Measurement of Maximal Permissible Cerebral Ischemia and a Study of Its Pharmacologic Prolongation*

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Hypothermia has been used effectively to protect tissues from irreversible damage caused by circulatory arrest, whether the latter is of accidental occurrence or induced in the course of an operative procedure. Relatively little attention, however, has been given to the possibility of providing such protection by chemical means. Chemical protection against ischemia might be expected to be more easily and quickly induced than hypothermia and to be free of some of the undesirable cardiovascular side effects of the hypothermic state. Three classes of potential protective agents can be envisaged: (1) agents designed to maintain the patency of the vasculature during the ischemia in order to insure complete perfusion of the tissue following its termination; (2) inhibitors of cellular activity that would lower the metabolic demands of the tissue during the ischemia; (3) physiological compounds, added in excess of their normal concentrations, to forestall the first irreversible changes in the cells. Beneficial effects from pretreating ischemic kidneys with heparin24 and studies demonstrating extension of the period of permissible cardiac standstill with compounds producing cardioplegia21,23,25,37 have suggested the feasibility of the first two approaches. Surprisingly little systematic study has been devoted to the third possibility.

Controlled study of cerebral ischemia and of factors modifying its effects has been hampered by the unavailability of suitable experimental preparations. Production of temporary but total arrest of circulation to the brain is difficult to achieve in commonly available laboratory animals because of the large vertebral-anterior spinal artery axis and the abundant muscular collateral vessels which communicate with the carotid system. Previously described methods have had the undesirable features of damage to the spinal cord, impairment of circulation to other organs, or problems of recovery from thoracotomy.

In the experiments described below, a relatively simple operative technique has been developed and tested for producing temporary cerebral ischemia in cats. This technique has been used to determine possible effects of several substances on the period of ischemia that can be reversibly sustained. The substances tested included two barbiturates, ethyl alcohol and solutes added to the blood to increase its osmolarity.

Methods

Technique for Producing Temporary Cerebral Ischemia. The procedure developed involved occlusion, by means of an initial operative technique, of all arterial inflow to the brain except that provided by the common carotid arteries. The animal then was allowed to recover. At the time of the experiment it was anesthetized again, this time very lightly, and both common carotid arteries were exposed and occluded temporarily with rubber-shod clamps.

In preliminary studies to identify the collateral circulation to the cat's brain, Micropaque* barium sulfate was injected into the left auricle with the basilar and both common carotid arteries occluded. The chief collaterals were found to be: (a) the superior thyroid arteries which anastomose with branches of the inferior thyroid; and (b) branches of a large muscular artery which arise laterally from the common carotid near the level of the superior thyroid artery and communicate with divisions of the thyrocervical trunk and ex-

* Made by Damancy and Co., Ltd., Ware, Herts., England. The size of the particle is between 0.5 and 3μ.
ternal carotid arteries (Fig. 1). The ramifications of these muscular vessels are largely in the posterior cervical region. The relative paucity of these arterial collaterals in the cat led to its selection, rather than the dog, for these studies.

At a first-stage procedure, using sterile precautions, the basilar artery, the superior thyroids and the ascending and descending branches of the large muscular arteries were occluded (Fig. 2). The technique for occlusion of the basilar artery was similar to that used in dogs by White and Donald. A longitudinal incision, approximately 4 cm. in length, was made just to the right of the thyroid cartilage and trachea. The fascial plane between trachea and carotid sheath was developed by blunt dissection; and, after placing a 15 gauge needle in the distal portion of the trachea, a self-retaining retractor was inserted to displace the trachea and esophagus to the left. This metal cannula was used to prevent suffocation from the necessarily forceful retraction of the trachea. The longus capitis muscle was detached from its origin at the base of the skull to expose the ventral rim of the foramen magnum. The dura mater was separated gently from the foramen magnum with a dental spatula, and a craniectomy was performed extending rostrally approximately 8 by 12 mm. between the tympanic bullae. The dura mater was opened in a cruciate manner with careful avoidance of the circular sinus just inferior to the foramen magnum. A segment of basilar artery free of branches was selected caudal to the posterior inferior cerebellar arteries (which in the cat arise from the basilar instead of the vertebral arteries). This was mobilized gently with fine forceps and occluded with a metal clip (Fig. 3). A small piece of gelatin foam was placed over the dural opening and the wounds were closed with interrupted sutures of 4-0 black silk.

In this preparation no bone is removed from the the convexity of the skull, and since the tentorium of cats is bony it seems unlikely that the small amount of bone removed over the ventral aspect of the medulla provided any significant decompression for the brain. It was not possible, however, to obtain a watertight closure of the small incision in the meninges at the site of craniectomy, so some drainage of cerebrospinal fluid into the soft tissues of the neck presumably occurred.

Each animal received penicillin, 300,000 units, by intramuscular injection at this initial procedure. The mortality for this first-stage operation was approximately 10 per cent. Anisocoria was frequent in the surviving animals, which otherwise seemed intact neurologically.

![Fig. 1. Angiogram of head and neck of cat made by injecting Micropaque into the left auricle after the basilar artery and both common carotid arteries had been occluded. Note that the contrast agent fills the distal carotid circulation by entering chiefly through the large muscular branch.](image1)

![Fig. 2. Sites of arterial occlusion in the cat whereby the brain is made dependent for its blood supply on both common carotid arteries. CCA=common carotid artery. MB= muscular branches. STA= superior thyroid artery. ICA= internal carotid artery. PA= pharyngeal artery. AB= anastomotic branch. IMA= internal maxillary artery. ACA= anterior cerebral artery. MCA= middle cerebral artery. PCA= posterior cerebral artery. PICA= posterior inferior cerebellar artery. VA= vertebral artery. ASA= anterior spinal artery.](image2)
The experimental procedure was performed 1 to 3 days later, after the animals were awake and ambulatory. They were anesthetized with intraperitoneal chloralose (40–50 mg./kg.), chosen because it has been shown to have no effect on metabolism of cerebral oxygen and glucose and because, at this dosage, the animals were very lightly anesthetized and, as a rule, had brisk corneal reflexes. A transverse incision was made low in the anterior cervical region and both common carotid arteries were exposed. The tracheal cannula was replaced, and, after the substances being evaluated had been administered by the appropriate route, both common carotid arteries were occluded for the desired period with rubber-shod occluding clamps. Respiratory arrest ensued immediately, but respirations were maintained artificially with a simple mechanical respirator using room air. After release of the common carotid arteries artificial respiration was continued until spontaneous respiratory movements resumed or until myocardial contractions ceased. During the period of unconsciousness an animal was given daily intraperitoneal injections of 30 ml./kg. body weight of 2.5 per cent dextrose in 0.45 per cent saline solution.

The surviving animals were examined to assess their neurological status and then were sacrificed. All animals were subjected to careful postmortem study. The occluding metal clip and silk ligatures were inspected. Brains were removed, fixed in 10 per cent formalin solution and sectioned. In all animals, 4 ml. of a 2 per cent solution of trypan blue were injected intravenously prior to the carotid occlusion. Sections were examined for evidence of breakdown of the blood-brain barrier as manifested by uptake of the dye.

In 16 animals, the completeness of vascular occlusion to the brain was tested by injecting Micropaque or Dionosil* into the left atrium of the heart while the carotid occluding clamps were in place. After cardiac arrest caused by occlusion of the coronary arteries with particles of barium, the brains were removed, fixed in formalin, and then radiographed.

Administration of Test Substances. The barbiturates were administered immediately prior to the period of ischemia by intracarotid injection, with the dose divided equally between the two arteries. Because these compounds cross the blood-brain barrier rapidly, it was expected that this route would provide increased brain levels. Both pentobarbital and Pentothal are soluble only in alkaline solution, and other experiments in this laboratory have shown alkaline solutions to be toxic when injected rapidly into the carotid arteries. For this reason, the injections were performed slowly over a period of 2–3 min. Sodium pentobarbital was administered as a solution containing 6 mg./ml. at a pH slightly above 8.5 and in a volume sufficient to provide a dose of 12 mg./kg. of body weight. The Sodium Pentothal was injected at a concentration of 10 mg./ml. in a solution of pH slightly above 10, to give a total dose of 10 mg./kg. of body weight. Because of the higher pH needed to maintain solubility of the Sodium Pentothal, the relative effective dose which could be administered via this route was appreciably less than with pentobarbital.

The ethyl alcohol was administered by intraperitoneal injection of 10–15 per cent solution 20–45 min. before producing cerebral ischemia. The dose used was 3 mg./kg. of body weight which has been shown to provide a blood level of greater than 250 mg. per cent for more than 2 hrs. and to result in a deep stupor lasting for 3–5 hrs.

Urea and mannitol were used to test the effect of increased osmolarity. The urea was given intravenously 10 min. before occlusion as a 30 per cent solution in invert sugar,* for a total of 50 mm./kg. of body weight. The mannitol was administered as 1 molar solution in a volume of 2 ml./kg. of body weight; equal portions of this were injected distally into the carotid arteries just after application of the temporary occluding clamps. Thus most of the blood was displaced with the hypertonic solution of mannitol during the period of ischemia.


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* Made by Glaxo Laboratories, Greenford, Middlesex, England; the contrast agent in Dionosil is propyliodone. Most particles are between 3 and 15μ in diameter, around 10 per cent are between 15 and 30μ, and 5 per cent are larger—up to 100μ.
Measurement of Cerebral Arteriovenous Differences for O₂ and CO₂. Before and immediately after intracarotid injection of sodium pentobarbital, as described above, a series of blood samples were obtained from the transverse sinus and compared with regard to the O₂ and CO₂ content with samples taken simultaneously from the femoral artery. The samples of the transverse sinus were obtained with a 22 gauge polyethylene catheter introduced via the internal jugular vein.

Results

Evidence of Completeness of Occlusion. Within a few seconds after application of the occluding clamps respirations ceased, an observation consistent with the findings of others, and the cats became unresponsive to noxious stimuli, and pupillary dilation and a rigid extensor posture developed. Examination of the optic fundi during occlusion revealed an extreme blanching of the retinal vessels. In a few cats, the cerebral cortex was exposed while the carotid clamps were in place; and, though the systemic blood pressure was well maintained, the brains were found to be pale and there was no bleeding when cortical vessels were incised. Of the 5 animals injected with Dionosil while the carotid arteries were occluded, none showed filling of the vessels of the brain on roentgen-ray examination. Only 3 of the 13 cats that were studied similarly with Micropaque showed radiographic evidence that it had entered the cerebral circulation, and, in each instance, there was only a small amount present. Two animals, found at autopsy to have incomplete occlusion of the basilar artery, were excluded from the study.

Survival of Controls. The data for the number of cats that survived as compared with those that succumbed following varying periods of cerebral ischemia are presented in Table 1, both for the control group and for the groups that had previously received one of the test compounds. For the control group, the critical period of ischemia in terms of survival fell between 5 and 7½ min. The surviving animals appeared normal except for possible visual impairment in a few instances. No attempt was made to evaluate their intelligence.

Effect of Sodium Pentobarbital on Arteriovenous O₂ and CO₂ Differences. Arteriovenous O₂ and CO₂ differences were measured before, and at intervals after, the intracarotid injection of 12 mg./kg. of sodium pentobarbital. The results for the 2 animals thus tested were similar and have been averaged and plotted in Fig. 4. Both the O₂ and CO₂ differences fell to less than 50 per cent of control values 5 to 10 min. after injection of the barbiturate but had returned nearly to normal by 13 min.

Effect of Sodium Pentobarbital and Sodium Pentothal on Survival. Sodium pentobarbital (12 mg./kg.), administered by intracarotid injection, just before the occlusion, approximately doubled the tolerable period of ischemia (Table 1). One of the animals that survived 12.5 min. of cerebral arterial occlusion was left with a moderate left hemiparesis, but the other surviving animals appeared
normal. Because of the characteristics of its solubility, the relative effective dose (10 mg./kg.) of the Sodium Pentothal was considerably less than that of the pentobarbital. Nevertheless, it appeared to prolong significantly the permissible period of ischemia (Table 1).

Effect of Ethyl Alcohol on Survival. In the small series of cats in which this was tested, the period of permissible ischemia did not differ significantly from that of the controls (Table 1).

Effect of Urea and Mannitol on Survival. In 6 cats, the intravenous injection of urea (3 gm./kg. of body weight) 10 min. before occluding the carotid may have provided some protection (Table 1). The survival of the 6 cats in which hypertonic mannitol was injected into the carotid arteries distal to the occluding clamps did not differ from that of the controls (Table 1).

Staining with Trypan Blue. Staining of the brain with trypan blue did not occur when the duration of ischemia was less than 10 min. About one fifth of the brains made ischemic for 10 min. or more showed some degree of staining with the incidence increasing as the duration of ischemia increased. The data are insufficient to permit any correlations to be made between staining and death or recovery or between staining and the type of therapy tried.

Discussion

The almost immediate reaction to ischemia of the central nervous system and its lack of ability to recover therefrom distinguish it from other tissues. Circulatory arrest produces a number of concurrent and progressive changes in the milieu of the cells, e.g. fall in O₂ and rise in CO₂ tension, fall in pH, fall in glucose, rise in metabolic end products, etc. Which of these are responsible for the limiting events that underlie loss of function and the development of an irreversible change is not well known. Other studies in our laboratories have suggested that anoxia is primarily responsible for loss of function with ischemia whereas deprivation of glucose seems to play a critical role in production of the irreversible lesion. Little is known presently of the cellular changes which first determine the point of irreversibility, and surprisingly little systematic study has been devoted to this problem. In the absence of more knowledge, it is difficult to predict to what extent the period of permissible ischemia might be extended by proper maneuvers, but there is some indication that it may not be as immutable as believed heretofore.

The paucity of experimental work on this important problem may be ascribable in part to the difficulty in achieving temporary cerebral ischemia in laboratory animals. A number of techniques have been described. Kabat and Dennis ligated the vertebral arteries of dogs, performed a laminectomy of the C2 vertebra, and later inflated a pressure cuff around the neck of the awake animal to occlude the remaining arterial supply to the brain. Sufficient damage to the cord resulted from the pressure cuff to make neurological evaluation of survivors difficult. Grant et al. and Weinberger et al. produced ischemia in cats by temporarily occluding the pulmonary artery. Negovski and Crile and Dolley rapidly exsanguinated dogs by arterial cannulae and then quickly replaced the blood after varying intervals. Wolfe produced temporary
ventricular fibrillation electrically and then revived the animals by manual pumping of the heart followed by electrical defibrillation. These latter 4 methods have the disadvantage of impairing circulation to vital organs other than the brain.

The method described in this paper would appear to have a number of advantages: (1) the final experimental occlusion can be performed by means of a simple operative procedure so that the postischemic period is not complicated by recovery from a major operation; (2) it can be performed under light anesthesia; (3) the rest of the body is circulated normally during the period of occlusion; (4) the spinal cord is not damaged. The above data indicate that a high degree of ischemia was achieved by this technique and that animals subjected to the procedure under controlled conditions gave reproducible responses.

The response of the control cats to varying periods of ischemia is consistent with the published results obtained by using the various methods cited above. Quite at variance, both with these results and our own, however, are the findings of Neely and Youmans who recently have reported recovery by dogs from periods of ischemia lasting up to 25 min. Their experiments differ both from ours and those cited above in that the ischemia was produced by temporary elevation of cerebrospinal-fluid pressure to 400 mm. Hg which resulted in almost complete exsanguination of the brain.

The reduction in cerebral arteriovenous O₂ and CO₂ differences following intracarotid pentobarbital cannot be interpreted unequivocally in terms of O₂ consumption and CO₂ production in the absence of data on blood flow. However, the response observed is highly suggestive of a reduction in cerebral metabolism. For it to have been caused by a change in blood flow, there would have to have been a twofold increase in flow following injection of the barbiturate. This seems unlikely in view of the results of Kety et al. who found no appreciable change in cerebral blood flow in humans after administration of barbiturates. Depression of cerebral metabolism by barbiturates has been demonstrated by a number of investigators using different techniques. Feitelberg and Pick found that the difference in temperature between warmer cat brain and carotid blood decreased in deep pentobarbital narcosis. Several laboratories have found barbiturate narcosis to decrease utilization of brain oxygen and glucose, whether studied in intact animals, in isolated or perfused brains or in brain slices.

Acute intoxication with ethyl alcohol appears to have less effect on cerebral metabolism. In human studies, Goldfarb et al. and Loman and Myerson found a small and inconstant reduction in utilization of cerebral oxygen and no significant effect on uptake of glucose. In vitro studies of Sutherland et al. have shown ethanol to have no effect on uptake of glucose or oxygen of human cerebral cortex. Battey et al. have shown, however, that utilization of O₂ and glucose is depressed somewhat in deep alcoholic coma.

The effect of the barbiturates and ethanol on the period of cerebral vascular occlusion which could be reversibly sustained as observed in the present studies, parallels in a general way the effect of these agents on cerebral metabolism. Although the mechanisms involved remain obscure, it may be postulated that uncompensated catabolic processes occurring during ischemia lead, after a certain interval, to irreversible cell damage. This interval may be extended by agents that reduce the metabolism of tissue.

Cerebral edema is known to result from cerebral ischemia and might be expected to contribute to the sequence of events leading to irreversible damage, particularly if it were to interfere with recirculation of the brain following release of the arterial occlusion. In the few previous studies of this possibility, the results have been equivocal. Kaupp et al. found that intravenous urea did not reduce significantly the mortality or morbidity of monkeys or dogs in which cerebral ischemia was produced by occlusion of the vena cavae and the brachiocephalic and left subclavian arteries. Young and Javid, however, found intravenous urea somewhat...
beneficial in dogs made ischemic by occlusion of the venae cavae and aorta. The unusually long survival times reported by Neely and Youmans may be consistent with the hypothesis that cerebral edema contributes to the irreversible changes consequent to ischemia. The almost completely exsanguinated brains of their dogs would have had no opportunity to become edematous by uptake of fluid from the vascular compartment.

The few experiments reported above with intravenous urea and intracarotid mannitol were obviously of a preliminary nature. Although the urea may have been of some benefit, the mannitol appears not to have been. However, the unphysiological nature of the mannitol solution, which was injected distal to the carotid clamps, may have counteracted any beneficial effect of its increased osmolarity.

It might be anticipated that, if agents acting in different ways could be found to extend the period of permissible ischemia, their effects could be additive. The problem would appear to be of sufficient importance to warrant further study.

Summary

A method has been described for producing temporary cerebral ischemia of a high degree in cats, without thoracotomy, impairment of circulation to other organs, or damage to the spinal cord. The maximum period of ischemia which could be reversibly sustained was between 5 and 7½ min. The period of permissible ischemia was approximately doubled by an intracarotid injection of sodium pentobarbital given just prior to the arterial occlusion. A protective effect was also found from intracarotid Sodium Pentothal and, in a few animals, with hypertonic urea administered intravenously. Coma induced with ethanol provided no apparent benefit, nor did hypertonic mannitol administered by intracarotid injection.

References


