Complete Cerebral Ischemia*

An Ultrastructural Study

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Summary. Neuronal, astrocytic, and oligodendrocytic elements in several brain loci of the cat were examined at the light and electron microscopic level immediately after periods of complete cerebral ischemia (CCI) uncomplicated by post-ischemic recirculation. Such CCI episodes ranged from 1.5–25 min duration and were methodically produced in a cat model employing rigorous physiological controls. Subsequent to these CCI insults, morphological alterations occurred in a homogeneous manner within each cell type of all loci examined; however, variation in the temporal onset and magnitude of alterations among the various cell types was observed. With brief ischemic insults all cell nuclei demonstrated pronounced nuclear alterations, while their cytoplasmic organelles displayed minimal change. Chromatin clumping and nucleolar condensation were observed in both neurons and glia subsequent to 1.5–5 min of CCI, respectively. With increasing durations of CCI such changes were more dramatic and conspicuous alterations of the cytoplasmic organelles were observed. On the basis of extensive morphological analyses the present study illustrates that nuclear alterations are the first to occur subsequent to CCI. The homogeneity of neuronal involvement seen subsequent to CCI uncomplicated by post-ischemic recirculation is inconsistent with the “selective vulnerability” purported to occur by others. The significance of this inconsistency remains to be assessed; yet, the suggestion is advanced that post-ischemic recirculation may be a factor in the genesis of such vulnerability.

Key words: Complete cerebral ischemia — Post-ischemic recirculation — Electron microscopy — Nuclear perturbations

Of all conditions which restrict blood flow to the brain, none is more controversial than complete cerebral ischemia (CCI). Experimental data from model systems mimicking this important clinical problem have resulted in major discrepancies (Plum, 1973) concerning the following issues: (1) the duration of CCI which results in irreversible brain injury, (2) the vulnerability of the various cell types of the brain to CCI (direct neural vs. glial vs. vascular injury), and (3) the pattern of neuronal injury after CCI (“selective vulnerability” vs. random heterogeneous or homogeneous neuronal responses). The threshold of irreversible brain injury, long believed to occur after 5–10 min of CCI (Brierley, 1973; Brierley et al., 1973), has been challenged (Arsenio-Nunes et al., 1973; Hossmann and Kleihues, 1973; Hossmann and Otsson, 1970). Hossmann has reported the recovery of structural integrity and the return of some neural functions after CCI of 60-min duration, if the occurrence of secondary vascular hypoperfusion was avoided. In contrast to the traditional view that neurons are primarily vulnerable to ischemic/hypoxic brain injury (Brierley, 1973; Brierley et al., 1973; Levy et al., 1975), some investigators have identified various vascular abnormalities as important limiting factors in the tolerance of the brain to ischemic episodes (Ames et al., 1968; Fisher, 1973; Nemato et al., 1975). Recent ultrastructural investigations have demonstrated that both neurons and glia manifest comparable, qualitative structural alterations subsequent to CCI (Garcia et al., 1975; Kalimo et al., 1977). Additionally, these neuronal alterations were observed to be homogeneous after CCI without post-ischemic recirculation. Such a finding is of interest in that it is at variance with the patterns of “selective vulnerability” of neuronal involvement classically reported after cardiac arrest or CCI (Scholz, 1949, 1963; Schneider, 1963; Brierley, 1973; Brierley et al., 1973). In view of such existing controversies surrounding the pathogenesis of CCI, the need for further studies

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aimed at resolving these issues is evident. Well controlled and systematic light and electron microscopic examinations of reversible CCI are well suited for addressing such issues and to this end we have utilized a recently developed model of reversible CCI which allows for a systematic examination of the morphopathologic substrates of CCI (Vries et al., 1976). This model produces in cat a pure square wave insult of quantifiable duration which can electively be followed by periods of post-insult recirculation at controlled blood pressure levels. Additionally, the model allows for excellent and consistent perfusion fixation of the brain for morphological analyses. The present investigation reports those structural alterations induced by CCI uncomplicated by post-ischemic recirculation. An enhanced understanding of those structural perturbations observed following the primary ischemic insult should provide an excellent basis for subsequent studies exploring how various periods of reperfusion may modify such structural changes and to what degree they are reversible.

Materials and Methods

Model System

In this investigation 30 male and female adult cats weighing 2.5–3.5 kg were utilized. All animals were initially anesthetized with i.v. Methohexitol Sodium (Brevital, 10 mg/kg) at which time the right femoral artery and vein and trachea were cannulated for measurement of systemic arterial blood pressure, infusion of various drugs and fluids, and mechanical ventilation. The animals were paralyzed with Gallamine Triethiodide (Flaxedil, 5 mg/kg) and respiration was controlled with a small animal respirator at a tidal volume of 10 cc/kg of body weight. The respiratory rate was adjusted to yield an end tidal CO₂ of between 4 and 4.5 % and all animals were ventilated on a 70 % O₂, 30 % N₂O mixture. In all subsequent surgical procedures the N₂O anaesthesia was supplemented with a 2 % Xylocaine solution as a local analgesic. Screws for recording both right and left hemispheric EEG were inserted into the right and left parietal bones over the region of the suprasylvian gyri and a reference screw was placed in the frontal bone rostral to the coronal suture. Care was taken not to pierce the dura or contuse the underlying brain. A complete spinalectomy was performed at the C₃–C₄ level to isolate the spinal arteries from the vertebrobasilar arterial system. Concomitant with the spinalectomy an increase in systemic arterial blood pressure (mean rise of 100 torr) was observed and persisted for approximately 5–10 min. Subsequent to the postspinalectomy blood pressure elevation, the systemic arterial blood pressure was maintained within normal limits by the continuous intravenous infusion of norepinephrine (Levophed).

A midline thoracotomy was then performed and the following arterial trunks and arteries were ligated to prevent collateral circulation to the brain: (1) left subclavian artery, (2) right and left internal thoracic arteries, (3) right and left costocervical arteries, (4) right and left thyrocervical arteries, (5) left vertebral artery, (6) left brachial artery, and (7) right and left upper six anterior and posterior intercostal arteries. Additionally, the right brachial artery was exposed and a cannula was passed into the right subclavian artery. The cannula was secured by a ligature just distal to the origin of the right vertebral artery. After the successful completion of the previous procedures, blood flow could reach the brain only via the brachiocephalic arterial trunk. The arterial blood pressure within this system was monitored via the right brachial arterial cannula and reflected not only anterograde pressure from the aortic arch but any retrograde pressure transmitted from the common carotid arteries and right vertebral artery.

Induction and Verification of Insult

After the surgical preparation, induction of CCI was accomplished by occluding the brachiocephalic arterial trunk at its origin from the aortic arch. Based upon the duration of the ischemic insult, two separate procedures for occluding the brachiocephalic artery were employed. In animals subjected to 5 or more min of CCI the brachiocephalic artery was occluded with a special clamp which could be released to end the ischemic period. In contrast, the brachiocephalic artery was occluded with a silk ligature in the animals receiving a 1.5 min insult of CCI. The physiological events which accompanied the onset of CCI were as follows: (1) the blood pressure monitored from the right brachial cannula fell immediately to zero upon clamping or ligating the brachiocephalic arterial trunk; (2) the right and left hemispheric EEG became isoelectric within 11 to 12.5 s following the onset of brachiocephalic occlusion; (3) both pupils became fully dilated within 30–60 s of the occlusion period (Fig. 1). Inclusion in this study, all experimental animals were required to fulfill the following criteria throughout each experiment: (1) arterial pH values of 7.34–7.44; (2) arterial HCO₃⁻ values of 14–24 mEq/l; (3) arterial pO₂ values above 90 torr; (4) arterial pCO₂ values of 29–42 torr; (5) body temperature of 36–38°C; (6) mean systemic blood pressure levels of 100 torr or more.

Verification of a complete cerebral ischemic insult with this preparation was determined in a pilot study of ten animals. In these animals the systemic arterial blood pressure was raised to 250 torr at the time of brachiocephalic clamping by infusing intravenous norepinephrine. Evans blue was then injected into the systemic circulation and after a 5 min period each animal was killed and examined. The macroscopic examination of all ten animals failed to reveal any evidence of the dye within the cerebrovascular system above the C₃ spinal segment. Additionally, in each of these animals the right brachial arterial blood pressure consistently remained at zero throughout the period of brachiocephalic occlusion despite systemic blood pressure values of 250 torr. In another pilot series of five animals various aspects of the collateral ligation procedure were omitted. In these animals both subclavian arteries and the brachiocephalic artery were occluded; however, either some cephalic vessels were left patent or a spinalectomy was not performed. When the systemic arterial blood pressure was maintained at a normal level in such animals, the brachial arterial pressure persisted as high as 70 torr throughout the period of brachiocephalic occlusion and dye was readily detected in all parts of the cerebral circulation. Thus, from such studies it was concluded that a zero brachial arterial blood pressure served as an accurate index of a complete cerebral ischemic insult.

Perfusion Fixation Procedures and Methods of Morphological Analysis

A different perfusion protocol was employed with each of the two methods of brachiocephalic occlusion. Animals subjected to 5 or more min of CCI were perfused transcardially. Saline perfusion was initiated immediately upon clamp release and the perfusion pressure and duration of the saline wash were monitored by the right brachial cannula (Fig. 1). These values ranged from 50–95 torr and 1.6–3 min, respectively, in all such animals. When the returning perfusate from the right atrium appeared clear of blood, the saline was discontinued and the perfusion of 4 % paraformaldehyde and 0.5 %