Blood Recirculation and Pharmacological Responsiveness of the Cerebral Vasculature Following Prolonged Ischemia of Cat Brain

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SUMMARY Normothermic adult cats were submitted to 30 min complete cerebral ischemia by arterial inflow occlusion, and the brains subsequently recirculated with blood for 4 hr. Cortical blood flow was recorded with a heated thermocouple, cortical oxygen pressure with a multiwire oxygen electrode, and the pial arterial tone by photomicroscopy using an implanted glass window. In addition, blood pressure, blood flow through the innominate artery and the electrocorticogram were continuously monitored.

During ischemia cortical oxygen pressure fell from 44 ± 5.8 to 0 torr (means ± S.E.), cortical heat conductance decreased from 15.1 ± 0.8 to 10.2 ± 0.5 × 10^{-4} cal × cm^{-1} × sec^{-1} × °C^{-1}, and the pial arteries constricted to less than 50% of their initial diameter. Recirculation of the ischemic brain initiated reactive hyperemia (heat conductance 23.6 ± 3.6 × 10^{-4} cal × cm^{-1} × sec^{-1} × °C^{-1}) which lasted for about 45 min. and which was accompanied by an increase in arterial P_{O_2} to 74 ± 14 torr, and a dilatation of the pial arteries by about 50%. Hyperemia was followed by postischemic hypoperfusion during which cortical P_{O_2} returned to, or slightly below, normal (34 ± 4 torr), cortical heat conductance decreased to 13.9 ± 0.4 × 10^{-4} cal × cm^{-1} × sec^{-1} × °C^{-1}, and pial arteries constricted by about 10%.

The pharmacological responsiveness of the pial vasculature was tested before ischemia and during postischemic hypoperfusion by intra-arterial administration of alpha- and beta-adrenergic stimulating and blocking agents, the antiserotoninergic agent metergolin, and papaverine. The autonomic drugs and metergolin induced only minor changes in cortical blood flow or oxygenation but there was distinct vasodilatation and an increase in blood flow with papaverin both before and after ischemia. It is concluded that treatment of postischemic hypoperfusion is more efficient with drugs which cause direct relaxation of the vascular smooth muscle than with those which act on the autonomic receptor sites.

REGULATION OF cerebral blood flow is a complex mechanism adjusted to yield a flow rate which precisely matches the metabolic requirements of the brain. Following a period of cerebral ischemia, this mechanism is severely disturbed. Immediately after ischemia, blood flow is increased (postischemic hyperemia) although brain metabolism initially is considerably reduced. After longer recirculation times, when the electroencephalogram begins to recover, blood flow gradually decreases despite the rise in the metabolic requirements of the brain (postischemic hypoperfusion syndrome). This situation may provoke a misrelationship between oxygen consumption and oxygen availability to the brain, and bears the risk of delayed metabolic disturbances.

Postischemic reactive hyperemia has been studied extensively before and appears to be the consequence of both a loss of cerebrovascular autoregulation and of CO_{2} responsiveness, resulting in total paralysis of the cerebral vasculature. The pathomechanism of the postischemic hypoperfusion syndrome is less clear. Factors which may be involved, are vasospasms, changes in blood viscosity, brain swelling, or functional disturbances such as the dissociation between an already recovered autoregulation and the still severed CO_{2} reactivity.

Numerous attempts have been made to improve cerebral blood flow pharmacologically after ischemia (for review see Refs. 9, 10), but there is considerable disagreement about the efficiency of such treatment. Some of the controversy may be due to the route of application because many of the drugs used have a more pronounced effect on the extracerebral than on the cerebrovascular smooth muscle which is protected by the blood-brain barrier. Systemic application, therefore, may provoke steep effects of the blood from the brain to extracerebral tissues. Another point of uncertainty is the fact that the permeability properties of the blood-brain barrier, as well as the responsiveness of the cerebral...
vasculature, may change after ischemia, and that the results obtained in healthy animals may not be applicable to a post-ischemic situation. Finally, increase in cerebral blood flow following ischemia may not necessarily be related to an improvement of cerebral function. It is conceivable that a rise in blood flow might be non-nutritive, and therefore without beneficial effect on brain metabolism. On the other hand, a higher oxygen extraction would be of importance even in the presence of an unchanged flow rate.

We have tried to clarify some of these questions by using an experimental setup which allowed us to obtain information on both extra- and intracerebral flow, on the tonus of the pial vasculature, and on the relationship between blood flow and cerebral oxygenation. With this approach spontaneous and pharmacologically induced changes in cerebral vascular circulation and oxygenation were investigated before and after a period of complete cerebral ischemia. It appeared that after ischemia the pharmacological responsiveness of the pial vasculature was similar to that of the intact animal; however, the induced changes were very small when compared to those occurring spontaneously after ischemia, indicating that pharmacological treatment of postischemic hypoperfusion is still far from satisfactory.

Methods

Adult cats of either sex were anesthetized by a single intraperitoneal injection of 30 mg/kg pentobarbital (Nembutal), immobilized with gallamine triethiodide (Flaxedil), and mechanically ventilated with room air. Body temperature, acid-base state and blood gases were maintained within normal limits throughout the experiments. Small polyethylene tubes were inserted into a femoral vein and a femoral artery for blood pressure recording, blood-sampling and intravenous infusions. Another catheter was placed into the innominate artery via the right subclavian artery for the application of the test substances. In the cat, the innominate artery gives rise to both carotid and the right subclavian artery; when the tip of the catheter is placed into this vessel, the test substances reach both hemispheres of the brain.

Craniotomies were made over the frontal and parietal cortex on both sides and, after removal of the dura, used for the implantation of a glass window, for electrophysiological recording, and for the placement of a thermocouple and an oxygen electrode (see below). Thoracotomy was performed on the left side, the aortic arch was exposed, and an electromagnetic flow probe (Statham Instruments) was placed around the innominate artery for continuous measurement of blood flow in this vessel.

Cortical blood flow was continuously monitored with a heated thermocouple placed on the exposed pia mater cortex.\textsuperscript{11} Heat conductance was calculated by

\[ \lambda = \frac{K \times I^4}{\delta} \text{cal} \times \text{cm}^{-1} \times \text{sec}^{-1} \times \text{degC}^{-1} \]

where \( I \) is the heating current, \( \delta \) is the temperature difference between the heated and non-heated thermojunction, and \( K \) is an instrument constant. \( K \) was determined experimentally using gelatine. \( \lambda = 12.5 \times 10^4 \) cal \( \times \text{cm}^{-1} \times \text{sec}^{-1} \times \text{degC}^{-1} \) as a standard.

The reactivity of the pial vasculature was monitored by taking photographs through the glass window with an operating microscope (Zeiss). Changes in the diameter of small pial arteries (50–250 \( \mu \)m) were measured on the micrographs, and expressed as percent of the control values. The cortical oxygen tension was determined using a combination electrode which consisted of eight small platinum wires (15 \( \mu \)m diameters) covered by a 12 \( \mu \)t f teflon membrane.\textsuperscript{12} The platinum wires were connected in parallel giving an average oxygen pressure for the 8 measuring sites.

The functional state of the brain was monitored by recording the pyramidal response to the electrical stimulation of the motor cortex, as has been described in detail before.\textsuperscript{13} In addition, the electrocorticogram was recorded continuously using silver ball electrodes placed bilaterally over the frontal areas.

Complete ischemia of the total brain was produced by intrathoracic clamping of the innominate and left subclavian arteries for 30 min. Collateral flow to the brain was interrupted by permanent ligation of the internal mammary arteries, and by lowering the blood pressure to below 80 mm Hg. The completeness of ischemia was ascertained by injecting \textsuperscript{133}Xenon into the innominate artery just prior to the clamping, and recording the radioactivity of the head with external scintillation detectors. Ischemia was considered complete only when radioactivity did not decrease by more than 10% during the whole length of ischemia.

Recirculation of the brain after ischemia was initiated by raising systolic blood pressure to more than 180 mm Hg with a sympathicomimetic drug (norfenefrin, Novadral, Goedecke, Berlin), and releasing the clamps on the peak of the pressure rise. Blood serum electrolytes and the acid-base state were adjusted to normal and kept within physiological limits until the end of the experiment.

The responsiveness of the cerebral vascular system to various vaso-active drugs was tested before and 2–3 hours after ischemia by infusing the respective compounds at constant speed into the innominate artery. For this purpose the drugs were dissolved in Ringer's solution and applied at a rate of 1 ml/min using a constant speed infusion pump. In every animal 3 drugs were tested, the order of which was changed systematically. Successive tests were performed after all recordings had returned to normal, but never earlier than 30 min after the preceding one. At the end of the experiments, the animals were killed by an intravenous injection of pentobarbital. The brains were removed and inspected for lesions at the recording sites, which, when present, led to the rejection of the experiment. Statistical differences were calculated using Student's \( t \)-test.

Materials

The following drugs were used for investigation of cerebral vascular reactivity:

- Norepinephrine (Arterenol): 2.5 \( \mu \)g/kg/min; phentolamine (Regitin): 250 \( \mu \)g/kg/min; propranolol (Dociton): 25 \( \mu \)g/kg/min; orciprenaline (Alupent): 12.5 \( \mu \)g/kg/min; papaverin (Boehringer, Mannheim): 625 \( \mu \)g/kg/min; metergolin (Liserdol): 125 \( \mu \)g/kg/min. (Liserdol was kindly supplied by Farmitalia, Milano).
Results

Reactive Hyperemia and Postischemic Hypoperfusion

Control recordings prior to ischemia were as follows: mean arterial blood pressure during and after 30 min complete ischemia of the cat brain. Ischemia was produced by arterial inflow occlusion and in-

- Cortical Po\(_2\) was 44 ± 5.8 mm Hg, cortical heat conductance, which is a function of cortical blood flow, was 15.1 ± 0.8 × 10\(^{-4}\) cal × cm\(^{-1}\) × sec\(^{-1}\) × °C\(^{-1}\), and the blood flow in the innominate artery was 41.4 ± 2.9 ml/min, and mean arterial blood pressure was 138.6 ± 6.1 mm Hg. When cerebral blood flow was interrupted by clamping the innominate and subclavian arteries, cortical Po\(_2\) decreased to 0 mm Hg within 40 sec, heat conductance decreased to 10.2 ± 0.5 × 10\(^{-4}\) cal × cm\(^{-1}\) × sec\(^{-1}\) × °C\(^{-1}\), and the pial arteries constricted to less than 50% of their initial diameter (fig. 1).

Releasing the clamps after 30 min of ischemia provoked considerable reactive hyperemia. Innominate flow rose to 113 ± 12 ml/min, heat conductance increased to 23.6 ± 3.6 × 10\(^{-4}\) cal × cm\(^{-1}\) × sec\(^{-1}\) × °C\(^{-1}\), and cortical Po\(_2\) to 74 ± 14 mm Hg. The pial arteries dilated by about 50% of the control level, accompanied by a reddening of the blood in the pial veins. The maximum of the changes related to hyperemia reached a peak within 5 min with the exception of cortical Po\(_2\) which attained its maximum after 20 min. The latter was due to the fact that in most animals respiratory distress developed after ischemia. Treating pulmonary atelectasis by hyperinflating the lungs, sometimes dramatically improved oxygen tension of the arterial blood and, in parallel, of the cerebral cortex.

Normalization of the various parameters following reactive hyperemia occurred in the following way: heat conductance returned to normal after about 45 min, pial arteries constricted after 1 hour, and cortical Po\(_2\) normalized after 1.5 hours. The latter was closely correlated with the return of the EEG which generally began to recover after the same time period. Hyperemia was followed by a phase of hypoemia which lasted for about 4 hours (fig. 1). During this time the diameter of the pial arteries was about 10% below normal, heat conductance decreased to 13.9 ± 0.4 × 10\(^{-4}\) cal × cm\(^{-1}\) × sec\(^{-1}\) × °C\(^{-1}\) and cortical oxygen pressure to 34 ± 4 mm Hg. Hypoemia was not related to cardiac insufficiency because the flow in the innominate artery, which gives rise to both carotid arteries, was increased throughout this period.

Pharmacological Treatment

Six drugs were tested before ischemia and during the phase of postischemic hypoperfusion: One alpha- and beta-adrenergic stimulating and blocking agent, respectively, papaverin and a compound which has pronounced anti-serotonergic effects. The dose of the drugs was adjusted to yield a slight but significant peripheral effect, either on systemic blood pressure or on the flow in the innominate artery. The mean values of the changes observed are demonstrated in figures 2 and 3.

Norepinephrine, an alpha-adrenergic stimulating agent with little influence on beta-receptors, induced changes which can be best explained by its systemic effects. Before ischemia, blood pressure rise was accompanied by pial arterial constriction, indicating an autoregulatory adjustment of the vasculature. Autoregulation was not fully efficient, however, because cortical blood flow and oxygen pressure slightly increased. After ischemia, pial arterial tone remained constant, but changes in cortical flow and oxygenation did not exceed those of the intact animals, and remained far below the values which occurred spontaneously during postischemic reactive hyperemia.

Phentolamine, a specific alpha-adrenergic blocking agent,
led to a decrease in systemic blood pressure and a reduction in cortical blood flow both prior to and after ischemia. Innominate flow, on the other hand, distinctly increased. This indicates that phentolamine had a vasodilatoratory effect predominantly on the extracerebral vasculature, whereas the cortical blood flow passively followed the blood pressure changes.

Orciprenalin, a beta-adrenergic stimulating agent, and propranolol, a beta-blocker, did not have a consistent effect on the pial vasculature, nor on cortical flow or oxygen tension. In some animals flow was pressure-dependent, but in others a dissociation between blood pressure and flow was observed.

Metergolin, a specific antisertotoninergic agent, slightly increased blood flow but did not influence the pial arterial tone or cortical oxygenation before or after ischemia.

Papaverin, in contrast, had a distinct vasodilatating effect, particularly during the state of postsischemic hypoperfusion. Vasodilatation was accompanied by an increase in cortical flow, whereas cortical Po₂ remained almost constant. This suggests that, unlike norepinephrine, the increased flow rate led to an increased oxygen uptake by the brain.

During drug application the electrocorticogram was recorded in order to assess a possible effect on the electrophysiological state of the brain. In none of the drugs could consistent changes be observed, even in those cases in which there was an increase in blood flow. It is, therefore, unlikely that with the present form of application any of the test substances substantially improved the functional performance of the brain following complete cerebral ischemia.

Discussion

The present investigation substantiates the earlier conclusion that complete cerebral ischemia of as long as 30 min is followed by reactive hyperemia, provided blood pressure is high enough to re-initiate blood circulation. Hyperemia apparently is a consequence of cerebral vasoparalysis as
reflected by the marked vasodilatation of the pial arteries. There is good evidence that vasoparalysis is due to lactacidosis of the brain which, during ischemia, builds up as a consequence of anaerobic metabolism. Vasoconstriction of pial arteries during ischemia in the present experiment does not contradict this conclusion because interruption of arterial blood supply leads to a loss of intraluminal pressure.

During hyperemia, the peak values of cortical blood flow and pial vasodilatation were reached almost at the same time, i.e. about 5 min after initiation of flow. Cortical PO$_2$, however, attained its maximum after 30 min when cortical blood flow had already returned to normal. Cortical oxygen pressure depends on 3 factors: oxygen content of the arterial blood, cortical blood flow and cortical oxygen consumption. It has been demonstrated before that postischemic oxygen consumption is lowest on the peak of reactive hyperemia. The fact that during this time cortical PO$_2$ was relatively low, must be explained by a reduced oxygen content of the arterial blood. The reason for this was postischemic respiratory insufficiency which is known to occur after prolonged ischemia, and which was also observed in the present study.

The time which elapsed after ischemia until normalization occurred, was quite different for the various recorded parameters. Heat conductance of the cortex normalized after about 45 min which is in line with earlier measurements of blood flow using the intraarterial $^{183}$Xenon injection method. Normalization of the diameter of pial arteries was delayed until one hour which may be explained by the fact that up to this time there was brain swelling, causing an increase in cerebrovascular resistance. Cortical oxygen pressure returned to normal after about 1.5 hours, i.e. at a time when spontaneous EEG activity began to reappear. This coincidence apparently is related to cortical oxygen consumption which increases in parallel with the recovery of EEG.

Reactive hyperemia was followed by a phase of reduced blood flow which lasted for about 4 hours. A similar finding has been reported by Nemoto et al. who referred to this state as the "postischemic hypoperfusion syndrome." Postischemic reduction in flow has been related to an increased intracranial pressure, venous congestion, vasospasms, or changes in blood viscosity. Our own results suggest that it is mainly due to an increased vascular tone because pial
arteries were constricted rather than dilated as one would expect in all the other conditions. It is reasonable to assume that vasoconstriction is due either to an increased sympathichotonicus or to the accumulation of vasoconstricting agents such as serotonin which reportedly increases in concentration during ischemia. However, the systemic application of the alpha-adrenergic blocking agent phentolamine or of the specific antiserotoninergic agent metergolin did not release the increased vasotonus although doses were used which had a distinct peripheral effect. This is in contrast to the topical application of alpha-blockers which both in the intact and the ischemic animal led to marked vasodilatation of the pial arteries and hyperemia of the cortical surface.

The other autonomic drugs, norepinephrine, orciprenalin and propranolol, had little or inconsistent effects on pial tone, cortical blood flow or oxygenation, particularly when compared to the much greater changes which occurred spontaneously after ischemia. Earlier studies in intact animals have also yielded conflicting results (for review see Ref. 9) depending on the dose, route of administration and the individual blood pressure response. This is not at variance with pharmacological evidence of a certain sympathetic control of the pial vasculature but rather indicates that the drug did not reach the alpha- or beta-adrenergic receptors of the vascular smooth muscle in sufficiently high doses to produce a pharmacological effect. In the present series of experiments the pial responsiveness to these drugs did not change during the phase of postischemic hyperperfusion but remained as low as in the intact animals. Consequently, there is no indication at the present that changes in the permeability properties of the blood-brain barrier might render the cerebral vasculature more accessible to circulating autonomic drugs than in the normal state.

With papaverin the situation was somewhat different. Papaverin causes relaxation of the vascular smooth muscle and apparently passes the blood-brain barrier in sufficiently high concentrations to evoke a pharmacological effect. In intact animals a short-lasting increase in cerebral blood flow and cortical oxygen tension has been observed following intravenous or intracarotid administration. In humans a similar effect has been described both in normal and in ischemic brain tissue. The present study demonstrates that the responsiveness of the pial vasculature to papaverin is preserved after ischemia, and that, in fact, an amelioration of postischemic hyperperfusion can be achieved. The recording of the EEG during papaverin infusion did not reveal an immediate improvement of ischemic functional capacitance of the brain. This may be due either to the relatively small increase in blood flow or to the fact that the drug was applied too briefly. However, at present, papaverin or papaverin-like substances which cause direct relaxation of the smooth muscle, seem to be the most promising candidates for a successful treatment of post-ischemic hypoperfusion, and further research is needed.

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