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FUNCTIONAL DISTURBANCES IN BRAIN FOLLOWING INJURY: Search for Underlying Mechanisms†

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It was shown previously that local cerebral glucose utilization is less than 50% of normal in all cortical areas of rat brain 3 days following a focal freeze-lesion and that this effect of trauma is significantly diminished by dexamethasone (0.25 mg/Kg/day), and by indomethacin (7.5 mg/Kg single dose). To elucidate the mechanism of action of steroids and non-steroidal antiinflammatory drugs in traumatized brain, the effects of dexamethasone and indomethacin on arachidonic acid release, malondialdehyde production and prostaglandin synthesis in the lesion area were investigated. Five seconds after a freezing lesion arachidonic acid was significantly increased in the lesion area of untreated animals. Neither dexamethasone nor indomethacin had any effect on this release. The thiobarbituric acid reaction, as an estimate of malondialdehyde and non-enzymatic free radical lipoperoxide formation from unsaturated free fatty acids showed no change in the control and lesion areas of untreated and both dexamethasone and indomethacin treated groups. There was a marked increase in $PGF_{2\alpha}$, PGE_2, PGD_2 in the lesion area of untreated animals. Indomethacin prevented the formation of prostaglandins by more than 90% while dexamethasone had no effect. These results suggest that some components of the arachidonic acid metabolism must be involved in functional disturbances resulting from trauma while steroid action is mediated in injured brain independently from the prostaglandin cascade.

INTRODUCTION

Cerebral edema has been generally accepted as an underlying cause of functional disturbances associated with injury to the brain. The best proof

† This issue is dedicated to Donald B. Tower.

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of this tacit assumption is the fact that all efforts to develop rational therapy for neurological complications resulting from trauma to the brain have been directed at modifying the edematous process (1). Furthermore, clinically beneficial effects of empirically developed treatment modalities, for example, of steroids have been ascribed to their control of cerebral edema despite the fact that experimental evidence for such effects was contradictory (2, 3). When studies with standard freezing lesions in the cat failed to show any correlation between the effects of steroid therapy on the resultant edema and on the associated EEG abnormalities it was postulated that injury to the brain may induce functional disturbances by mechanisms not related to cerebral edema (4). Further progress in the elucidation of this concept was hindered by lack of a suitable model for sensitive assessment of the functional state of the traumatized animal brain (5).

The development of the deoxyglucose method (6) for the measurement of local cerebral glucose utilization (LCGU) has allowed the mapping of functional cerebral activity in awake animals under a variety of conditions since metabolism and function in brain are closely coupled (7). Applying this approach in studies on injured rat brain, unilateral focal freezing or heat lesions were shown to depress LCGU in all areas of the cortex in the traumatized hemisphere and, to a lesser extent, in the contralateral hemisphere and bilaterally in subcortical structures and in the white matter (8). This metabolic depression which reached its peak 3 days after the lesion was made was not associated with diminished blood supply, as no corresponding changes in blood flow were observed. The results were interpreted as indicating a widespread depression in the functional state of the traumatized rat brain (8). Furthermore, there were both spatial and time-course discrepancies between changes in LCGU and the edematous process, since many studies with the freezing model have demonstrated vasogenic edema to be more circumscribed, unilateral, and diminishing in extent after 48 hours (2, 8). Thus the functional consequences of brain injury, as reflected by a widespread metabolic depression, did not appear to be mediated by cerebral edema.

In contrast to its equivocal effects on cerebral edema, dexamethasone given either before or after the lesion was made significantly ameliorated the effects of trauma on LCGU in rat brain (9). Furthermore indomethacin, which had been shown to have no effect on the development of vasogenic edema in response to a standard freezing lesion (10), was even more effective than dexamethasone in diminishing injury-induced changes in LCGU in traumatized rat brain when given before the lesion was made (unpublished observation). Since indomethacin is an inhibitor of prostaglandin synthetase (11) and corticosteroids have been reported to inhibit

arachidonic acid release from membrane phospholipids in non-neuronal tissues (12) in our search for mechanisms underlying the effect of trauma on cerebral function several components of the arachidonic acid "cascade" were measured in the area of the cortical lesion immediately after injury in untreated and both dexamethasone- and indomethacin-treated animals. The results of these experiments form the basis of the present report.

EXPERIMENTAL PROCEDURE

General Procedure. Freezing lesions standardized to produce superficial focal cortical injury in the rat were made in the left parietal region of halothane anesthetized Sprague-Dawley male rats (280–320 g) by applying a freezing probe (−50°C) to the dura for 5 seconds through a 4×4mm opening in the skull. The animal was killed exactly 55 seconds later (60 seconds after start of lesion) by decapitation, the head being dropped immediately into liquid N_2. A sharp chisel was used to remove the brain from the cranial vault and to separate as much of the lesioned area of the cortex as possible from the rest of the hemisphere. The sample was then weighed rapidly. Because of the limited amount of tissue affected in each brain the lesion areas from 3 to 4 animals were pooled, care being taken that no thawing had occurred until the pooled sample was homogenized in chloroform–methanol 2:1 by volume. Samples of comparable size were also chiseled out from the non-lesioned right hemispheres of each lesioned brain. These served as controls together with unlesioned brains from rats decapitated into liquid nitrogen.

Drug Treatment. In the Dexamethasone-treated group each rat received 0.25 mg Decadron phosphate (Merck Sharp and Dohme, Kirkland, Quebec) per kg in two divided doses 18 hours and 1 hour before the lesion was made. In the Indomethacin-treated group each rat received 7.5 mg Indocid (Merck Sharp and Dohme, Kirkland, Quebec) per kg in two divided doses on a schedule as above. The doses and the regimen were chosen to match the treatment which was found to be effective in experiments in which LCGU was measured in traumatized rat brain (9 and unpublished results).

Estimation of Edema and LCGU. The difference in weight between the lesioned and the control hemisphere was taken as the weight of the edema fluid, as previously described (4). LCGU was measured by the deoxyglucose method (6), as previously described (8, 9).

Determination of Arachidonic Acid. Frozen pieces of cerebral cortical tissue (100–300 mg) taken from the lesion area and from the non-lesioned control hemisphere were homogenized in 20 volumes of chloroform–methanol 2:1 by volume to which had been added 10 μg of heneicosanoic acid as internal standard. The chloroform-methanol extract was filtered, evaporated to dryness and the lipids redissolved in a small volume of chloroform and applied to small silicic acid columns (Bio-sil HA minus 325 mesh, Biorad Labs). The free fatty acids and neutral lipids were eluted with chloroform and after concentration applied to silica gel 0.25mm thin layer plates and developed in hexane- diethylether-acetic acid, 112:35:2.5. The free fatty acid zone identified by brief exposure to iodine vapor was scraped off and the silica gel extracted with chloroform–methanol 2:1. The solvent was evaporated and the fatty acids methylated in small tubes with ethereal diazomethane in 10% methanol. The arachidonic acid was quantitated by gas liquid chromatography on 6 ft. columns of 10% Silar 10C (Applied Science Labs., Pennsylvania) in a Hewlett Packard 5730A gas chromatograph connected to a 3390A integrator.

Thiobarbituric Acid Reaction. Malondialdehyde formed during the peroxidation of unsaturated fatty acids or from the breakdown of endoperoxides was determined fluorimetrically by the methods outlined by Yagi and Schimizu, Rondo and Hayaishi (13, 14).

Determination of Prostaglandins. Prostaglandins $F_{2\alpha}$, E_2 and D_2 were extracted and purified by the method of Powell (15) utilizing ODS Sep Pak reversed phase cartridges (Waters Associates, Milford, Massachusetts). Tetradeuterated internal standards (1 μg) of $PGF_{2\alpha}$ and PGE_2 were added to initial extracting solvents. The prostaglandins were quantitated by gas chromatography-mass fragmentography on a LKB-9000 instrument as previously described (16, 17). Prostaglandin D_2 was quantitated by the method of Abdel-Halim et al (18) after reduction with sodium borohydride and correction for the PGE_2 contributions to $PGF_{2\alpha}$ and $PGF_{2\beta}$ peaks. The ions monitored were m/e 423 and 427 for the methyl ester—TMS derivatives of $PGF_{2\alpha}$ and m/e 321 and 325 for the methyl ester—TMS derivative of PGE_2 converted to PGB_2.

RESULTS

Effect of Injury and Drugs on Edema and on Local Cerebral Glucose Utilization. The striking difference between effects of dexamethasone and of indomethacin on cerebral edema and on LCGU in traumatized brain is demonstrated by the results presented in Table I.

Pretreatment by either drug had no statistically significant effect on the extent of edema which developed in response to a standard lesion, although in animals on dexamethasone a decrease was observed. On the

TABLE I

EFFECTS OF DEXAMETHASONE AND INDOMETHACIN ON CEREBRAL EDEMA AND ON CORTICAL GLUCOSE UTILIZATION IN TRAUMATIZED BRAIN

	Extent of Edema[a] (g)	Cortical LCGU[b] % of normal
Untreated	0.66 ± 0.39 (49)	49 ± 8 (7)
Dexamethasone (0.25 mg/Kg/day)		
Started before lesion	0.56 ± 0.19 (29)	77 ± 2* (6)
Started 24 hours after lesion	—	71 ± 2* (4)
Indomethacin (7.5–15 mg/Kg)		
Given before lesion	0.71 ± 0.30 (3)	87 ± 3* (7)
Given 24 hours after lesion	—	84 ± 3* (6)

Averages ± SD. Numbers of animals in bracketts.

[a] Edema measured as difference in weight between traumatized and contralateral hemisphere 24 hours after a standard freezing lesion in the cat. Data from ref. 10. See also ref. 4.

[b] Average LCGU in six cortical areas of the traumatized hemisphere 3 days after a standard freezing lesion in the rat, expressed as percent of normal. Calculated from data in refs. 8, 9 and unpublished results.

* Statistically significantly different from untreated $p < 0.01$.

other hand statistically highly significant increases in LCGU were present in both dexamethasone and indomethacin pre-treated animals, as compared to untreated in which cortical glucose utilization was depressed to more than half of normal. As can be seen in Figure 1 the difference in LCGU between untreated and treated animals was such as to be easily discernible by visual inspection of representative autoradiographs.

Both drugs were equally effective in ameliorating the effects of trauma on cortical LCGU whether given before or 24 hours after the lesion.

Arachidonic Acid Release. Results summarized in Table II show that arachidonic acid content of the cortical lesion area increased sharply within 60 seconds of injury but that this release was not affected by either dexamethasone or indomethacin treatment. Thus dexamethasone did not affect arachidonic acid release from phospholipids in the lesion area of the rat brain and its effects in injured brain cannot be explained in terms of such inhibition.

Lipoperoxide Formation Assessed by Thiobarbituric Acid (TBA) Reaction. It will be seen from data in Table III that no difference in TBA reaction products was demonstrable between the area of the lesion and control tissue. Furthermore, neither dexamethasone nor indomethacin had any effect on the TBA reaction. Since TBA reaction is only an indirect method for estimation of free radical formation and lipoperoxidation these results do not unequivocally rule out a role for such processes in functional disturbances in injured brain but they do not provide any support for such a role, nor for effects of steroids being mediated through such a mechanism.

Formation of Prostaglandins. Results of prostaglandin (PG) analysis in the three groups of animals are presented in Table IV. Within 60 seconds of injury there was a sharp increase in $PGF_{2\alpha}$, PGE_2 and PGD_2 content of the lesion area as compared to cerebral cortex tissue in the unlesioned hemisphere in the untreated rat. The accumulation of $PGF_{2\alpha}$, the only PG measured in the two treated groups, was not affected by dexamethasone treatment but was more than 90% inhibited in the indomethacin-treated rats.

DISCUSSION

The processes underlying the widespread functional disturbances which develop in rat brain as a consequence of injury and which are reflected by extensive decreases in LCGU remain to be elucidated. The ameliorating effect of pre-treatment and post-treatment with indomethacin, a

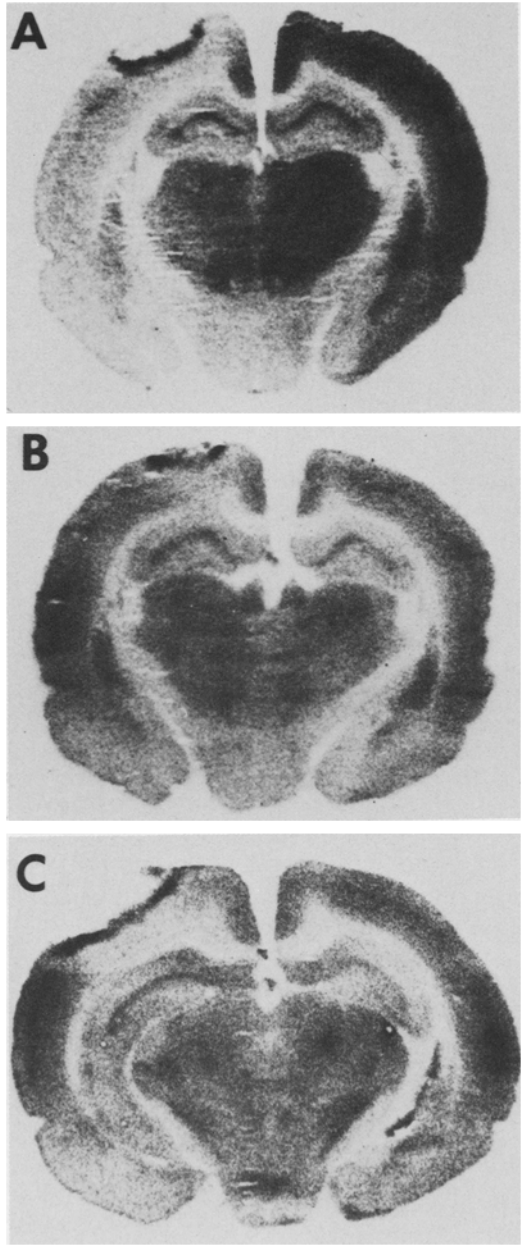
A
B
C

TABLE II

ARACHIDONIC ACID RELEASE IN RAT CEREBRAL CORTEX AFTER FREEZING LESION

Cerebral tissue	Arachidonic acid μg/g wet weight
Control hemisphere	2.6 + 1.4 (20)
Lesion area	
Untreated	15.4 + 7.1* (13)
Dexamethasone treated (0.25 mg/Kg)	12.4 + 9.0* (8)
Indomethacin treated (7.5 mg/Kg)	10.4 + 5.6* (6)

Averages + S.D. Number of pooled samples in brackets. See Experimental Procedure for details.
* Statistically significantly different from control hemisphere $p < 0.01$
No statistical difference between lesion areas in treated and untreated groups.

non-steroidal anti-inflammatory drug, on LCGU in lesioned brain indicates that some component or components of the prostaglandin system are involved. Whether the prostaglandins originate from blood elements in the lesion area or from the brain tissue itself is not clear from the available data.

TABLE III

LIPOPEROXIDE FORMATION IN RAT CEREBRAL CORTEX DETERMINED BY THIOBARBITURIC ACID REACTION

Cerebral tissue	TBA Reaction Product nmol/g tissue
Control hemisphere	27.3 + 3.8 (7)
Lesion area	
Untreated	26.9 + 8.8 (4)
Dexamethasone treated (0.25 mg/Kg)	27.1 + 9.2 (4)
Indomethacin treated (7.5 mg/Kg)	22.1 + 7.9 (4)

Averages ± SD. Number of pooled samples in brackets. See Experimental Procedure for details.

FIG. 1. [^{14}C]Deoxyglucose autoradiographs at the level of the freezing lesion in rat brain 3 days following injury. A. Untreated. Note gross asymmetry in cortical areas reflecting severely depressed glucose utilization in the traumatized hemisphere. B. Animal treated with dexamethasone 0.25 mg/kg/day i.p. starting 18 hours before lesion. C. Animal given single dose of indomethacin 7.5 mg/kg i.p. 6 hours before lesion. Note that in B and C side-to-side differences are not obvious on visual inspection.

TABLE IV
PROSTAGLANDIN FORMATION IN RAT CEREBRAL CORTEX AFTER FREEZING LESION

Cerebral tissue from	$PGF_{2\alpha}$	PGE_2	PGD_2
	ng/g wet weight		
Control hemisphere			
Untreated	1.4 ± 1.0 (8)	1.1 ± 0.1 (4)	2.0 ± 1.8 (4)
Dexamethasone treated (0.25 mg/Kg)	1.2 ± 0.9 (4)	—	—
Indomethacin treated (7.5 mg/Kg)	1 (3)[a]	—	—
Lesion area			
Untreated	57.5 ± 19.8 (6)*	29.5 ± 12.6 (4)*	111.5 (2)*
Dexamethasone treated (0.25 mg/Kg)	60.0 ± 11.5 (4)*	—	—
Indomethacin treated (7.5 mg/Kg)	1.8 ± 0.3 (3)**	—	—

Average ± SD. Number of pooled samples in brackets. See Experimental Procedure for details.
[a] Below limits of detectability of the method.
* Statistically different from control hemisphere $p < 0.01$.
** Statistically different from untreated $p < 0.01$.

The mechanism of action of steroids also remains unexplained. However, the present experiments have shown that the effects of dexamethasone in traumatized brain are not mediated by an inhibition of the release of arachidonic acid in cerebral tissues and the results also strongly suggest that pathological free radical reactions are not involved (19). On the other hand glucocorticoids have been shown to affect protein synthesis and neurotransmitter metabolism in the brain (20) so a specific and direct effect on neuronal function can be envisaged. It is of interest in this connection that changes in serotonin and catecholamine metabolism with cold injury and head trauma have been described (21–23). Any role of serotonin in the development of cerebral edema associated with a freezing lesion has been ruled out (21). The results with indomethacin show clearly, however, that failure to modify cerebral edema by manipulation of any process does not preclude the involvement of that process in functional consequences of injury. Thus the possibility remains that some of the functional changes observed in traumatized brain may be mediated through a neurotransmitter system. Both prostaglandins and dexamethasone could then exert their effects by modifying the response of the system in question to injury. The serotonergic system is a good potential candidate for such

a role, particularly since serotonin innervation is widely distributed to the cerebral cortex of the rat (24), the area most affected in traumatized brain.

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