Actinomycin D Suppresses The Protective Effect Of Dexamethasone In Rats Affected By Global Cerebral Ischemia

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SUMMARY Simultaneous occlusion of both common carotid arteries in female Sprague-Dawley CFY rats produced characteristic symptoms of global cerebral ischemia, such as staggering, circling, convulsions, followed by coma and death. A close correlation existed among these symptoms and the elevation of water and Na⁺ content, appearing at the stage of staggering; Evans blue extravasation and diminution of K⁺ content, detected at circling; and the increase in Ca²⁺ content in the total brain tissue, manifesting itself at the phase of convulsions, indicating the development of cerebral edema due to ischemia. Dexamethasone given subcutaneously in a single 2.0 mg kg⁻¹ dose 5 hours prior to the induction of global cerebral ischemia reduced considerably the morbidity and mortality, the alterations in water and electrolyte content, and albumin leakage in the brain tissue. Actinomycin D, in a dose of 0.5 mg kg⁻¹ injected intravenously 1 hour before steroid treatment, abolished the beneficial effect. This finding suggests that de novo protein synthesis is involved in the cerebroprotective effect of dexamethasone.

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SEVERAL CLINICAL TRIALS HAVE shown that glucocorticoids exert a beneficial effect in patients with cerebral ischemia and brain injuries. On the other hand, Bauer and Tellezz‡ have described opposite results. Brain disorders of other types than trauma, (e.g. cerebral malaria) do not provide any evidence for the benefit of steroid treatment. Experimental results obtained in different animals with various types of brain injuries have supported the assumption that ste-

roid treatment evokes a cerebroprotective effect. Local freezing-induced brain edema has been found to be suppressed by cortisone in cats by but not by dexamethasone in rats and Rhesus monkeys. Regional reduction in blood supply causing cerebral ischemia has been shown to be alleviated by dexamethasone treatment. In contrast, some other data with experimental animals have indicated the ineffectiveness of glucocorticoids in prevention of cerebral ischemia. Finally, it appears, therefore, from the data available that the therapeutic value of steroids in the treatment of cerebral ischemia and trauma still remains a subject of debate.

Various types of deleterious events, such as ischemia and hypoxia, disturbing O₂ supply and glucose transport in the brain, lead to characteristic morphological changes, modifications in the function of

References
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blood brain barrier resulting in brain edemas of cytoxic, and vasogenic types. In the light of experiences with steroid therapy, the conclusion may be drawn that the functional disturbances associated with injury to the brain do not necessarily develop as a consequence of edematous processes. The controversial data concerning cerebral ischemia may lead us to a similar conclusion.

For a more effective therapeutic intervention in the ischemic disorders of the brain, the animal model used and the principles of causal therapy should be better outlined. This requires a more exact knowledge of their pathogenesis and a deeper insight into the mechanism of drug action.

Although bilateral common carotid artery occlusion is known to be insufficient to induce progressive global cerebral ischemia in the great majority of rat strains, in a preliminary study we have found, surprisingly, that this intervention could lead to fatal brain damage provided that the surgery was performed on Sprague-Dawley rats of CFY strain. Detailed pathophysiologic and neuropathologic characterization of the model is in progress in our laboratory.

We report here that the simultaneous occlusion of both common carotid arteries in female Sprague-Dawley rats of CFY strain produces characteristic symptoms of global cerebral ischemia which correlate well with changes in water, electrolyte, and albumin content of brain tissue. Dexamethasone provides marked protection against these alterations, and its effect may be related to de novo synthesis of some cerebroprotective protein factors.

Methods

A close colony of randomly-bred female Sprague-Dawley CFY rats weighing 200–220 g, fed commercial food pellets and tap water ad libitum, was used. The rats proved to be normotensive on the basis of blood pressure measurement from the cannulated left common carotid artery under pentobarbitone anesthesia (30 mg kg\(^{-1}\) intraperitoneally, Mebubarbital sodium, Rhone-Poulenc) and on the cuffed tail artery test ensued at 268 ± 5.6 min, followed by convulsions at 146 ± 4.7 min (table 1). Male rats were less sensitive to carotid artery clamping than females. Sham-operated rats without carotid artery occlusion served as controls in this study. The rats were killed by decapitation at the time when the different symptoms developed. The whole brain was removed and its wet weight was immediately measured. The tissue was then dried at 110°C for 40 hours to obtain its dry weight. Water content was expressed as a percent of the total dry tissue weight. To determine electrolyte contents, the dry tissue was ashed at 550°C for 20 hours. The ash was dissolved in 5 ml 3 M nitric acid (Merck, Suprapur\textregistered), and diluted 10-fold with deionized water. Na\(^+\) was measured at the wavelength of 330.3 nm K\(^+\) at 404.4 nm, and Ca\(^{2+}\) at 422.7 nm in an air-acetylene flame by a Perkins-Elmer 306 atomic absorption spectrophotometer. The slit width was 0.7 mm in all measurements. The value determined at the time of the appearance of the symptoms of cerebral ischemia after various pretreatments of the rats were expressed mmol kg\(^{-1}\) dry weight.

The determination of Evans blue dye was performed according to Rossner and Tempel. In brief, the rats were injected intravenously with Evans blue (Sigma, 2.0% w/v) in a dose of 20 mg kg\(^{-1}\) body weight 2 hours before the appearance of characteristic symptoms. They were then sacrificed by opening the chest wall under ether anesthesia. In order to remove the intravascularly localized dye, a polyethylene tube was inserted into the aorta through the left ventricle, and a perfusion with isotonic saline until colorless perfusion fluid was obtained from the right atrium was made. The whole brain was removed and weighed, then homogenized in 1 ml 50% trichloroacetic acid (w/v), and centrifuged with 10,000 g for 30 min. The supernatant was determined at 615 nm by a Unicam SP 1800 ultraviolet spectrophotometer. The results were expressed as \(\mu g\) \(g^{-1}\) wet weight.

Statistical analysis of the mortality rate was made by the Chi-square method. The appearance of staggering and the alterations in water, electrolyte, and Evans blue content in the brain tissue were statistically evaluated by the unpaired Student’s \(t\)-test.

Results

Preliminary observations in our laboratory have indicated that simultaneous ligation of both common carotid arteries in female Sprague-Dawley CFY rats (n = 325) produces death within 12 hours in two-thirds of the animals. This period was long enough to detect behavioral changes and clearly distinguishable symptoms developing as a result of global cerebral ischemia. The first characteristic symptom was staggering that appeared at 146 ± 4.7 min (table 1) circling ensued at 268 ± 5.6 min, followed by convulsions at 361 ± 16.6 min, then deep coma developed. As the first symptom occurred, the process was irreversible, and the rats died. Male rats were less sensitive to carotid artery clamping than females. Sham-operated animals without bilateral common carotid artery ligation did not develop any symptoms. Under pentobarbi-
tal anaesthesia, there was a slight increase in blood pressure in the carotid artery ligated rats until 20 minutes, then it returned to normal and remained constant throughout the observation period.

In the present study, the lethality index was 67% in the control group. This was markedly reduced in rats treated with 2.0 mg kg⁻¹ dexamethasone given subcutaneously 5 hours prior to the induction of global cerebral ischemia, and the appearance of staggering when developed was also slightly delayed (table 1), while the other symptoms occurred at the same time as in the controls and their severity was unchanged. Significant protection was seen neither after 1.0 mg kg⁻¹ dexamethasone nor when the steroid was given at the time of carotid artery occlusion. Six hours after intravenous administration of 0.5 mg kg⁻¹ actinomycin D, the mortality rate was unchanged as compared to the control, while extravasation of Evans blue dye was detected only at the second stage of ischemia. K⁺ changed in the opposite direction, and its decrease was less obvious at the beginning of ischemia, while in later stages it became excessive (table 3). Ca²⁺ concentration was only slightly decreased during the appearance of the first two symptoms, when convulsions appeared however there was a sharp elevation in its level (table 3). The alterations in water, electrolyte and Evans blue content of brain tissue provided evidence for a gradually and continually developing severe cerebral edema. In the dexamethasone treated group in which the samples were taken at the time when the symptoms appeared in the controls, all metabolic and blood brain barrier alterations were markedly suppressed (tables 1 and 2). In the rats treated with actinomycin D alone, the water, electrolyte, and Evans blue contents exhibited alterations of the same direction and almost of the same extent as measured in controls (tables 1 and 2). Actinomycin D treatment prevented the dexamethasone-induced protective effect on these parameters.

**Discussion**

The results reported here indicate that bilateral carotid artery occlusion in female rats, originated from a closed colony of Sprague-Dawley CFY strain, produces characteristic symptoms of global cerebral ischemia that correlate well with the development of brain edema. These findings are in accordance with that found by Lespinase et al.¹⁸ with bilateral internal carotid artery clamping in Long Evans rats. Nakatomi et al.¹⁹ have shown that spontaneous hypertensive male

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**Table 1**  
Protective Effect of Dexamethasone Against Fatal Cerebral Ischemia and Its Prevention by Actinomycin D

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats died by 12 hours</th>
<th>Lethality, %</th>
<th>Appearance of staggering, min mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control*</td>
<td>30</td>
<td>20</td>
<td>67</td>
</tr>
<tr>
<td>Dexamethasone†</td>
<td>24</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>Actinomycin D‡</td>
<td>26</td>
<td>17</td>
<td>65</td>
</tr>
<tr>
<td>Actinomycin D‡ plus Dexamethasone†</td>
<td>25</td>
<td>15</td>
<td>60</td>
</tr>
</tbody>
</table>

*Control rats were given 1.0 ml kg⁻¹ isotonic saline subcutaneously 5 hours before the induction of global cerebral ischemia. †Dexamethasone was also injected subcutaneously in a dose of 2.0 mg kg⁻¹ (the dose refers to the base) 5 hours before the induction of global cerebral ischemia. §Values are means ± SEM. **p < 0.05 and ***p < 0.001. Symbols designate statistically significant differences compared to sham-operated groups at the level of **p < 0.01, ***p < 0.001. **p < 0.01 and ***p < 0.001. Symbols designate statistically significant differences in corresponding stages of ischemia as compared to the saline-treated control groups at the level of €p < 0.01 and §§p < 0.001.

In the saline-treated group, carotid artery ligation significantly elevated the water, Na⁺ and Evans blue content of total brain tissue as compared to sham-operated animals, and these changes showed a close correlation with the symptoms which appeared at different stages of global cerebral ischemia (tables 1 and 2). Water and Na⁺ content was already increased in the rats affected by staggering, while extravasation of Evans blue dye was detected only at the second stage of ischemia. K⁺ changed in the opposite direction, and its decrease was less obvious at the beginning of ischemia, while in later stages it became excessive (table 3). Ca²⁺ concentration was only slightly decreased during the appearance of the first two symptoms, when convulsions appeared however there was a sharp elevation in its level (table 3). The alterations in water, electrolyte and Evans blue content of brain tissue provided evidence for a gradually and continually developing severe cerebral edema. In the dexamethasone treated group in which the samples were taken at the time when the symptoms appeared in the controls, all metabolic and blood brain barrier alterations were markedly suppressed (tables 1 and 2). In the rats treated with actinomycin D alone, the water, electrolyte, and Evans blue contents exhibited alterations of the same direction and almost of the same extent as measured in controls (tables 1 and 2). Actinomycin D treatment prevented the dexamethasone-induced protective effect on these parameters.

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**Table 2**  
Water Content* and Extravasation of Evans Blue† in Brain Tissue at Different Stages of Global Cerebral Ischemia. Effect of Dexamethasone and Its Prevention by Actinomycin D

<table>
<thead>
<tr>
<th>Groups‡</th>
<th>Stages of ischemia§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham operation</td>
</tr>
</tbody>
</table>

*Water content was expressed as percent of total dry tissue weight. †Evans blue content was expressed as µg g⁻¹ wet brain tissue weight. §Pretreatments were given as indicated in table 1. Values are means ± SEM. | The time of the killing of these rats was established by the occurrence of symptoms in untreated animals. Asterisks denote significant differences as compared to sham-operated groups at the level of *p < 0.05, **p < 0.01, and ***p < 0.001. Symbols designate statistically significant differences in corresponding stages of ischemia as compared to the saline-treated control groups at the level of €p < 0.01 and §§p < 0.001.
rallies, therefore, likely that macrorcin induces cardioprotection by preventing the formation of arachidonate
metabolites. These metabolites were found to be produced in isolated brain microvessels as well. It has
also been demonstrated that platelet hyperaggregability contributes to the development of ischemic cerebrovascular diseases. Although direct evidence for the role of glucocorticoid-induced mediators in the control of stroke has not been available, the present study suggests that second messengers of steroid action might provide protection also against fatal cerebral ischemia.

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References
CEREBRAL ISCHEMIA due to vasospasm, thrombi or emboli results in a series of events leading to neuronal necrosis and failure of synaptic transmission. A number of studies point to a role for catecholamines in the pathophysiology of brain injury following cerebral ischemia. In fact, massive amounts of catecholamines are released by ischemic neurons, as suggested by histochemical studies and by the increased level of biogenic amines in the cerebrospinal fluid of humans affected by cerebral infarction. This event may contribute to the development of ischemic brain damage. Alterations in catecholamine content and metabolism associated with changes in receptor number, affinity and coupling to adenylate cyclase have been demonstrated in various cerebral areas after experimental vascular lesions. These biochemical and histological findings support the hypothesis that catecholamines may modulate the development of brain injury following cerebral ischemia.
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