Cerebral Blood Flow Immediately Following Brief Circulatory Stasis

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SUMMARY  Cerebral blood flow was studied in rabbits immediately following complete circulatory stasis of varying duration. Systemic arterial pressure was measured continuously. The postischemic circulation was examined both by an infusion of carbon black and, in separate experiments, by injection of "C-antipyrine into the blood. We examined the relationship between the duration of stasis, the postischemic arterial pressure, and the amount of cerebral reperfusion.

As stasis increased from 5 to 30 min the pressure required to achieve reperfusion of the entire brain rose from 20 to 100 torr. Following even temporary exposure to arterial pressures above 110 torr all areas of the brain were generally reperfused. Blood flow in reperfused brain varied directly with arterial pressure, indicating failure of autoregulation. At normal (preischemic) arterial pressure, postischemic cortical flow was twice the normal rate.

The data indicate that the pressure required to initiate flow in ischemic brain increases as the duration of stasis is lengthened and that once flow occurs there will be a significant hyperperfusion unless systemic arterial pressure is lowered to the low normal or hypotensive range.

SOME STUDIES of the cerebral microcirculation immediately following ischemia have consistently demonstrated localized or confluent areas in which perfusion failed to resume ("no reflow"). Other studies have shown either no immediate impairment of reperfusion or hyperperfusion. Factors which promote failure of the resumption of flow in the brain are the occurrence of stasis of blood during the ischemia and low arterial pressure. The precise relationship between "no reflow" and arterial pressure has not been delineated because in most earlier experiments the pressure was either not determined or it was indirectly inferred from the pressure at which perfusate was delivered from a reservoir.

The study reported here had 3 objectives. The first was to obtain data on the relationship between arterial pressure and the resumption of flow in ischemic brain. The second was to examine cerebral reperfusion under more physiological circumstances than those occurring in a carbon black infusion, by injecting "C-antipyrine into the blood of a resuscitated animal. The third was to use the "C-antipyrine to quantitate blood flow once circulation had been reestablished in ischemic brain.

Methods

Ischemic Model

New Zealand White rabbits weighing 1.5 to 2.5 kg were anesthetized with pentobarbital (30 mg/kg), paralyzed with succinylcholine (0.1 mg/kg), ventilated by a mechanical respirator and anticoagulated with sodium heparin U.S.P. (500 units/kg). Both femoral arteries and one femoral vein were catheterized for arterial pressure monitoring, arterial blood sampling and intravenous drug administration. Sternotomy and pericardiotomy were performed to expose the heart and great vessels. Global ischemia was achieved by cross clamping the entire pedicle of the heart: the aorta, pulmonary artery, superior and inferior vena cava and pulmonary veins.

Carbon Infusion Experiments to Detect Areas of Non-reperfusion

After instituting circulatory stasis, an 18-gauge Teflon catheter (Cathlon IV Catheter, Jelco Lab, Raritan, NJ) was inserted in a retrograde direction into the descending thoracic aorta above an occluding ligature and was connected to a pressure transducer. A filtered suspension of carbon black biological ink (as provided by the manufacturer John Henschel Co., 141 Albertson Avenue, Albertson, NY) was introduced without bubbles into the delivery system consisting of an adjustable reservoir connected by ½" plastic tubing to a tapered piece of ¼" glass tubing. The glass tubing was inserted through the apex of the left ventricle into the proximal ascending aorta. The forelegs and ears were ligated to restrict the vasculature being perfused largely to the head, and the jugular veins were opened. After the specified period of ischemia the carbon suspension was infused into the proximal aorta for 1.5 min. The height of the carbon black reservoir was varied from experiment to experiment to provide intra-aortic pressures from 22 to 110 torr as recorded by the transducer. At the end of the infusion, the brain was removed from the calvarium, fixed in 10% formalin, cut coronally into 200 µ thick sections, dehydrated, cleared and mounted on slides. Using a grid of points and a dissecting microscope, 6 standard coronal sections of the brain were assessed for completeness of infusion of the carbon into the microvasculature. Each section of brain was scored by...
counting as "no reflow" points the points falling on or touching areas of the capillary vasculature not filled with carbon, and counting as "reflow" points the points falling completely within a filled capillary bed. By combining the scores for all sections of the brain the percentage of reflow or "no reflow" was estimated for each animal.

Quantitative Blood Flow Studies Using the Diffusible Tracer Technique to Detect Graded Abnormalities of Flow

After 15 min of circulatory arrest animals were resuscitated by an intravenous injection of 0.05 to 0.1 cc of 1:1,000 (1 mg/ml) epinephrine and direct cardiac massage. Spontaneous cardiac action was continuously monitored. Once the heart was restarted, the pressure sometimes rose gradually to the level at which cerebral flow was to be studied and sometimes made an abrupt rise, often to well above the preischemic level of 88 to 103 torr, and then gradually fell to the pressure at which flow was studied. The determination of cerebral blood flow was begun 1-8 min after resuscitation by rapidly injecting 250 μCi 14C-antipyrine dissolved in 0.3 cc of saline into the exposed pulmonary artery. Simultaneously, constant rate arterial sampling was begun from the right femoral-iliac arterial catheter using an infusion-withdrawal pump. Twelve seconds later the pedicle of the heart was reclamped and the pump was stopped. Within 4-7 min after restopping the circulation the brain was removed from the calvarium, cut into coronal sections with razor blades, mounted on cardboard squares with double-edged Scotch tape, covered with carbon, and counting as "reflow" points the areas where carbon was present completely within a filled capillary bed. Twenty sections later the pedicle of the heart was reclamped and the pump was stopped. Within 4-7 min after restopping the circulation the brain was removed from the calvarium, cut into coronal sections with razor blades, mounted on cardboard squares with Tissue Tek II (O.C.T. Compound, Lab Tek Products, Miles Lab. Div., Naperville, IL) and immediately immersed in a beaker of 2-methylbutane (Eastman Kodak Co., Rochester, NY) suspended over liquid nitrogen in a thermos. Autoradiographs were prepared from the coronal sections of the brain and small regions were excised, weighed and assayed for 14C activity together with the arterial sample. The rationale and calculations used for determining local cerebral blood flow by this technique are described in detail elsewhere.

Assay of Blood Samples

Arterial blood samples were centrifuged at 1500 rpm for 30-45 min in a Sorvall GLC-2 centrifuge. Fifty μl of supernatant were pipetted into scintillation vials containing 10 ml Instagel scintillation cocktail (Packard Instrument Co., Downers Grove, IL) and counted in a Beckman LS-800 scintillation counter. The concentration of 14C-antipyrine in serum was counted in a Beckman LS-800 scintillation counter.

Assay of Brain Samples

Areas of brain were cut from frozen coronal slices in a cold room (0°C), placed in weighing vials, and brought to room temperature for weighing. The samples were then homogenized in 0.5 ml of HC104 at 0°C, centrifuged at 1500 rpm for 30-45 min, and the supernatant counted in the same fashion as the serum. Corrections were made for mild color quenching.

Autoradiography

Twenty μ sections were cut from the coronal brain slices in a Jung cryotome (−20° to −25°C), picked up on cold coverslips and dried on a warm plate (heat tolerable to finger touch). Drying did not result in significant loss of detail as judged by comparison with our own autoradiographs and with those in the literature prepared at constant, below freezing temperatures. The coverslips were attached to sheets of cardboard with double-edged Scotch tape, covered with Saran wrap, and exposed for 10-20 days in an x-ray cassette on SB-5 Medical X-ray film blue sensitive for photofluorography (Safelight — 6B filter, 7/2 watt bulb), and developed.

Results

Carbon Infusion Experiments to Detect Areas of Non-reperfusion

Following 5 min of circulatory arrest, 95% of the microvasculature filled with carbon even when the aortic pressure was reduced to 22 torr. After 15 min of circulatory arrest only 30% of the microvasculature filled with carbon when the pressure was 37 torr, but there was almost complete filling when the infusion pressure was increased to 66 torr. After 20 min of stasis, an infusion pressure of 66 torr resulted in only 50% filling of the microvasculature. The greatest increase in difficulty reperfusing the ischemic brain appeared between 10 and 15 min of stasis. These results demonstrate an inverse relationship between aortic pressure and the incompleteness of reperfusion, as well as a progressive difficulty in reperfusing the microcirculation as the period of circulatory stasis is lengthened (fig. 1).

Experiments Using 14C-Antipyrine to Measure Blood Flow During Reperfusion

In 13 rabbits that had been subjected to 15 min of circulatory arrest, cerebral blood flow was measured at different arterial pressures and within 8 min of restoration of cardiac function. Autoradiography in 4 animals showed gross areas of brain unmarked by 14C-antipyrine, and in 2 of these blood flow measurements were successfully completed. The frontal cortical flows of 0.46 and 0.11 ml/g/min were well below those of the other animals (fig. 2). These 4 animals had relatively low arterial pressures following the ischemia: in 3, the 14C-antipyrine was injected with the pressure at 44 torr after peaks of 59, 74, and 103 torr; in the fourth, the antipyrine was injected at 66 torr.
PERCENT OF BRAIN REPERFUSED

FIGURE 1. Reflow as a function of duration of stasis and perfusion pressure. The percent of the brain's microvasculature that filled with carbon has been plotted against the arterial pressure during the carbon infusion. Duration of stasis varied from 5 to 30 min, as indicated by numbers at beginning of curves. Values are means ± SE; number of animals in parenthesis.

following a peak of 118 torr, but the period of circulatory stasis had been prolonged to 22 min because of difficulty resuscitating the animal.

Nine of the 13 animals studied with antipyrine had relatively high arterial pressures in the post ischemic period; 8 had peaks of 110 to > 147 torr before flow was measured at 44 to 96 torr, and the ninth had a peak of 88 torr with flow measured at 74 torr. None of these animals showed definite regions of failed uptake by autoradiography and all had flow rates equal to or greater than the non-ischemic control animals, measured at comparable pressures (fig. 2).

In the non-ischemic control animals, frontal cortical blood flow was constant over a wide range of pressure (fig. 2), falling only from 0.59 ml/g/min at the normal arterial pressure (96 ± 5 SE torr) to 0.55 ml/g/min with moderately severe hypotension (42 ± 2 torr). In animals resuscitated after 15 min of circulatory arrest, however, there was a direct relationship between blood pressure and cortical flow throughout the same range of pressure. In hypotensive (44 torr) animals, the immediate postischemic blood flow was near the level for non-ischemic hypotensive animals. In normotensive (96 torr) animals, postischemic blood flow was more than twice the level for non-ischemic animals with the same pressure (cf. fig. 2).

Discussion

The carbon infusion studies showed, as previously reported, that there is difficulty reinstituting flow following a period of stasis. Perfusion at pressures that gave complete blackening of normal brain left regions of failed reperfusion in the postischemic brains. The volume of brain involved varied directly with the length of the stasis and inversely with the pressure of the reperfusion. As shown in figure 1, after stasis of only 5 min, reperfusion was virtually complete at a pressure of only 22 torr. But, when there was
stasis for 10 min, reperfusion was incomplete at pressures up to 50 torr; with stasis for 15 min, reperfusion was incomplete at pressures up to 70 torr; and, with stasis for 30 min, reperfusion was incomplete at pressures up to 103 torr.

These results are similar to those previously reported (e.g. 11) except that the pressure range over which the difficulty with reperfusion was manifested is lower than previously thought. It appears on the basis of the present data that, if stasis is followed by low-normal or hypotensive pressures, there may be difficulty in reinitiating flow. But, if stasis is followed by high-normal or hypertensive pressures, reperfusion will occur in all areas of the brain.

The use of a carbon suspension to assess reperfusion depends on the assumption that the limiting event is the displacement of the animal's own blood from the microvasculature and that the hydrodynamic characteristics of the carbon suspension are therefore not of critical importance. This may be a reasonable assumption with respect to looking at factors concerned with restarting flow, but not with respect to examining the nature of flow once started. It was primarily for the latter purpose that we studied animals resuscitated after a period of global circulatory arrest, using $^{14}$C-antipyrine to measure cerebral blood flow. In 4 of the antipyrene experiments, postischemic pressures fell within the range in which there is difficulty in starting flow. Autoradiographs of these brains showed regions of failed reperfusion that were similar in appearance but somewhat more extensive than those revealed by carbon infusions at comparable combinations of duration of stasis and perfusion pressure. This indicates that the difficulties in reflow revealed by the carbon infusions are also demonstrable in a more physiological situation and that factors such as pulsatile flow are not of critical importance.

It was originally proposed that failure of reflow following stasis was due to swelling of perivascular glia with capillary narrowing. However, subsequent morphological studies using precautions to insure prompt fixation showed a relatively small amount of glial swelling and luminal narrowing. In vitro studies by Merrill have shown that stasis causes a reversible increase in blood viscosity; and experiments in rabbits have shown that moderate hemodilution with saline, prior to the stasis, greatly facilitates the reinitiation of flow. The present study clearly indicates that the resistance to restarting flow is a graded one that can be completely broken through at higher perfusion pressures (fig. 1). These several findings taken together point strongly to increased blood viscosity as an important cause of the difficulty in initiating flow. The finding that it becomes progressively more difficult to initiate flow, as stasis is extended from 5 to 20 min (fig. 1), is quite consistent with this mechanism; the red cell aggregation that causes the increased viscosity is a function of the third power of the hematocrit, and progressive local increases in hematocrit are to be expected as red cells sediment in dependent portions of the vasculature.

The ischemia-induced loss of autoregulation, as demonstrated by these experiments (fig. 2) and those of others (vide infra), may be an important secondary factor in the etiology of the no-reflow phenomenon and may explain its patchy distribution. If some portions of the ischemic vasculature are more susceptible to the difficulty in reinitiating flow, they will then be subjected to a generalized "steal effect" by the surrounding areas of ischemic brain where the moving blood, now with normal viscosity, courses through vessels lacking in autoregulatory function and offering less than normal overall resistance.

In the animals resuscitated after 15 min of global ischemia and in which postischemic pressures rose into the high normal or hypertensive range, $^{14}$C-antipyrine radioautography did not reveal regions of impaired reperfusion. The flow rates, calculated from antipyrine uptake, were normal when measured at subsequently low arterial pressure and twice normal when measured at normal pressures.

Loss of autoregulation following ischemia is a well-established phenomenon. How extensively the mechanism is damaged by various ischemic insults has not been defined. In humans hyperemia may be observed for up to 5 days following cardiac arrest and for several weeks after both middle cerebral artery occlusion and stroke without vascular occlusion. As noted by Nemoto, this secondary fall in blood flow does not represent recovery of autoregulation, as data suggest the cerebral blood vessels are still in a state of vasospasm. Postischemic hyperemia has been noted as a transient phenomenon in several experimental models of global ischemia and is generally followed by hypoperfusion. As noted by Nemoto, this secondary fall in blood flow does not represent recovery of autoregulation, as data suggest the cerebral blood vessels are still in a state of vasospasm. There is some evidence that progressive edema may contribute to secondary hypoperfusion. It is conceivable that postischemic hyperemia due to loss of autoregulation may play a role in this pathophysiological sequence. Optimal management of an ischemic episode due to temporary stasis may include a brief period of elevated pressure to insure reflow in all vessels, followed by protection of the brain against excessive perfusion during the period of lost autoregulation.

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