BILATERAL HEMISPHERIC ISCHEMIA IN THE RAT/Kameyama et al


A Model of Selective Experimental Ischaemia in the Primate Thalamus

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SUMMARY A model for studying changes in local CBF and evoked potentials in selective thalamic ischaemia has been developed. The arterial supply to the posterior thalamus (mainly from the posterior choroidal arteries) was occluded in the baboon using a transorbital approach to the region of preoptic and ambient cisterns. Local CBF was measured by the hydrogen clearance method using electrodes introduced into the nucleus ventralis posterior lateralis of thalamus as well as cortex on both sides. The production of focal ischaemia was demonstrated by a significant decrease in thalamic CBF and confirmed by examination of the brain perfused with carbon particles.

DESPITE CONTINUING ADVANCES in cerebrovascular research and increased understanding of brain function under ischaemic conditions, there is still a fundamental need for reliable animal models of focal cerebral ischaemia. The first models were developed to investigate the most common clinical cerebrovascular lesions and used occlusion of the relevant arteries in different animals. Carotid and particularly middle cerebral occlusions made it possible to achieve a better understanding of changes in brain function in response to ischaemia such as development of brain oedema, failure of autoregulation and the threshold of different dysfunctions occurring after cerebral ischaemia. MIDDLE CEREBRAL OCCLUSION IN PARTICULAR SIMULATES A CLINICAL STROKE IN MOST RESPECTS. THE PREDOMINANT INJURY, HOWEVER, IS CortICAL WITH AN ADMIXTURE OF CAUDATE AND PUTAMINAL DAMAGE AND CONSIDERABLE LOSS OF SUBSTANCE IN

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the internal capsule. The middle cerebral model, therefore, shows an admixture of disability resulting from deep and cortical brain ischaemia, and in the chronic circumstance it is difficult to fractionate the degree of disability induced by the various portions of the lesion. The need to concentrate on selected aspects of brain function as, for example, differences in ischaemic sensitivity along the somatosensory pathway, prompted us to attempt the development of a model of hypoperfusion in isolated brain centres.

This study was designed to establish focal unilateral ischaemia in the ventroposterolateral nucleus (VPL) of the thalamus in the primate and demonstrates the feasibility of occluding arteries involved in the posterior thalamic circulation via the transorbital approach.

Methods

Ten baboons (Papio cynocephalus) of either sex weighing between 7 and 10 kg were anaesthetized initially with intramuscular ketamine hydrochloride while the short saphenous vein was catheterized for administration of thiopentone (5 mg/kg) for tracheal intubation and cannulation of the femoral vessels. Anaesthesia was then maintained with alpha chloralose (60 mg/kg iv) with immobilization by gallamine triethiodide (1 mg/kg iv, repeated as necessary). Arterial pCO₂ was maintained in the range of 38–40 torr during electrode placements and 34–36 torr during microsurgical manipulations, controlled by pump ventilation with O₂ at appropriate stroke volume. Blood pH, PaCO₂, hematocrit, systemic arterial pressure and rectal temperature were monitored, with compensation if required. The animal was maintained on a thermostatically controlled heat blanket. The interspinous ligament was removed via a posterior cervical approach at the level of C-II and a silver ball electrode was placed over the dura. Stimulating electrodes were placed over both median nerves so that somatosensory evoked potentials (SEP) could be recorded. The skull was exposed and bilateral central craniectomies were performed. After removal of the dura and with the animal in a stereotactic frame (Narashigi), electrodes for measuring local cerebral blood flow (ICBF) by the hydrogen clearance method and SEP simultaneously were inserted2 into the VPL and medial lemniscus. Within the exposed regions, additional flow electrodes were placed in the cortex and the cortical SEP electrodes also positioned over the area of maximal response at this stage. The closed skull was reconstituted by acrylic cement.

The animal was then turned into a face-up position with the neck extended to use the spontaneous movement of the brain to achieve greater exposure. The head was turned about 15° to the left for a proper view of the prepontine region, and subsequently turned back and slightly to the right while the course of the posterior or cerebral artery was followed along the ambient cistern. A circular incision was made around the bony margins of the orbit and, with dissection back on the orbital walls towards the apex of the muscle cone, the contents of the orbit were excised. We found that the easiest way to control bleeding from both ethmoidal anastomoses and ophthalmic branches was to remove the orbital peristeum completely with dissection and clipping of the ophthalmic artery as close to its origin as possible.1 Leakage from the medial orbital wall was prevented with bone wax. A high speed drill was used to make a craniectomy of 15 mm diameter involving the anterior temporal fossa, the sphenoidal wing and the subfrontal bone. The craniectomy extended to the most medial area of the temporal fossa, the trunk of the middle meningeal artery being coagulated to prevent further insidious bleeding from the dural edges. It was necessary to remove the lateral two thirds of the superior orbital rim to facilitate the approach to the basal arteries. Removal of the optic foramen, except for its medio-inferior corner, together with the anterior clinoid process was also necessary. The dura was easily opened along the Sylvian fissure and removed in the medial two thirds of the exposure, together with the dural sheath of the optic stump, thus giving the widest possible access to the base of the brain. The arachnoid was opened over the middle cerebral trunk. The anterior cerebral artery was identified and the optic nerve was cut flush with it. The optic stump was diminished further by suction in an oblique plane towards the interpeduncular cistern and the oculomotor nerve. After the bony exposure and extended dural excision the internal carotid became mobile enough to permit visualisation back to the basilar artery (fig. 1).

For the necessary permanent retraction of the medial surface of the temporal pole, which covered the tentorial edge and ambient cistern, a new kind of retractor was constructed. Instead of an ordinary metal retractor which would have reduced the already narrow surgical field, a 17-gauge catheter united with an elongated rubber inflatable balloon was used. The temporal pole was initially protected by being covered by the empty balloon (fig. 2A). Very slow inflation of the balloon with 0.6–0.7 cc air then gradually retracted the temporal pole. The catheter was moved out of the field of view and fixed outside the head. The oculomotor nerve was dissected from the dural plica and then cut as close to the posterior cerebral artery, which became visible after the third nerve had been cut and mobilized. Care was taken to avoid the superior cerebellar artery at this stage. The arachnoid covering the posterior cerebral artery was opened after additional retraction of the internal carotid first medially and then laterally as the remnant of the optic nerve was retracted with the sucker. The arachnoid of the interpeduncular cistern was removed and the thalamoperforators from the P1 segment of the posterior cerebral artery were identified.

Previous experience has confirmed that the transorbital approach by itself has no influence on cerebral blood flow or SEP, and control blood flow and SEP were therefore recorded from all electrodes following the transorbital preparation. Occlusion of thalamic vessels was then initiated starting with the posterior communicating artery at its origin from both the internal carotid and the posterior cerebral arteries. The ascending thalamic arteries from the posterior communi-
The posterior cerebral artery was followed by dissection to the posterior communicating complex. In two animals, however, the posterior cerebral artery could not be explored as far as its proximal bifurcation and the posterior communicating complex. In some experiments systemic arterial pressure was lowered by controlled exsanguination via the femoral artery. Electrophysiological and detailed blood flow data, and the relationships of the SEPs to ICBF, will be described in a subsequent paper. The animal was heparinized and the chest opened, the descending aorta clamped, the ascending aorta cannulated via the left ventricle, and the animal perfused with 3000 cc saline followed by formalin-saline (1:9) solution at 120 mm Hg pressure. Perfusion was halted, and about 25 ml of carbon ink-gelatine solution (16 cc carbon ink, 8 cc saline, 800 mg gelatine mixed at 36° C) injected into the ascending aorta at a similar pressure. The brain was removed after two days and, following gross examination, coronal sections were made to demonstrate the changes of thalamic perfusion.

Results

Blood Flow and Technical Considerations

Using the appropriate exposure and microtechniques we found it feasible to occlude sufficient thalamic arteries to achieve selective hypoperfusion in the VPL on the operated side as shown by ICBF recordings, and also to produce a selective infarct in the thalamus on the same side as shown by the sections from carbon perfused brain (fig. 3).

In all cases it was possible to coagulate and cut the posterior communicating complex. In two animals, however, the posterior cerebral artery could not be explored as far as its proximal bifurcation and the posterior choroidal arteries were accordingly left intact. In one animal, additional frontolateral retraction became necessary which resulted in temporary compression of the middle cerebral trunk. In the other 9 animals cortical perfusion was unaffected.

The first ICBF values following coagulation of the vessels were measured 30–40 minutes after the lesion had been completed. In four cases, the reduction in ICBF in the right VPL was not appreciable, and further ICBF measurements were done after the arterial pressure had been lowered by controlled blood removal. Following this, the decrease of the ICBF appeared predominantly in the operated thalamic sites, with other brain areas unaffected. This difference is explained by the relatively impaired autoregulatory capacity on the operated side.

Experiments showing decrease of ICBF in thalamic sites were divided into two groups. The first group comprised those six animals showing immediate evidence of thalamic hypoperfusion. Local CBF data from this group were averaged and displayed in a histogram to compare the right and left sides (fig. 4). In the remaining group, histograms were constructed using ICBF data obtained from ML, VPL, and sensory cortex on the right and left sides, both immediately after occlusion of the thalamic afferent vessels and
after subsequent controlled systemic hypotension (fig. 5).

Relationships between ischemia and electrophysiological changes will be presented in a subsequent paper.

**Anatomical Considerations**

To occlude the afferent ventrolateral thalamic vessels we were able to rely on the thorough descriptions of the thalamic arterial supply available in the literature. In assessing the proportion of anterior choroidal participation in the posterior thalamic circulation in the baboon, we performed the initial experiments using occlusion of the anterior choroidal arteries in addition to the posterior communicating, thalamoperforating and thalamogeniculate arteries. Sections from the carbon-perfused brains revealed a densely ischaemic area involving the internal capsule and basal ganglia, but the ventrolateral nucleus, in which we were primarily interested, was not significantly affected. In the remainder of the series we decided to keep the anterior choroidal artery intact provided the posterior choroidals were entirely occluded.

In gross examinations of the perfused brains, the thalamic afferent vessels and their anastomoses in those brains from the group requiring hypotension to
FIGURE 3. Sections from the brain of one animal after occlusion of right thalamic afferents and carbon-black perfusion. Abbreviations: A: (top left) Posterior to the level of the thalamus there is no sign of hypoperfusion. B: (top right) There is a marked infarct (ischaemic area) corresponding to most of the right thalamus, red nucleus and substantia nigra. Disappearance of the anterior thalamoperforators from the right interpeduncular cistern is clearly demonstrated. C: (bottom left) the level of the basal ganglia shows minimal differences between the two sides. Calibration = 10 mm.

achieve a decrease in CBF were further investigated by dissecting them in the ambient cistern. Connections were found there between the anterior and lateral posterior choroidal arteries as well as between small vessels from the proximal segments of the superior cerebellar artery and medial posterior choroidal arteries. In one case a rather interesting significantly developed anastomosis was observed behind the rich collateral circulation of the posterior thalamus which must have prevented selective VPL hypoperfusion following the occlusion of the afferent vessels alone. In this brain the left-sided medial posterior choroidal artery gave off branches across the midline over the tela choroidea of the third ventricle to the suprapineal segment of the right medial posterior choroidal artery. In these unusual cases, not only were the anastomotic twigs identifiable, but there was also evidence of functioning collateral circulation in these arterial branches, as shown by the presence of carbon particles in the distal segment of the afferent arteries divided after coagulation while the
proximal segments were filled only up to the point of section.

We found some important differences between the baboon and man in the distribution and topography of the posterior cerebral arteries. Our material showed that the posterior choroidal arteries are separated by a greater distance and leave the parent posterior cerebral artery in quite a different plane (fig. 6). The medial posterior choroidal artery takes its origin more proximally, characteristicly opposite the origin of the posterior communicating artery (fig. 6), and takes its course along the peduncle below the posterior cerebral trunk, more caudally than in man, having a long free segment in the ambient cistern hidden between the posterior cerebral and superior cerebellar trunks rather than between the posterior cerebral artery and the peduncle. In the latter case, the medial posterior choroidal artery can be seen directly in the transorbital approach. It seems likely that this topography, characteristic of baboons, is a result of the ratio of hemisphere to brainstem size being less than in humans.
In many cases the posterior choroidal vessels were doubled or even tripled. The picture was further complicated by the presence of small arteries running from the posterior cerebral artery to the peduncle itself. Careful distinction was needed to ensure coagulation of the former with preservation of the latter. All vessels to the thalamus were observed to have long, often tortuous, cisternal course before entering the mid-brain. The small arteries with a short, subarachnoid extraparenchymal course were always left intact.

There were significant variations in the arterial contribution to the brainstem circulation from the anterior choroidal artery. In 2 animals we found the anterior choroidal artery well developed, possibly giving exceptional branches not only to the temporal pole but to the lateral posterior choroidal complex and along the ambient cistern. As we infer from the observation that when the arteries from the peripeduncular posterior cerebral segment were sacrificed, the decrease of ICBF in thalamic sites was minimal. It was clear that in those cases the middle cerebral and the posterior cerebral arteries were less developed and their supply apparently replaced in part by branches from the prominent anterior choroidal artery. Following anterior choroidal coagulation, a reduction of thalamic ICBF could then be achieved.

The oculomotor nerve is much bigger in the baboon relative to adjacent structures than in the human and it fills the space between the tentorial edge and internal carotid. As a result, a large segment of the posterior cerebral artery is covered by the bulk of the third cranial nerve in the transorbital approach, requiring removal of the entire subarachnoidal part of the third nerve (fig. 1).

Discussion

This study demonstrates that in the baboon a satisfactory reduction in perfusion to a local CBF of less than 15 ml/100g/min requires occlusion of the complete functioning posterior thalamic arterial hemi-circle. This arterial system is made up of five separate sources from the posterior cerebral segment, the distribution of these sources being determined by the individual anatomical development of the arteries. The present preparation satisfies the need for a more selective brain infarct in the study of experimental focal cerebral ischaemia which up to now has relied upon techniques such as the production of emboli through the carotid using spheres or cylinders. The disadvantage of such techniques was that the arterial occlusion was only occasional.

Yonas et al. in 1981 reported a model involving occlusion of the lateral lenticulostriate arteries in baboons, in which infarction of the caudate, putamen and the anterior limb of the internal capsule was consistently produced. The model was designed to study focal cerebral ischaemia and to provide evaluation of proposed therapy for cerebral ischaemia. Yoshimoto et al. produced experimental thalamic infarction in dogs by occluding four cerebral arteries (internal carotid, anterior cerebral, middle cerebral, and posterior communicating) for 1–2 hours. They claimed that this method produced thalamic infarction with high frequency, but one may feel the method to be inappropriate for use in baboons. Neither the model of Yonas nor that of Yoshimoto have been combined with blood flow measurements or electrophysiological studies other than EEG recordings.

The transorbital approach, introduced by Hudgkins and Garcia in 1970, has the advantage of reduced brain retraction for manipulation at the base of the brain. Indeed, in using this approach for the purpose originally intended for it, occlusion of the middle cerebral trunk, there is no need for any brain retraction. In the present study, however, internal retraction of the temporal pole was necessary and was achieved using an inflatable balloon; the vessels running in the ambient cistern were thus made accessible for selective coagulation.

Galloway and Greitz described the medial and lateral posterior choroidal arteries in humans as completely different entities without any relationship. This is at variance with our present observations in baboons of significant anastomoses between the two groups of arteries. In the majority of our experiments, however, these anastomoses did not prevent major thalamic hyperperfusion after all other arising vessels had been occluded.

The Arterial Supply to the Thalamus

Abbie stated that perforating vessels from the anterior choroidal artery pass to the upper parts of the red nucleus and substantia nigra, and frequently to the...
ventrolateral nucleus of the thalamus and to the corpus subthalamicum. He described the posterior choroidal system as a complex of three or sometimes as many as five large vessels from the posterior cerebral artery at a variable point, usually anterior to and near the sulcus lateralis of the cerebral peduncle. He noted that the condition of the posterior choroidal arteries in the ape conforms fairly closely to that in man. Giliain described the thalamic arterial supply in the primate, showing the great importance of the perforating and thalamogeniculate branches. The posterior choroidal arteries are described as having at least two rami, a medial and a lateral, usually arising separately from the posterior cerebral artery. She found that the lateral posterior choroidal artery arose from the proximal posterior cerebral, the medial posterior choroidal originating more posteriorly. She also described conspicuous anastomoses between the two posterior choroidal arteries in the vicinity of the foramen of Monro.

According to Plets, the posterior half of the nucleus lateralis thalami obtains its arterial supply from the anterior thalamoperforating vessels, and from thalamogeniculate and choroidal arteries of the lateral ventricle (e.g. from the lateral posterior and anterior choroidal vessels). He also mentioned one premamillary artery derived from the posterior communicating artery running to the ventrolateral nucleus. Margolis, Newton and Hoyt described an anterior and a posterior group of thalamic branches from the posterior cerebral artery. The anterior group consisted of short perforators from the posterior communicating artery. Again, they divided the posterior thalamic group into two groups. One is the complex of interpeduncular thalamoperforating vessels including small arteries from the P-1 segment penetrating the thalamus via the paramedian portions of the posterior perforated substance. The other group of posterior branches of thalamic affere nts is made up from three to five thalamogeniculate perforating branches originating from the ambient segment of the posterior cerebral artery. As a further arterial supply of the thalamus, they mentioned the anterior choroidal artery which has branches that may continue through the peduncle to reach the substantia nigra, upper parts of the red nucleus, and a portion of the ventral anterior and ventrolateral nuclei.

In Zeal and Rhoton's description, thalamic branches originate as anterior thalamoperforators from the posterior communicating artery, and as posterior thalamoperforators from the P-1 segment entering the midbrain through the posterior perforated substance and its vicinity, arising from the posterior and superior aspect of P-1. Up to seven thalamogeniculate arteries also arise directly from P-2 beneath the lateral thalamus, and as branches of the medial posterior choroidal artery arising from either the postero medial aspect of the proximal P-2 or one of its branches and encircling the midbrain. Lastly, the lateral posterior choroidal is mentioned as having thalamic affere nts and arising from P-2 or its cortical branches.

Galloway and Greitz in 26 human autopsies found the medial choroidal artery to originate from the posterior cerebral artery within the interpeduncular or crural cisterns and to run parallel to that artery closely adherent to the peduncle. They described two lateral choroidal arteries in most of the cases, the larger (more posterior) one originating from the posterior cerebral artery within the ambient cistern with twigs to the ventral and lateral thalamus.

There are several references to anastomoses between the thalamic affere nts vessels such as communication between the two choroidal systems in the surface of the choroid plexus. Interesting connections between these thalamic affere nts have been reported, with occasional lack of some branches. In the majority of cases arteries from the cortical branches of the posterior cerebral artery can take over underdeveloped medial posterior choroidal branches.

A high specificity is attributed to the affere nts arteries by some authors. Each of these vessels is described as supplying only a selective portion of the thalamic nuclei and functions virtually as an end-artery. However, our present observations on those brains in which there was a small decrease in blood flow after partial coagulation of thalamic arteries in the ambient and interpeduncular cisterns suggest a lack of such specificity of thalamoperforating and thalamogeniculate arteries. In addition, the presence of numerous anastomoses between the circumflex and choroidal arteries in both directions at a higher level (closer to the thalamus) made it impossible to select successfully between the various vessels and their origins in attempting to achieve a specific regional infarct within the thalamus, for instance in the VPL.

On the basis of these observations we consider that while the posterior communicating and the doubled posterior choroidal systems are very important to the posterior thalamic supply, the contribution of the anterior choroidal branches joining the posterior choroidals in the choroidal tissue is also considerable. To explore and occlude the thalamic branches of the anterior choroidal near the lateral geniculate is difficult and, if the choroidal trunk had been occluded more anteriorly during the experiments, extended lesions in the anterior or basal ganglia would have been expected.

In the present series, the measurement of decreases in local flow in the explored thalamic sites together with that of relatively stable local CBF in the brainstem (medial lemniscus) and cortex on both sides has demonstrated that a proper series of coagulations of the ascending thalamic arteries from the peripeduncular posterior cerebral segment can result in selective hypoperfusion in the unilateral posterior and ventral thalamus.

In such cases, there was an immediate disappearance of the somatosensory evoked response in the homolateral sensory cortex after the vessels had been occluded, despite continued normal cortical flow. This thalamic infarct model thus appears suitable for studying the electrophysiological function of the thalamus and its involvement in the generation of somatosensory evoked potentials.
Actinomycin D Suppresses The Protective Effect Of Dexamethasone In Rats Affected By Global Cerebral Ischemia

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SUMMARY Simultaneous occlusion of both common carotid arteries in female Sprague-Dawley CFY rats produced characteristic symptoms of global cerebral ischemia, such as staggering, circling, convulsions, followed by coma and death. A close correlation existed among these symptoms and the elevation of water and Na⁺ content, appearing at the stage of staggering; Evans blue extravasation and diminution of K⁺ content, detected at circling; and the increase in Ca²⁺ content in the total brain tissue, manifesting itself at the phase of convulsions, indicating the development of cerebral edema due to ischemia. Dexamethasone given subcutaneously in a single 2.0 mg kg⁻¹ dose 5 hours prior to the induction of global cerebral ischemia reduced considerably the morbidity and mortality, the alterations in water and electrolyte content, and albumin leakage in the brain tissue. Actinomycin D, in a dose of 0.5 mg kg⁻¹ injected intravenously 1 hour before steroid treatment, abolished the beneficial effect. This finding suggests that de novo protein synthesis is involved in the cerebroprotective effect of dexamethasone.

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SEVERAL CLINICAL TRIALS HAVE shown that glucocorticoids exert a beneficial effect in patients with cerebral ischemia and brain injuries. On the other hand, Bauer and Telliez have described opposite results. Brain disorders of other types than trauma, (e.g. cerebral malaria) do not provide any evidence for the benefit of steroid treatment. Experimental results obtained in different animals with various types of brain injuries have supported the assumption that steroid treatment evokes a cerebroprotective effect. Local freezing-induced brain edema has been found to be suppressed by cortisone in cats but not by dexamethasone in rats and Rhesus monkeys. Regional reduction in blood supply causing cerebral ischemia has been shown to be alleviated by dexamethasone treatment. In contrast, some other data with experimental animals have indicated the ineffectiveness of glucocorticoids in prevention of cerebral ischemia. It appears, therefore, from the data available that the therapeutic value of steroids in the treatment of cerebral ischemia and trauma still remains a subject of debate. Various types of deleterious events, such as ischemia and hypoxia, disturbing O₂ supply and glucose transport in the brain, lead to characteristic morphological changes, modifications in the function of...
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