INFLUENCE OF AMBIENT TEMPERATURE AND DRUG TREATMENTS ON BRAIN OEDEMA INDUCED BY IMPACT INJURY ON SKULL IN RATS

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Summary : The progression and persistence of oedema development following impact-injury on closed skull was studied in anaesthetised as well as in unanesthetised rats. The degree and rate of oedema development, following trauma, was aggravated in anaesthetised hypothermic animals but was reduced/or delayed by maintenance of body temperature at euthermic level. In general, the unanaesthetised animals showed a greater accumulation of oedema fluid than the corresponding anaesthetised group. The development of oedema corresponded more or less with the accumulation of 5-HT level in plasma and brain. This development of oedema was completely prevented following pretreatment with p-CPA, indomethacin paracetamol and aminophylline in unanaesthetised animals; whereas these drugs were able only to partially reduce the oedema development in euthermic anaesthetised animals. On the other hand the cyproheptadine pretreatment aggravated the oedema development which was more pronounced in unanaesthetised animals. The probable mechanism of the action of these drugs has been discussed.

Key words : ambient temperature, brain oedema, 5-HT, blood-brain barrier, prostaglandin cAMP, indomethacin, paracetamol, aminophylline, cyproheptadine, hypothermia, p-CPA

INTRODUCTION

The occurrence of oedema following traumatic injury to brain is recognised as a common feature and the involvement of several neurohumoral substances such as 5-HT, prostaglandins and histamine have been emphasized in the pathophysiology of trauma-induced brain oedema (2, 4, 5, 9). We earlier reported a presumptive role of 5-HT and prostaglandins in the development of oedema following acute stab injury in exposed cerebral cortex of anaesthetized rats as well as the influence of ambient air temperature on the extent of oedema developed following brain trauma (6).

It has been reported that trauma inflicted on intact closed skull is considered to be more harmful than trauma inflicted on brain after opening the skull (1,3,10). Therefore,

further investigation was undertaken to study the oedema development following impact head-injury in both anaesthetized and unanesthetized rats with respect to involvement of 5-HT and prostaglandins, and the influence of ambient air temperatures in the development of oedema.

MATERIAL AND METHODS

Experiments were carried out on inbred CF rats of either sex (200–300 g). The rat feed (Hindustan Lever Ltd., India) and tap water were supplied *ad libitum*.

Infliction of trauma :

The trauma was inflicted on exposed right parietal bone of the skull under urethane anaesthesia (1.5 *g/kg*, i.p.) or during a short period of light ether anaesthesia by dropping a weight of 114.6 g (impact area 12 46 mm^2) from 20 cm height through a metal guide tube. This weight delivers an impact of 0.224 N (kinetic energy) on skull surface which was transmitted to brain via the fluid compartment. The physical characteristics of skull (e.g. thickness, density, elasticity and compliance) in a particular strain at similar age group, was assumed to be constant (5).

The measurement of brain oedema, 5-HT level in brain and plasma, and changes in blood-brain barrier (BBB) permeability was carried out as described earlier (6).

Experimental design :

Group I : Chronic exposure to low ambient temperature (17° 20°*C) :* In a group of 10 urethane anaesthetized traumatised rats, the brain water content (5 rats) and plasma and brain 5-HT (5 rats) were measured at 1, 2 and 5 hr after trauma. The anaesthetized animals remained continuously exposed to an ambient temperature of 17°–20°C till the time of sacrifice without any artificial warming; a fall of 4° to 5°C rectal temperature was seen before sacrifice.

Group II : Chronic exposure to low tembient amperature (17°–20°*C) with subsequent placement in B.O.D. incubator at* 30°*C :* Whether the prevention of hypothermic condition of the animals influences the oedema development as well as 5-HT level in plasma and brain tissue was studied in a group of 10 anaesthetized rats which immediately after brain trauma, were placed in B.O.D. incubator at 30°C till the time of sacrifice. The measurement of brain water as well as plasma and brain 5-HT was carried out at 1, 2 and 5 hr after injury. The rectal temperature in this group of animals showed a fall of 0.5° to 1.5° at the end of 5 hr post-trauma.

Group III : Chronic exposure to high ambient temperature (25°–29°C) : The anaesthetized traumatised rats remained exposed to 25° to 29°C ambient air temperature and the experiments were carried out in the same manner following 1, 2 and 5 hr intervals after brain trauma. In this group of animals, the rectal temperature did not fall at the end of 1 and 2 hr after injury; only at the end of 5 hr post-trauma a mean fall of 0.8°C in rectal temperature was noted.

Group IV : trauma under ether anaesthesia : To understand the progression and persistence of oedema development following trauma in conscious animals, in another separate groups of rats (n=10), the trauma was inflicted under light ether anaesthesia. These traumatised animals revived within 1–2 min following trauma impact and these animals did not show fall of rectal temperature. One set of experiments (n=10) was carried out with such traumatised rats continuously exposed to low ambient temperature (17°–20°C) and another set (n=10) exposed to high ambient temperature (25°–29°C) as described in group I and III.

Control group :

The non-traumatised animals in each group (n=10) served as control.

Drug treatments :

The influence of various drugs (viz. indomethacin, paracetamol, p-CPA, cyproheptadine and aminophylline) were studied in group II and group IV. The dosage and schedule of drug treatments was described earlier (6). Each set of experiments consisted of five animals

Statistical analysis :

The unpaired Students 't' test was used to analyse the significance of data obtained.

RESULTS

The results are shown in Table I and II.

Group I : The urethane anaesthetised animals, chronically exposed to low ambient temperature, showed 4°-5°C fall in body temperature by the end of 1 hr post-trauma period and remained hypothermic throughout the whole 5 hr post-trauma period. These animals showed a sustained increased level of 5-HT in plasma and in both halves of the brain (injured and uninjured) throughout 5 hr post-trauma period, although plasma 5-HT

slowly declined from the initial peak level observed at the end of 1 hr post-trauma period. Correspondingly, the brain oedema developed in both injured and uninjured half of brain to the same extent at 1 hr post-trauma period, and oedema continued to persist over 5 hr post-trauma period (Table I).

TABLE I : Effect of ambient temperature on trauma-induced increse in brain water content (n=5) and 5-HT (n=5) in urethane anaesthetised rats.

Treatment	% swelling from intact control		5-HT		
	Injured right hemisphere	Uninjured left hemisphere	Plasma µg/ml	Injured right hemiphere µg/g	Uninjured left hemisphere
Ambient temperature 17°–20°C					
Intact control	—	—	0.25±0.02	1.61±0.06	1.45±0.07
Injury 1 hr	+ 9.58***	+9.25***	0.96±0.16*** (+284)	5.77±0.97*** (+253.38)	3.51±1.07*** (+142.06)
Injury 2 hr	+10 76***	+8.05***	0 78±0.12*** (+212)	4.16±0.26*** (+158.38)	3.68±0.43*** (+153.79)
Injury 5 hr	+ 8.17***	+7.91***	0.69±0.09*** (+176)	3.82±0.28*** (+173.26)	3.30±0.39*** (+127.58)
Ambient temperature 17°-20°C : B.O.D. temperature 30°C					
Intact control	—	—	0.22±0.02	1.24±0.06	1.12±0.10
Injury 1 hr	+4.30***	+4.94***	0.50±0.05*** (+127.27)	2.02±0.30*** (+62.90)	1.73±0.10** (+54.46)
Injury 2 hr	+5.28***	+5.42***	0.59±0.05*** (+168.18)	2.28±0.20*** (+83.27)	2.27±0.18*** (+103.57)
Injury 5 hr	+7.65***	+7.98***	0.86±0.08 (+290.90)	3·0±0.14 (+206.45)	4.16±0.23 (+271.42)
Ambient temperature 25°–29°C					
Intact control	—	—	0.29±0.04	1.47±0.03	1.42±0.98
Injury 1 hr	+7.00**	+6.79**	0.86±0.08*** (+196.55)	4.86±0.54*** (+230.61)	3.97±0.68*** (+179.57)
Injury 2 hr	— 0.84	—1.45	0.36±0.02 (+24.13)	1.67±0.24 (+13.60)	1.32±0.24 (+7.04)
Injury 5 hr	— 0.59	—2.38	Not done		

Values are expressed as Mean±S.D. *P<0.05, **P<0.01, ***P<0 001.
Figures in parentheses indicate % change from intact control.

Group II : When the trauma inflicted on anaesthetised animals, instead of exposed at low ambient temperature, were kept in B.O.D. chamber at 30°C, the body temperature fell to about 0.5°–1.5°C over 5 hr post-trauma period.

These non-hypothermic anaesthetised animals, showed a slow increase in 5-HT in plasma as well as in both injured and uninjured halves of the brain at the end of 1 and 2 hr post-trauma period. Similarly, the oedema in both halves of the brain also slowly developed and reached a peak level at 5 hr post-trauma period (Table I).

Group III : When the closed injury was inflicted on anaesthetised animals chronically exposed to high ambient temperature (25°–29°C), the body temperature fell to only 0.5° to 1°C over the 5 hr post-trauma period.

These animals exposed to high ambient temperature showed a short-lived effect following brain trauma. Thus, a sharp rise in 5-HT in plasma and in both halves of the brain alongwith oedema in both halves of brain have occurred at the end of 1 hr post-trauma period, but by the 2 hr post-trauma period, the increased 5-HT level and oedema were no longer observed (Table I).

TABLE II : Effect of ambient temperature on trauma-induced increase in brain water content (n=5) and 5-HT level (n=5) in unanaesthetised rats.

Treatment	*% swelling from intact control*		*5-HT µg/g*		
	Injured right hemisphere	*Uninjured left hemisphere*	*Plasma µg/ml*	*Injured right hemisphere*	*Uninjured left hemisphere*
Ambient temperature 17°–20°C					
Intact control	—	—	0.29±0.03	1.27±0.13	1.15±0.08
Injury 1 hr	+16.38***	+15.45***	1.13±0.23*** (+289.65)	2.50±0.61*** (+96.85)	1.26±0.17 (+9.56)
Injury 2 hr	+13.38***	+12.88***	1.48±0.12*** (+410.34)	3.40±0.12*** (+167.71)	1.76±0.19 (+53.04)
Injury 5 hr	+13.92***	+13.62***	1.61±0.31*** (+300)	2.76±0.22*** (+117.32)	2.00±0.11* (+73.91)
Ambient temperature 25°–29°C					
Intact control	—	—	0.27±0.04	1.57±0.16	1.67±0.25
Injury 1 hr	+8.83**	+ 6.90**	0.98±0.04*** (+262.96)	2.63±0.76** (+67.51)	1.94±0.27 (+16.16)
Injury 2 hr	+9.23***	+ 5.42***	0.95±0.12*** (+251.85)	1.89±0.46 (+20.38)	1.86±0.28 (+11.37)
Injury 5 hr	+5.23***	+ 1.61***	1.16±0.24*** (+329.62)	2.04±0.52* (+29.93)	1.78±0.43 (+6.58)

Values are expressed as mean±S.D. *P<0.05, **P<0.01, ***P<0.001.
Figures in parentheses indicate % change from intact control.

Group IV : In contrast to the urethane-anaesthetised animals, when the trauma was inflicted on animals (chronically exposed to low ambient temperature) under light ether anaesthesia, and the animals were brought to consciousness by immediate withdrawal of ether anaesthesia, the 5-HT level and oedema showed somewhat different pattern of changes over the 5 hr post-trauma period.

Thus there was profound increase in plasma 5-HT, much higher than that observed in comparable anaesthetised group (group I) throughout 5 hr post-trauma period. And the increase in 5-HT in brain was limited mainly to injured half of brain. But, the oedema developed in both halves of the brain to the same degree and was of much higher degree than the anaesthetised group (Table II). The body temperature did not fall in this group.

When the trauma was induced on unanaesthetised animals but chronically exposed to high ambient temperature (25°–29°C), the plasma 5-HT level again showed sustained profound increase throughout 5 hr post-trauma period and the increase in 5-HT occurred only in injured brain but lasted only for 1 hr post-trauma period. These animals also showed oedema development in both halves of the brain, but the degree of development was much less compared to previous group. Further, the oedema declined faster in uninjured brain than the injured one (Table II). The body temperature did not fall in this group.

Drug treatments :

The repeated administration of pCPA (5-HT synthesis inibitor), indomethacin and paracetamol (prostaglandin synthetase inhibitors) or aminophylline (phosphodiesterase inhibitor) have shown remarkable protective effect against the trauma-induced oedema development. The effect of drug treatments on oedema formation following trauma was investigated both in nonhypothermic anaesthetised animals (group II) as well as in unanaesthetised animals (group IV). Repeated administration of drugs in these groups did not show any oedema development at 5 hr post-trauma period. Such prevention of oedema, however, was not observed with cyproheptadine (5-HT receptor blocker) rather it aggravated the development of oedema (Table III). The administration of PG synthetase inhibitors and aminophylline leads to complete prevention of oedema development in unanaesthetised animals (group IV) and partial prevention in euthermic anaesthetised animals (group II), at 5 hr post-trauma period (Table III). Interestingly, the administraion of PG synthetase inhibitors was not effective in suppressing oedema formation at 2 hr post-trauma period in group II (Table III). These results indicate, in general, that 5-HT and PGs are involved in a temporal manner in the dynamic processes of oedema formation. In anaesthetised group (group II), following trauma, the plasma and brain 5-HT were also determined following drug treatments. The treatment with PG synthetase inhibitors (indomethacin and paracetamol) and aminophylline showed

TABLE III : Shows the effects of drug treatments on brain oedema (n=5) as well as on 5-HT in plasma and brain (n=5) in euthermic anaesthetised animals (A) and the same on brain oedema in unanaesthetised animals (B).

Treatment	*% brain swelling from intact control*		*5–HT level*		
	Right injured half	*Left uninjured half*	*Plasma µg/ml*	*Brain µg/g*	
				Right injured half	*Left uninjured half*
(A) *Ambient temperature 17°-20°C : B.O.D. temperature 30°C*					
Intact conrol	—	—	0.22±0.02	1.24±0.06	1.12±0.10
Injury 2 hr	+5.28***	+5.42***	0.59±0.05*** (+168.18)	2.28±0.20*** (+83.87)	2.28±0.18 (+103.57)
Indomethacin+ 2 hr Injury	+8.00***	+4.59***			
Paracetamol+ 2 hr Injury	+8.10***	+8.84***			
5 hr Injury	+7.65***	+7.98***	0.86±0.08*** (+290.90)	3.80±0.14*** (+206.45)	4.16±0.22** (+271.42)
Indomethacin+5 hr Injury	+3.70	+4.28**	0.90±1.03 (+309.09)	2.60±0.33*** (+109.67)	2.34±0.22*** (+108.92)
Paracetamol+5 hr Injury	+4.70**	+5.04**	0.72±0.33** (+227.27)	2.25±0.42** (+81.45)	2.46±0.34*** (+119.64)
pCPA+5 hr Injury	—0.17	—0.08	0.29±0.03** (+31.81)	1.08±0.13 (—12.90)	1.13±0.09 (+0.89)
Cyproheptadine+5 hr Injury	+9.55***	+10.35***	3.60±0.89*** (+1536.36)	6.15±2.25*** (+395.96)	3.13±0.47*** (+179.46)
Aminophylline+5 hr Injury	+5.76**	+6.58***	1.78±0.74*** (+709.09)	3.44±0.43*** (+177.41)	2.82±0.13*** (+151.78)
(B) *Ambient temperature 25°–29°C (unanaesthetised) (n=5)*					
Intact control	—	—			
Injury 5 hr	+5.23	+1.61			
Indomethacin+5 hr Injury	—4.81***	—6.34***			
Paracetamol+5 hr Injury	—2.20*	—2.68*			
pCPA+5 hr Injury	—4.05***	—5.22***			
Cyproheptadine+5 hr Injury	+14.95***	+13.31***			
Aminophylline+5 hr Injury	—3.35**	—2.48**			

*** $P<0.001$ Figures in parentheses indicate % change from intact control

** $P<0.01$ Values are expressed as Mean ± S.D.

* $P<0.05$

reduction of brain oedema accompanied with less increase in brain 5-HT, although the increase in plasma 5-HT level was not attenuated. But in case of pCPA treatment, the complete prevention of oedema development was closely associated with complete prevention of increase in 5-HT in brain as well as in plasma.

In case of cyproheptadine treatment, the aggravation of oedema development was closely associated with profound increase in plasma and brain 5-HT.

Changes in BBB permeability : Mild diffused extravasation of Evans blue was noted across pial vessels below the impact zone which extended to over all the dorsal surface of cerebral cortex in a few cases. This increased extravasation of Evans blue was not modified following exposure of animals to different ambient temperature or drug treatments.

DISCUSSION

The present results indicate that the development of oedema following closed injury in anaesthetised animals appears to be aggravated (following exposure to low ambient temperature) by allowing the fall in core body temperature, the prevention of which, led to the marked attenuation of oedema development or the oedema remained for a short period. This observation may have some clinical significance in the management of cerebral oedema in unconscious persons showing signs of hypothermic state.

A close association between the oedema development and the temporal pattern of rise in brain and plasma 5-HT level has been observed. Thus, the animals which showed slow development of oedema, the plasma and brain 5-HT have also shown a gradual increase. The animals in which the oedema continued to persist for over 5 hr post-trauma period, the brain and plasma 5-HT level also remained at a high sustained level throughout the period of oedema. Such relationship has earlier been also reported by us in open injury (6). The development of oedema following closed injury is more severe in unanaesthetised animals as compared to euthermic anaesthetised animals. Interestingly, increase in brain 5-HT only occurred in injureh half of brain unlike in anaesthetised one, in which the 5-HT was increased in both halves of brain. The reasons for such selective increase of 5-HT in brain in unanaesthetised animals following closed injury is not clear at present.

The absence of oedema formation alongwith no increase in brain and plasma 5-HT level following infliction of closed injury in animals pretreated with pCPA further strengthens the view that the accumulation of 5-HT may be one of the causative factors

for initiating the processes of oedema formation which has also been supported with our earlier findings (6) and by other workers (2, 5, 9, 11, 12).

The remarkable prevention of oedema formation at 5 hr post-trauma period in euthermic unanaesthetised animals has been observed following administration of indomethacin, paracetamol and aminophylline. It is also interesting to note that, following these drug treatments, there was not only complete prevention of oedema development, rather there was even some degree of reduction in water content of brain even from the control value. But such complete protective effects of these drugs were only observed in unanaesthetised injured animal, while in euthermic anaesthetised injured animals, a partial reduction of oedema was observed which corresponded more or less closely with the partial diminution of the increased 5-HT level in both halves of brain, rather than with the plasma 5-HT level.

Further it was observed that the administration of PG synthetase inhibitors at an earlier period after trauma (i.e. 2 hr post-trauma period) was unable to reduce the oedema formation. Similar observations have been reported (6) earlier and recently Iannotti *et al.* (7) suggested the effectiveness of PGs in inhibiting the development of ischaemic oedema in its late phase, but not in the early phase, as observed in gerbil.

Cyproheptadine (5-HT receptor blocker) has shown deleterious effect in terms of aggravation of oedema development. Thus in euthermic anaesthetised animals the oedema development exceeded by 25 and 30% more in right injured and left uninjured halves respectively, the increase of 5-HT in plasma and in right injured brain was excessively high e.g., 528 and 192% respectively, from the control injured group. The uninjured half of brain showed no increase beyond the control injured group This untoward effect of cyproheptadine administration was more pronounced in euthermic unanaesthetised injured group. Thus, the development of oedema in right injured brain increased by 161% more than the control injured, and in case of left uninjured half of brain, the increase in oedema was 611% as compared to control uninjured group. These results appear to indicate that cyproheptadine exerts more action on the uninjured half of brain relative to the injured one. The similar enhancement of oedema development during cerebral ischaemia in gerbils has been reported at the end of 3 hr and 6 hr ischaemic period but was associated with significant diminution of 5-HT in both halves of brain (12). Thus, the mechanisms of action of 5-HT receptor blocker drug seems to be difficult to explain in relation to changes in brain 5-HT.

The effect of administration of aminophylline in preventing or suppressing the oedema development observed at 5 hr post-trauma period was comparatively much less than with indomethacin or paracetamol, and was associated correspondingly with much less diminution of increased 5-HT level of injured half of brain, moreover, the plasma 5-HT level remained high by 145% more than the control injured group. Earlier Kogure *et al.* (8) and Welch *et al.* (12) studied the effect of aminophylline, and another PDE inhibitor

BL-191 on the development of oedema in gerbil following one common carotid artery occlusion. The former have shown that the oedema development occurring 5 min after cerebral ischaemia produced by occlusion of one carotid artery in gerbil could be prevented with aminophylline administration but the brain 5-HT level was attenuated to only 20% from the increased level observed in control occluded group. But when the effect of aminophylline was examined at the end of 24 hr period following carotid occlusion, it rather aggravated the oedema development (8). The effect of BL-191 was also found to aggravate the oedema development in occluded hemisphere, although the 5-HT concentration in the same occluded hemisphere was diminished to a low level even from the control value (12). Thus the mechanisms of action of PDE inhibitors, in general are not consistently related with the brain 5-HT level.

At present, the relative difference observed with regard to the efficacy of PG synthetase inhibitors and PDE inhibitors between unanaesthetised and anaesthetised group is ill-understood.

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