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CENTRAL SEROTONERGIC AND HISTAMINERGIC MODULATION OF PERIPHERAL INFLAMMATION AND NOCICEPTION IN RATS

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Abstract : Possible central serotonergic and histaminergic modulation of acute peripheral inflammation was investigated in rats, adopting the formaldehyde-induced acute pedal inflammation as an experimental model. Intracerebroventricular (icv) administration of central inhibitory neurotransmitter, serotonin and its precursor, 5-hydroxytryptophan (5-HTP) attenuated the oedema volume and exudate protein content alongwith augmentation in pain threshold. On the contrary, cyproheptadine, a 5-HT-receptor antagonist and selective serotonin synthesis inhibitor, parachlorophenylalanine (PCPA) produced oedema augmenting and pro-nociceptive effects besides elevating the protein content of the exudate. Centrally administered histamine attenuated pedal oedema, nociception as well as protein concentration in oedema fluid. Cimetidine, an H_2 histaminergic receptor blocker did not produce any significant effect on inflammation.

Key words : serotonergic histaminergic formaldehyde inflammation intracerebroventricular

INTRODUCTION

The mechanisms underlying the initiation, maintenance and termination of inflammation are now well established (1). However, little is known about the role of central nervous system (CNS) in this regard. Central stimulants antagonise the inhibitory effects of general anaesthetics and hypnotics in inflammation (1). Thalamic and spinothalamic lesions in the patients have been associated with reduced flare response to histamine and mid-spinal transection abolished the early phase of carrageenin or formaldehyde-induced rat hind paw oedema, indicating that vasodilator component of inflammation is mediated by CNS (2). Central neural changes have been suggested to occur during the early phase after subcutaneous formalin injection (3). Evidence is now accumulating to show the existence of central neurogenic regulatory mechanism for peripheral inflammation (4). Since apparently, very little information is available regarding central influences mediating peripheral inflammation, this study was undertaken to evaluate the significance of the role played by central serotonergic and histaminergic neurotransmitters, if any, in the modulation of formaldehyde-induced inflammation in rats.

METHODS

The studies were conducted on adult male Sprague Dawley rats (150-200 g). The animals were caged individually at an ambient temperature of 25 ± 2°C with free-access to clean drinking water and balanced feed. Experiments were conducted at this temperature between 9 and 16 h. ICV cannulation was

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performed stereotaxically (5) under pentobarbitone sodium (40 mg/kg, ip) anaesthesia. Polyethylene cannulae were implanted into right lateral ventricles.

The following drugs with doses and pre-treatment times given in parenthesis were administered centrally : Histamine (20 µg, 15 min); Cimetidine (10 µg, 30 min); Serotonin (50 µg, 15 min); Cyproheptadine (50 µg, 30 min); 5-HTP (250 µg, 15 min) and PCPA (100 µg, daily for 3 days, 72 h). All the drugs were dissolved in sterile artificial cerebrospinal fluid (Sodium chloride -135.8 mM; Magnesium chloride -1.16 mM; Sodium bicarbonate- 21.0 mM; Sodium dihydrogen orthophosphate- 0.5 mM; Urea -2.2 mM and Glucose -3.4 mM, dissolved in distilled water) and a constant volume of 10 µL of the drug solutions was administered by icv injection. The control animals received an equivalent amount of artificial cerebrospinal fluid (CSF) through the same route.

Pedal inflammation was induced by injecting 0.1 ml of formalin (4% solution in 0.9% saline) below the planter aponeurosis of the hind paw of rats (2). The paw volume was recorded plethismographically, immediately prior to formalin injection, and then at 15 min, 30 min, 1 h and thereafter, at hourly interval upto 5 h. The increase in paw volume (expressed in ml) was taken as the index of inflammation. The pressure (expressed in g) in formalin injected paw, was recorded as pain threshold by Randal Selitto Assay before and at 1, 3 and 5 h post-formalin injection. Oedematous fluid was collected from the inflammed paws 6 h post-formalin injection and its protein content was determined (6).

After the termination of experiment, all the rats were administered 10 µL of 1% Evan's Blue dye solution icv and the brain was removed, sectioned and examined in order to ascertain the correct position of the cannulae in the ventricles.

Statistical analysis of the data was initially performed using Analysis of Variance (ANOVA). When the overall ANOVA was significant, Student's 't' test was applied to study the differences amongst the means (7).

RESULTS

Results of centrally administered drugs, influencing serotonergic and histaminergic systems on formaldehyde-induced pedal oedema are summarised in Table I. Serotonin significantly attenuated paw oedema throughout the observation period showing greater effect initially i.e. 15 min. 5-HTP also attenuated the oedema, the effect being significant from 3 h onwards. A significant increase in oedema volume was observed with cyproheptadine only

TABLE I : Effect of centrally administered drugs influencing serotonergic and histaminergic systems on formaldehyde-induced pedal oedema in rats.

Drug	*Oedema volume in ml (mean ± S.E.)*						
	15 min	*30 min*	*1 hr*	*2 hrs*	*3 hrs*	*4 hrs*	*5 hrs*
Serotonergic system							
Control	0.42 ± 0.03	0.50 ± 0.02	0.57 ± 0.03	0.61 ± 0.03	0.67 ± 0.04	0.74 ± 0.03	0.87 ± 0.03
Serotonin	0.27 ± 0.02***	0.34 ± 0.03**	0.43 ± 0.02**	0.47 ± 0.02**	0.55 ± 0.03*	0.64 ± 0.03*	0.77 ± 0.02*
5-HTP	0.41 ± 0.01	0.44 ± 0.03	0.50 ± 0.02	0.54 ± 0.03	0.56 ± 0.03*	0.61 ± 0.02**	0.74 ± 0.02**
Cyproheptadine	0.59 ± 0.04**	0.60 ± 0.04*	0.64 ± 0.02	0.67 ± 0.04	0.74 ± 0.03	0.83 ± 0.05	0.92 ± 0.03
PCPA	0.52 ± 0.02*	0.60 ± 0.04*	0.67 ± 0.03*	0.73 ± 0.03*	0.84 ± 0.05*	0.91 ± 0.05*	1.00 ± 0.04*
Histaminergic system							
Control	0.41 ± 0.02	0.46 ± 0.03	0.51 ± 0.02	0.59 ± 0.04	0.66 ± 0.03	0.71 ± 0.03	0.85 ± 0.04
Histamine	0.26 ± 0.03**	0.33 ± 0.03**	0.41 ± 0.03*	0.48 ± 0.03*	0.63 ± 0.04	0.71 ± 0.04	0.80 ± 0.04
Cimetidine	0.39 ± 0.02	0.43 ± 0.01	0.50 ± 0.03	0.58 ± 0.03	0.66 ± 0.02	0.68 ± 0.02	0.76 ± 0.03

n=6 animals in each group; *P<0.05; **P<0.01; ***P<0.001

for a brief period of 30 min, post-formaldehyde injection. PCPA significantly augmented the pedal oedema throughout the observation period.

Histamine, significantly attenuated the pedal oedema upto 2 h of formaldehyde injection. Cimetidine did not affect the formaldehyde-induced paw oedema.

Central effects of the drugs influencing serotonergic and histaminergic systems on formaldehyde-induced pain and on protein content of oedema fluid are summarised in Table II. Serotonin increased the pain threshold throughout the observation period whereas, anti-nociceptive effect of 5-HTP was clearly evident only at 3rd and 5th h. Cyproheptadine induced significant hyperalgesia at 1 h while PCPA showed the same effect through the entire period of observation. Serotonin and 5-HTP both decreased the formaldehyde-induced protein out flow into the oedema fluid significantly. On the contrary, cyproheptadine and PCPA significantly augmented the protein content of the exudate.

Histamine significantly increased the pain threshold whereas, cimetidine did not alter the formaldehyde-induced nociception. Histamine also decreased the protein concentration of the inflammatory exudate significantly. Cimetidine, on the other hand, failed to produce any change in the protein content of the exudate.

DISCUSSION

The formaldehyde model for inducing pedal inflammation was chosen for this study because it has been widely used as a noxious stimulus in tonic pain research and the development of oedema (8). Furthermore, evidence has been presented for the central neuronal changes to occur following subcutaneous formalin injection (3) and the supraspinal regulation of formalin-induced inflammation has been suggested (8).

Serotonin is thought to play a critical role as a central inhibitory neurotransmitter in brain (9). It has been reported to be a neurotransmitter in the neural tracts that inhibits pain signals (10). Centrally administered serotonin and its precursor, 5-HTP, significantly attenuated the paw oedema, nociception as well as the protein content of the exudate. Similarly, anti-inflammatory actions of central serotonin have been demonstrated on carrageenin-induced paw oedema (11) and formalin-induced nociception (12). On the other hand, the 5-HT antagonist, cyproheptadine and serotonin synthesis blocker, PCPA significantly augmented pedal oedema and protein content of the exudate and decreased pain threshold of formaldehyde-inflammed paws. These observations are substantiated by the reports of the oedema augmenting effects of PCPA, a 5-HT synthesis blocker and metergoline, a post-synaptic 5-HT receptor antagonist (11).

TABLE II : Effect of centrally administered drugs influencing serotonergic and histaminergic systems on formaldehyde-induced nociception and protein content of inflammatory exudate in rats.

Drug	*Pain threshold in g (mean ± S.E.)*				*Protein content of inflammatory exudate in mg/ml (mean ± S.E.)*
	0 hr	*1 hr*	*3 hr*	*5 hr*	
Serotonergic system					
Control	102.50 ± 6.92	61.67 ± 5.43	53.33 ± 4.01	44.17 ± 4.90	48.63 ± 2.02
Serotonin	104.17 ± 6.51	85.83 ± 5.39**	75.00 ± 5.92*	63.33 ± 4.22*	36.47 ± 2.68**
5-HTP	105.83 ± 6.51	70.83 ± 7.24	68.33 ± 4.94*	64.17 ± 2.71**	38.04 ± 1.85**
Cyproheptadine	100.83 ± 7.90	42.50 ± 4.23*	41.67 ± 5.58	38.33 ± 4.94	57.56 ± 1.97*
PCPA	99.17 ± 5.83	45.83 ± 4.36*	37.50 ± 4.23*	31.67 ± 2.47*	57.54 ± 2.80*
Histaminergic system					
Control	97.50 ± 8.04	60.00 ± 6.58	50.83 ± 6.11	45.00 ± 7.30	47.25 ± 2.66
Histamine	103.33 ± 6.91	85.83 ± 3.96**	63.33 ± 6.79	55.83 ± 7.00	36.38 ± 2.41*
Cimetidine	97.50 ± 8.64	67.50 ± 8.24	56.67 ± 6.01	49.17 ± 4.73	45.98 ± 2.15

n=6 animals in each group; *P<0.05; **P<0.01; ***P<0.001

Histamine has been reported to have several neuromodulatory effects in the CNS (13). In the present study, icv administered histamine significantly reduced pedal oedema and the protein content of the oedema fluid producing a corresponding increase in pain threshold. Our results support the earlier finding of the analgesic (14) and anti-inflammatory (15) action of centrally administered histamine. Central administration of cyproheptadine, a 5-HT antagonist, possessing, in addition, H_1 histaminergic receptor antagonistic property, significantly increased the paw volume and protein content of the inflammatory exudate and decreased the pain threshold. On the other hand, H_2-receptor antagonist, cimetidine, failed to alter the inflammatory response to formaldehyde. These observations are in accordance with the pro-inflammatory (15) and hyperalgesic (16) actions of H_1-receptor antagonist, mepyramine, on carrageenin-induced inflammation. Cimetidine was not found to produce any effect on the inflammatory response in both the studies. Thus, our results favourably suggest the central modulatory role of histamine H_1-receptors on peripheral inflammation, which needs further elucidation.

In conclusion, the present study demonstrates that the central serotonergic and histaminergic systems exert a modulatory anti-inflammatory and nociceptive actions on formaldehyde-induced peripheral inflammation in rats. The study further reveals conclusively that CNS is capable of modulating peripheral inflammation.

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