ELECTRON MICROSCOPIC OBSERVATIONS OF THE EFFECTS OF DEXAMETHASONE AND MANNITOL IN THE HYPOXIC RAT BRAIN (PART-II)

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SUMMARY :

In this part of our study, the neuronal changes of the cerebellum in hypoxic insult and on those alterations the effects of mannitol and dexamethasone (alone and in combination) were studied by electron microscopy and it was concluded that dexamethasone alone was much more effective than mannitol or its combination with mannitol in reducing cytotoxic edema and protecting the neuronal integrity.

KEY WORKS :

Brain edema, dexamethasone, electron microscopy, hypoxia mannitol, rat.

INTRODUCTION

In this second part, the effects of hypoxia in the cerebellum of the same rats used in the first part of study (7), with the effects of MANNITOL, DEXAMETHASONE (alone and in combination) on those hypoxic alterations were studied by electron microscopy (EM) and the compared results of these two agents are reported with the pertinent literature.

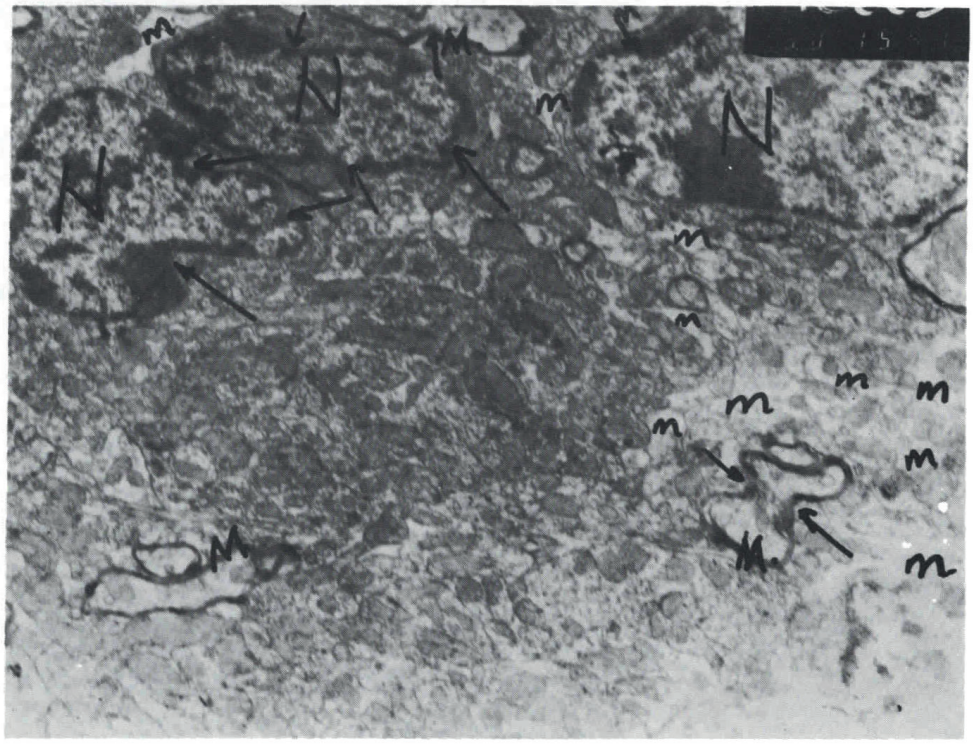
MATERIALS AND METHODS

In the first part of the study, the rats used in the experiment were divided into CONTROL-hypoxic/ischemic/reprefused-and TREATED-mannitol treated/dexamethasone treated/mannitol-dexamethasone treated-groups (7). Also the rats in that study were exposed to hemispheric ischemia by ligating the left common carotid artery for different time intervals which was followed by hypoxia at 320 torr in a low pressure camera, in aiming to induce the ischemic effect of the carotid ligation. As cerebellar tissue is fed by posterior circulation, it should have been affected only by hypoxia during the experiment (7), to which it is known to be most sensitive (1, 5). Therefore in this part of the study, only cerebellar tissue was taken into consideration for EM study in aiming to compare the effects of MANNITOL and DEXAMETHASONE (alone and in combination) on hypoxic brain insults. In the same manner, the cerebellar samples taken from rats in control and treated groups of the experiment (7) were employed as CONTROL (A) and TREATED (B) groups of this part of study respectively, having used three samples in each. (Details of the procedure applied in the experiment can be obtained from the first part).

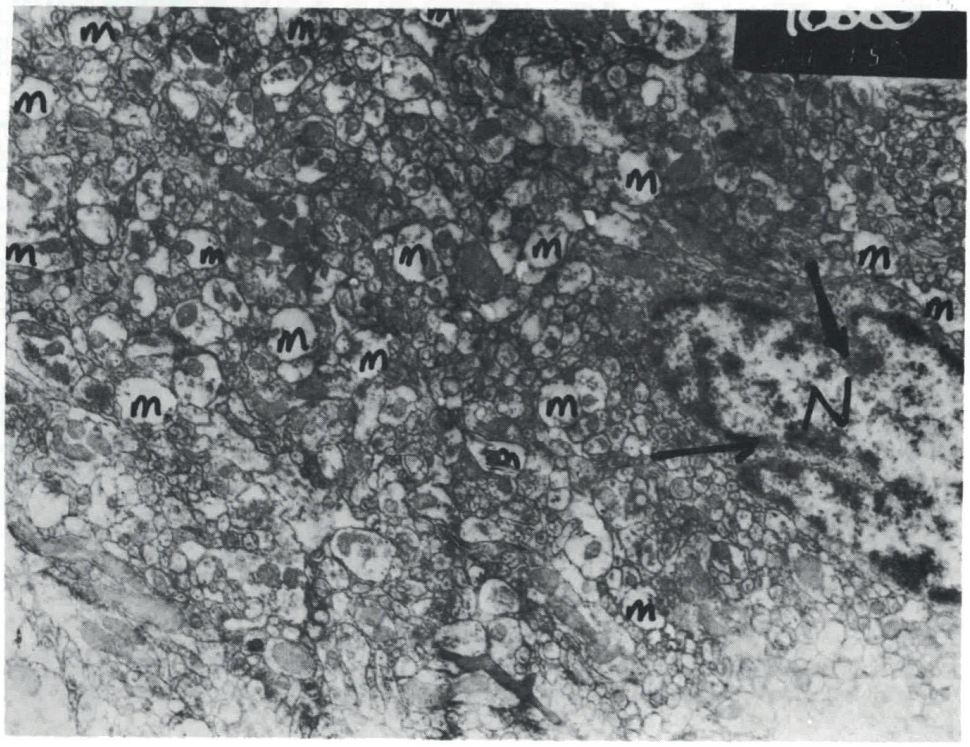
All samples were fixed as soon as possible with phosphate buffered (pH: 7.35) 2.5 % gluteraldehyde followed by post-fixation with 1 % osmium tetroxide. After the necessary follow up, samples were embedded in Vestopal-W and then cut into ultra thin sections which were stained with Uranyl acetate and Lead citrate (Reynol's) technique. All samples were then studied under JEOL-1000C transmission electron microscopy.

RESULTS

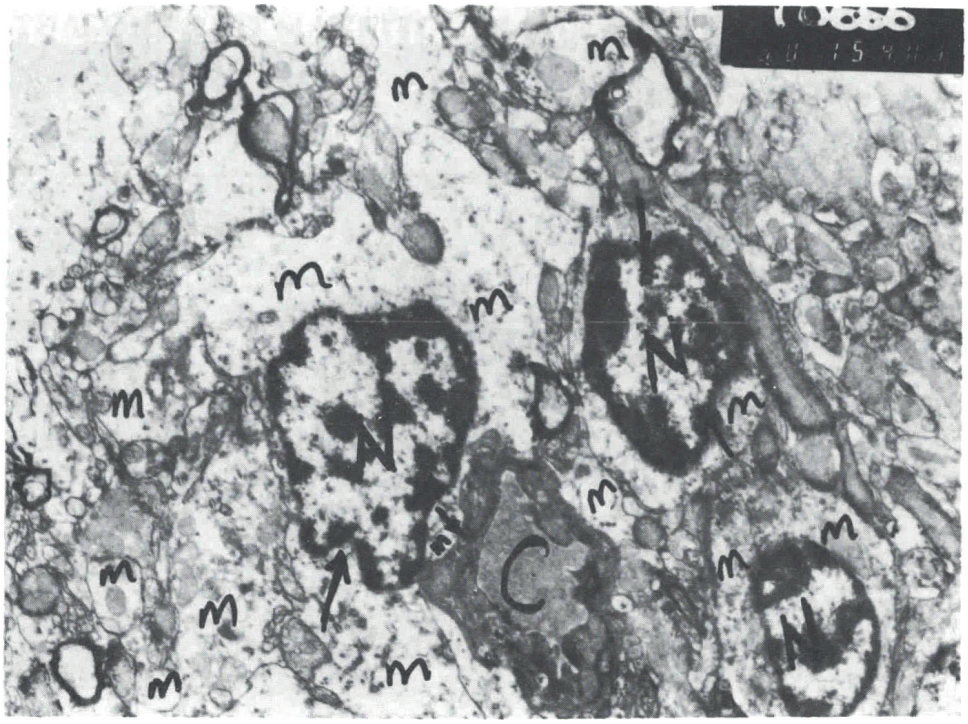
EM findings are presented in figures.



EM figure-A/1(x8500):



EM figure-A/2 (x8500):
In these CONTROL-EM figures of the experiment, in the stratum granulosa widespread microvacuolations (m), disruption of myelin tissue and disconfigurations (arrows) of the nuclei ard myelin (M) tissue are obviously seen.



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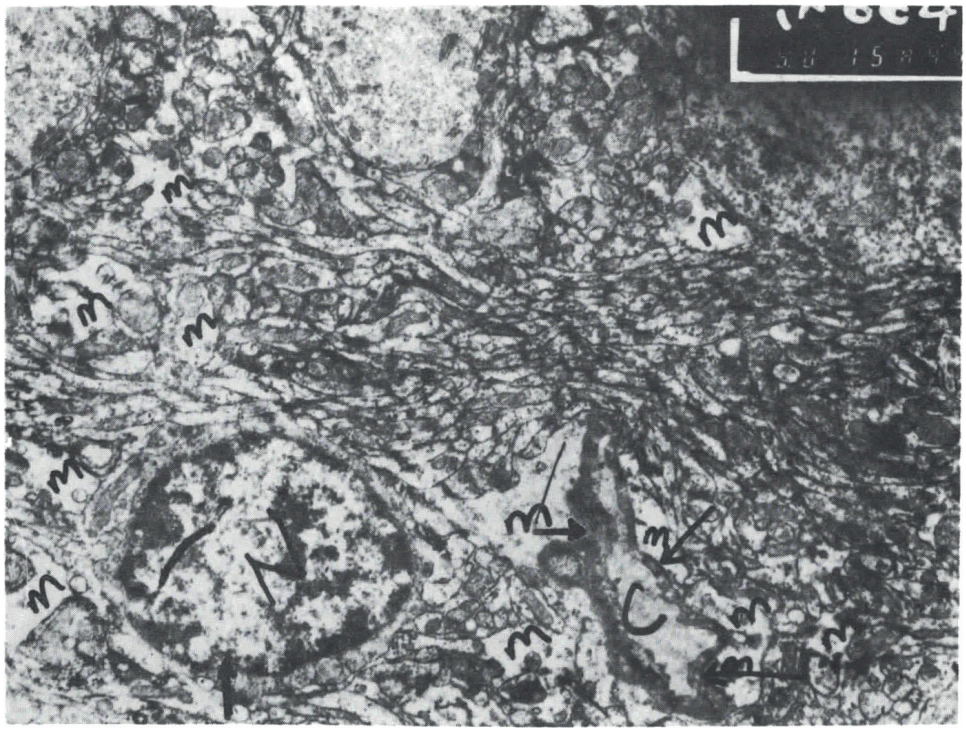
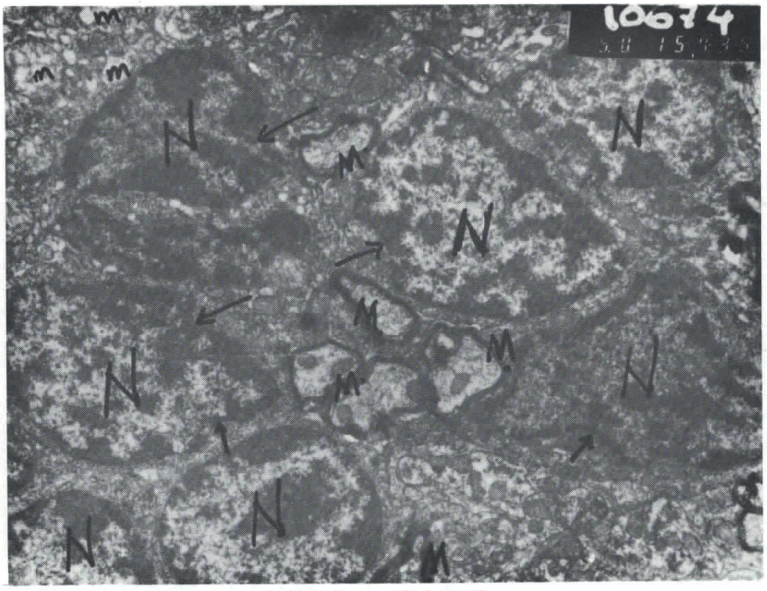


EM figure-B/2 (x8500) :

In these MANNITOL TREATED-EM figures of the experimental group mannitol appears unable to reduce hypoxic edema in the stratum granulosa and also its secondary of disconfigurations (arrows) of the nuclei (N) and capillaries (C).
(m:widespread edema-microvacuolations).



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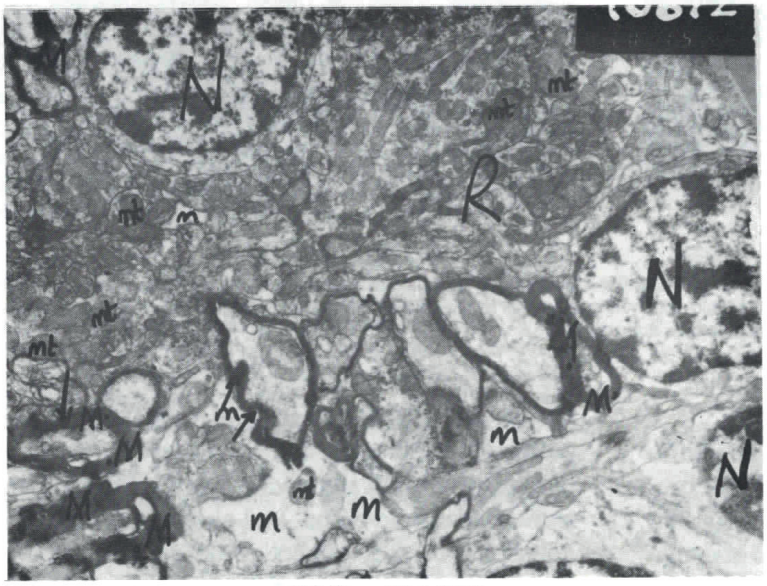

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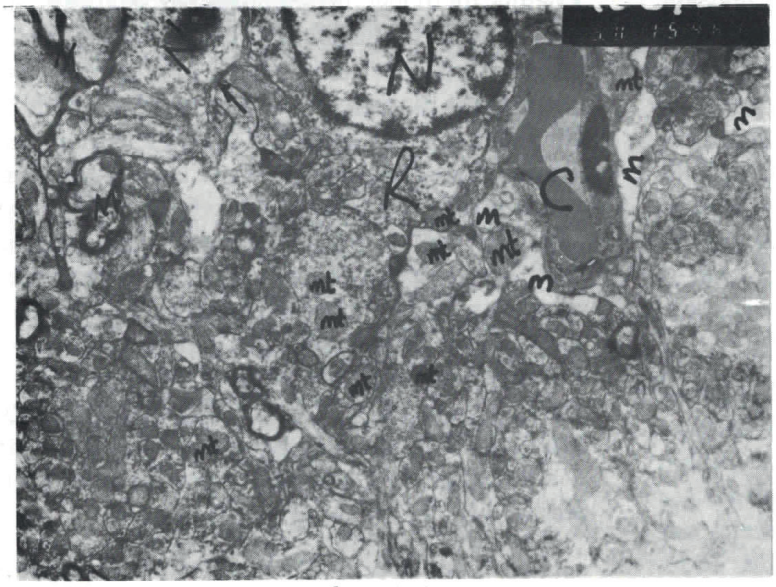
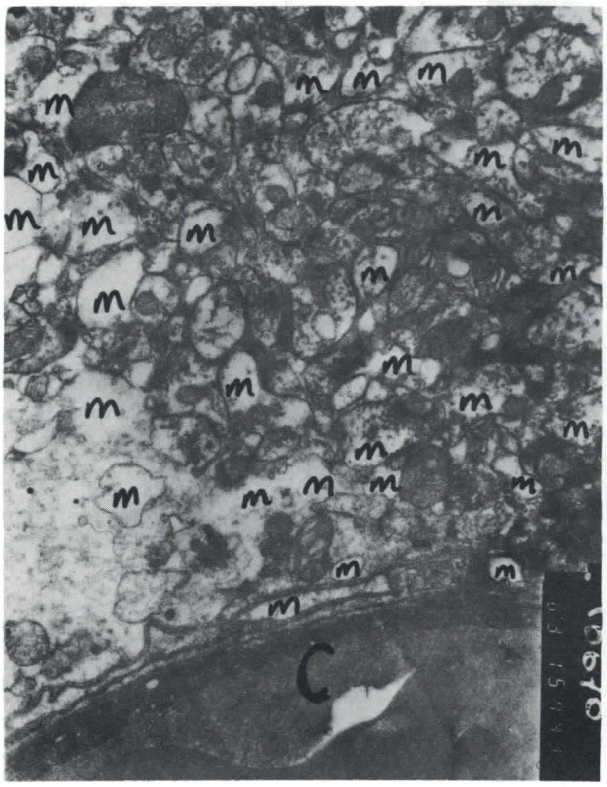
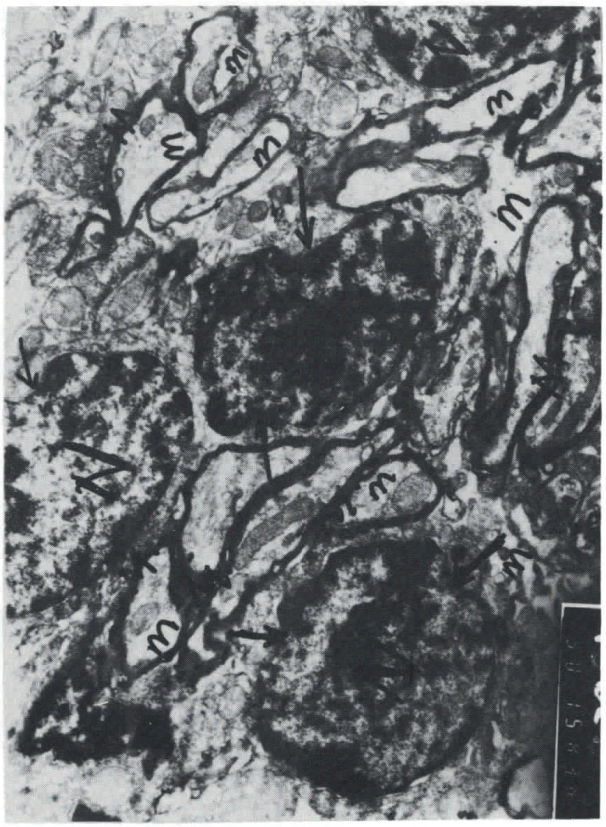


EM figure-C/3 (x8500):

In these DEXAMETHASONE TREATED-EM figures of the experimental group, it appears that dexamethasona was effective in reducing hypoxic edema in the stratum granulosa and therefore on its secondary effects, i.e, disconfigurations (arrows) of the nuclei (N), microvacuolations (m) and distortion (arrows) of myelin tissue (M). (When compared to the MANNITOL treated group, secondary effects of the edema appeared to be lessened). Also free ribosomes (R) are obviously increased and mitochondrias appeared to be in order (mt:mitochondria).



EM figure-D/1 (x8500):



EM figure-D/2 (x8500):

In these MANNITOL-DEXAMETHASONE TREATED EM figures of the experimental group; combined treatment seems not to be effective in reducing hypoxic edema in the stratum granulosa and on its secondary effects, as in the DEXAMETHASONE treated group (arrows: distortions, N:nuclei, M:myelin tissue, C:capillary m:microvacuolations-edema).

DISCUSSION

Types of brain hypoxia were classified by BRIERLEY (5) who showed that brain damage can be produced by hypoxic-hypoxia brought about by atmospheric decompression but the pattern of damage was found to be indistinguishable from the pattern produced by ischemic (oligemic)-hypoxia. Therefore, in the hypoxic brain damage there must be an oligemic (ischemic) component as well. Upon his study it was assumed that the terms, hypoxia and ischemia could be taken as synonymous (1, 5). Since the neuronal tissue have an abligatory aerobic glycolytic metabolism, they are the most sensitive structures to hypoxia in the body (1, 34). The ultrastructural study was restricted to the cerebellum because of its known vulnerability to hypoxic-hypoxia or oligaemic-hypoxia (1).

As in the both ischemia and hypoxia the basic pathological mechanism is the same, which is inadequate supply of oxygen to the neural structures, within several minutes an aneorobic metabolism of glucose appears which results in depletion of the energy stores, excessive lactic acid accumulation (cellular acidosis) and finally in the alteration of the blood-brain barrier (BBB) (32, 34). Therefore, normal cell ion homeostasis is disrupted and secondary excessive intracellular Na and water uptake and K release into the extracellular fluid occur. And also during these pathological processes, catabolic products increase. Both increased catabolic products and extracellular hyperkalemia are thought to stimulate astroglial swelling by increasing the metabolic demands and begets more ischemia (in the neuronal tissue) by preventing oxygen delivery through the swelling of the elements of the tissue (12). The change in extracellular ion concentrations, that is, hyperkalemia leads to concentration gradients between extracellular fluid, blood and cerebrospinal fluid. This causes a loss of potassium and an uptake of sodium into the brain tissue, which in turn is accompanied by water increase. Therefore, in the primary phase of ischemia, hypokalemia, hypernatremia and increased water content are the main chemical alterations (CYTOTOXIC. HYPOXIC, ISCHEMIC EDEMA). In the hypoxic or ischemic condition the other pathological processes are in effect as well. (6, 13, 14, 22, 27, 28, 33, 37).

Experimentally, it was also shown that the threshold of ischemia for the edema formation and che-

mical alterations is not the same (14). Neuronal alterations in ischemia were graded by LITTLE et al. in their experimental study (17). EM findings in our study changed from grade-I to grade-II (see EM figures). The effects of mannitol and dexamethasone on ischemic edema are still not resolved either experimentally or clinically, possibly due to the differences in administration time, doses and mode (IV, bolus etc), therefore, results of reports are contradictory (2, 3, 4, 8, 9, 10, 11, 15, 16, 18, 19, 20, 21, 23, 25, 26, 29, 30, 31, 35, 36).

In our experiment, it was electron microscopically observed that upon hypoxic neuronal changes which occurred at 320 torr, both mannitol and dexamethasone were effective in reducing cytotoxic edema and therefore protecting the neuronal integrity to some extent but when combination treatment with mannitol-dexamethasone was compared, it is obvious that dexamethasone was found to be more effective alone than combined with mannitol. (see the relevant EM figures). This conclusion was also supported by the study in part-I. (7).

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