid occlusion and hypotension at the lower limit of the autoregulation curve in halothane anaesthetised rats (Group 1). The control for this experiment was therefore considered to be hypotension to the same level (40–50 mmHg) without carotid occlusion (Group 2).

Group 1: Haemorrhagic hypotension (n = 5 CBF; n = 6 neuropathology):
After establishing stable normoxic and normocapnic conditions, systemic hypotension (40–50 mmHg) was induced by rapid but controlled withdrawal of blood from one of the femoral arterial catheters into a heparinised syringe. Mean arterial blood pressure was strictly regulated within these predetermined limits (40–50 mmHg) by means of repeated intra-arterial withdrawal or re-infusion of blood in order to ensure a stable period of hypotension. This was best performed manually to avoid blockage or stasis of catheters. CBF was measured 15 minutes after the blood pressure had stabilised at the hypotensive level.

Group 2: Right common carotid occlusion and hypotension (n = 6 CBF; n = 13 neuropathology):
CBF was measured following carotid occlusion and 15 minutes of the standard hypotensive insult (40–50 mmHg) described above.

Neuropathology
The brains of 19 rats were examined by light microscopy (6 in control group 1, and 13 following carotid occlusion with hypotension (Group 2). Two hours after reversing a thirty minute period of hypotension but with the carotid still ligated the animals were sacrificed by transcardiac perfusion with 100 ml–150 ml. of FAM fixative (40% formaldehyde/glacial acetic acid/absolute methanol = 1 : 1: 8) after a brief wash out with heparinised saline. After perfusion the brains were decapitated and the head was stored in the same fixative at 40°C for at least 4 hours. After its removal from the skull, the brain was immersed in the fixative for 24 hours. Thereafter the hind brain was detached by a cut through the midbrain and the cerebral hemispheres were cut into five coronal slices. The brainstem was cut perpendicular to its long axis into three slices and the cerebellum into two slices perpendicular to the folia of the dorsal surface of each hemisphere. Blocks of the brain were embedded in paraffin wax and sections (7–8 µm thick) stained by a method combinin-
TABLE 1

Local Cerebral Blood Flow (ml/g/min) in Various Regions with Hypotension (40-50 mm Hg) and with Right Carotid Occlusion (RCO) plus Hypotension (40-50 mm Hg)

<table>
<thead>
<tr>
<th>Region</th>
<th>Group 2 RCO + hypotension (n = 6)</th>
<th>Group 1 hypotension alone (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>Cerebellar hemisphere</td>
<td>0.75 ± 0.05</td>
<td>0.72 ± 0.05</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>1.77 ± 0.17</td>
<td>1.69 ± 0.19</td>
</tr>
<tr>
<td>Visual cortex II</td>
<td>1.35 ± 0.26</td>
<td>0.72 ± 0.15*</td>
</tr>
<tr>
<td>IV</td>
<td>1.58 ± 0.23</td>
<td>0.88 ± 0.26*</td>
</tr>
<tr>
<td>VI</td>
<td>1.34 ± 0.26</td>
<td>0.76 ± 0.27*</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>1.34 ± 0.18</td>
<td>0.84 ± 0.17</td>
</tr>
<tr>
<td>Auditory cortex</td>
<td>1.80 ± 0.20</td>
<td>0.91 ± 0.17*</td>
</tr>
<tr>
<td>Medial geniculate</td>
<td>1.75 ± 0.23</td>
<td>1.13 ± 0.16</td>
</tr>
<tr>
<td>Lateral habenular</td>
<td>1.68 ± 0.20</td>
<td>1.39 ± 0.18</td>
</tr>
<tr>
<td>Parietal cortex I</td>
<td>1.37 ± 0.07</td>
<td>0.86 ± 0.14*</td>
</tr>
<tr>
<td>IV</td>
<td>1.35 ± 0.09</td>
<td>0.85 ± 0.12*</td>
</tr>
<tr>
<td>VI</td>
<td>0.81 ± 0.11</td>
<td>0.50 ± 0.08*</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>0.82 ± 0.07</td>
<td>0.73 ± 0.05</td>
</tr>
<tr>
<td>Thalamus (ventrolateral)</td>
<td>1.24 ± 0.07</td>
<td>0.88 ± 0.09*</td>
</tr>
<tr>
<td>Sensorimotor cortex</td>
<td>1.24 ± 0.09</td>
<td>0.80 ± 0.13*</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>1.22 ± 0.11</td>
<td>0.82 ± 0.07*</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>1.29 ± 0.09</td>
<td>0.84 ± 0.10*</td>
</tr>
<tr>
<td>Genu</td>
<td>0.48 ± 0.04</td>
<td>0.35 ± 0.05</td>
</tr>
</tbody>
</table>

All values x ± SEM: *p < 0.05.

Results

Cerebral Blood Flow

Group 1: Hypotension (40–50 mmHg) alone:

Haemorrhagic hypotension (MAP 40–50 mmHg) without carotid occlusion did not produce any significant asymmetry in the distribution of flow (table 1, fig. 1).

Group 2: Right carotid occlusion with hypotension 40–50 mmHg:

From visual inspection of the autoradiograms optical density, and thus CBF, was clearly reduced in the hemisphere ipsilateral to the occlusion (fig. 2) and the reduction in CBF was quantified. CBF was reduced significantly throughout the neocortex (particularly the deeper layers) (fig. 3) as well as the thalamus and caudate nucleus (table 1). There was no significant
alteration of CBF in the cerebellum, hypothalamus, and lower brainstem.

Neuropathology

As judged by the uniform hardening of the specimens and by the absence of blood in the vessels, perfusion fixation appeared to be good in all animals. There was no evidence of postischaemic brain swelling and internal herniation was not seen. The cytological artefacts “dark cell” and “hydropic cell or water change” were not seen.12

No histological abnormalities were seen in the Group 1 animals (hypotension 40–50 mmHg alone). In the majority of the animals in Group 2 microscopy revealed foci of ischaemic brain damage that consisted of small areas of selective neuronal necrosis comprising microvacuolation and ischaemic cell change as described previously in FAM fixed material (fig. 4).13,14

The anatomical distribution of the ischaemic damage is given in table 2. Ischaemic damage was seen in 12 of the 13 Group 2 animals. Of these, the damage was ipsilateral to the carotid artery occlusion in 10 animals and bilateral in 2. In all 12 animals there were lesions within the neocortex, the highest incidence being found in the frontal and parieto-temporal regions with a greatly reduced incidence in other areas. In 3 (bilateral in 2) the cortical lesions were accentuated within the arterial boundary zones between the distributions of the anterior and middle cerebral arteries. Whereas in some animals the lesions affected all layers of the cortex there were others in which the ischaemic damage was undoubtedly accentuated within the deep-
er layers, affecting in particular layers 5 and 6. In many of these instances lesions were restricted to layers 5 and 6, the more superficial layers appearing entirely normal. In 7 animals there were lesions within the caudate nucleus, mostly in the lateral segment. In 6 animals there were lesions in the hippocampus and in 4 animals lesions were seen in the thalamus. In a small number of animals lesions were seen within septal nuclei, in the hypothalamus, the cerebellum and in the upper brainstem.

In summary, unilateral ischaemic neuronal damage was seen in 10 of 13 animals subjected to right carotid occlusion and hypotension (40–50 mm Hg) (table 3). Hypotension alone at 40–50 mm Hg did not produce ischaemic neuronal damage.

Discussion

In the rat carotid occlusion on its own does not result in ischaemia of sufficient severity to produce neuronal damage. A systemic insult must be added to produce changes. This may either be by hypotension15 16 or hypoxia.9 17 This is explained by the adequate collateral circulation through the Circle of Willis following carotid occlusion in rats where models of carotid occlusion have differed from models of middle cerebral artery occlusion1–5 in which ischaemic neuronal damage can be produced without a systemic insult. By contrast, in gerbils carotid occlusion frequently produces infarction without a systemic insult in about 50% of animals.18 All of these models of ischaemia have their clinical counterparts, but they may not entirely replicate all the mechanisms which produce transient cerebral ischaemia and which will subsequently lead to ischaemic damage with stroke in man.

There are numerous clinical situations in which it is clear that reduction in cerebral perfusion pressure results in focal ischaemia.5 19–24 Furthermore, there is much evidence that blood pressure, cardiac output and CBF fall by as much as 30% during normal sleep.25–29 Whether these reductions may precipitate cerebral ischaemia when there is occlusive cerebrovascular disease has yet to be determined, but it may be significant that up to 60% of ischaemic strokes are reported to occur during sleep, and are recognised only on waking.30

Experimental evidence for haemodynamically induced cerebral ischaemia is extensive. Sengupta et al4 showed that although carotid ligation in the baboon did not reduce CBF, it did render the circulatory reserve of the brain insufficient to meet physiological challenges. The same workers31 went on to confirm that, after carotid ligation, haemorrhagic hypotension produced a fall in CBF, i.e. that flow had become passively dependent on pressure. An hypoxic model was described by Levine17 and more recent autoradiographic studies9 have demonstrated the heterogeneity of rCBF with hypoxia following carotid ligation. In our study the greatest reductions in CBF occurred in the deeper layers of the cortex which are known to be most vulnerable to hypoxia and ischaemia. Figure 3 indicates that flow in the deepest layers of the cortex, where the pathological changes were maximal, was just below 50 ml/100g/min. The distribution of these neuropathological lesions in our study is similar to that described by Pulsenelli et al42 who used a 4 vessel occlusion model, except that our changes were predominantly unilateral. The levels of flow contralateral to the occluded carotid were marginally reduced when compared with non-occluded animals. While this change was in no way significant, it may reflect a steal from the non-occluded to the occluded hemisphere. This may also account for the occasional finding of bilateral ischaemic neuronal damage.

Morphological details of ischaemic nerve cell change have been well documented by light and electron microscopy.13 14 33 51 The earliest morphological change, microvacuolation of the neuronal cytoplasm, was observed in many nerve cells in the present study, although dark shrunken ischaemic nerve cells constituted the predominant pathological change. Ischaemic
cell change with encrustations was not a feature, probably because this change requires a longer time period to develop.13, 14, 34 There was no evidence of infarction. There is some controversy about what distinguishes reversible and irreversible brain damage, but most regard the stage of microvacuolation as being reversible. On the other hand, ischaemic cell change, and certainly ischaemic cell change with encrustation formation is probably irreversible. Some of the appearances of the animals in this series therefore are probably reversible. Because the animals subjected to neuropathological examination survived a subsequent two-hour reperfusion period compared with the autoradiographic animals, the ischaemic brain damage may be a consequence of the harmful effects that occurred during such reperfusion. The mechanisms of ischaemic brain damage during reperfusion include the no-reflow phenomenon, lactacidosis, and the accumulation of free radicals and fatty acids.

There was a clear relationship between reduced CBF and neuropathological abnormality, especially in the caudate, lateral neocortex and thalamus. Lesions were seen in the hippocampus of 6 animals, although there was not a significant drop in flow in this region. This represents the only 'mismatch' between CBF reduction and distribution of ischaemic cell change. The reason for this discrepancy is not clear; it is a well-known area of diminished local resistance and a higher metabolic rate in the limbic system and hippocampus may lead to damage in a relatively well perfused area. The match between CBF and neuropathology was otherwise good. The flow values in both groups of animals with hypotension at the lower limit of autoregulation for halothane anaesthetised rats (groups 1 and 2) are on average 12% less than those previously reported by Tamura et al.35 This suggests that with this degree of hypotension, in both our groups there was a trend towards reduced flow, although not of sufficient magnitude to reach statistical significance. This adds weight to the argument that the level of hypotension selected (40-50 mmHg) is at the lower limit of autoregulation. With this type of oligaemia it was the deeper layers of the cortex which were most vulnerable: CBF was lowest in these areas (less than 50 ml/100 g/min) and it was here that ischaemic cell changes were found most frequently. In general these changes were seen in areas where the reduction in flow was to approximately 50% of the control value (fig. 3). If this figure is compared with the control values in the non-occluded animals, then it can be appreciated that the reduction may be to more than 50% of control, because of a possible steal phenomenon in the contralateral hemisphere of animals with occlusion. While this value is higher than that given as the threshold for loss of electrical function in baboons using hydrogen clearance methods;36 it must be emphasized that in our experiments the control levels for CBF in the parietal cortex were 3 times higher than those reported by Astrup et al.34 Our threshold for morphological changes in discreet deep areas of the cortex at flows of just under 50 ml/100 g/min in rats subjected to a thirty minute hypotensive period represented a reduction in flow to one third of the control value. In a recent review,37 most of the studies quoted for determining the threshold for morphological change were from experiments where hydrogen clearance methods were used to determine CBF, and where control values were much lower than in the present study. The absolute levels of flow at which morphological changes appear must therefore be compared with the resting control values in similar brain regions.

We therefore conclude that in the presence of an obstructive lesion of the inflow tract, a reduction in cerebral perfusion pressure can produce focal ischaemia of sufficient severity that ischaemic cell changes appear at a level of systemic arterial pressure that would be tolerated in a normal subject. This supports the clinical evidence that with occlusive or stenotic cerebrovascular disease, haemodynamic mechanisms may be responsible for transient cerebral ischaemia and cerebral infarction in some circumstances.

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