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CENTRAL NORADRENERGIC AND CHOLINERGIC MODULATION OF FORMALDEHYDE-INDUCED PEDAL INFLAMMATION AND NOCICEPTION IN RATS

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Abstract : Possible central noradrenergic and cholinergic modulation of acute peripheral inflammation was investigated in rats, adopting the formaldehyde-induced pedal inflammation as the experimental model. Intracerebroventricularly (icv) administered noradrenaline (NA), α-adrenoceptor agonist, L-phenylephrine, α-2 adrenoceptor agonist, clonidine and non-selective β-adrenoceptor blocker, propranolol, suppressed formaldehyde-induced inflammation producing a decrease in oedema volume and increase in pain threshold. Conversely, both noradrenergic neuron degenerator, 6-hydroxydopamine (6-OHDA) and non-selective α-adrenoceptor antagonist, phenoxybenzamine produced an increase in paw oedema alongwith an augmentation of pain. Significant oedema augmenting effects were also produced by central excitatory neurotransmitter, acetylcholine (ACh) on icv administration. ACh also produced pro-nociceptive action. An ACh antagonist, scopolamine and ACh synthesis inhibitor, hemicholinium-3 (HC) reduced pedal oedema and produced analgesia.

The results of this study indicate that central NA exerts an inhibitory effect on peripheral oedema and pain whereas, ACh has an augmenting effect on formaldehyde-induced peripheral inflammation.

Key words : formaldehyde-induced oedema pain cholinergic systems noradrenergic

INTRODUCTION

The role played by autonomic nervous system in peripheral inflammation is now well established and the mechanism underlying it has been studied in detail (1). Unlike the peripheral influences modulating inflammation, very little is known about the role of the central nervous system (CNS) in this regard. Central stimulants antagonise the inhibitory effects of general anaesthetics and hypnotics on inflammation (2). Thalamic and spinothalamic lesions in patients have been associated with reduced flare response to histamine. In rats, the mid-spinal transection abolished the early phase of carrageenin/or formaldehyde-induced hind paw oedema. These clinical and experimental observations indicate that vasodilator component of inflammation is mediated by CNS (3). Central neural changes have been suggested to occur during early phase of inflammation after subcutaneous formalin injection (4). Evidence is now accumulating to show the existence of central neurogenic

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mechanism for peripheral inflammation (5). In a relatively recent study, it has been observed that terminals of primary afferents are important in the development of acute joint inflammation since dorsal rhizotomy attenuated inflammatory response in knee joint and sympathetic nervous system is not involved in acute inflammatory phase of kaolin-carrageenin-induced arthritis (6). Further, it has been demonstrated that medullary dorsal horn α-2 adrenoceptors participate in the process of perception of intensity of noxious thermal stimulation in monkeys (7). 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 noradrenergic and cholinergic neuro-transmitters, if any, in 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 with free access to clean drinking water and balanced feed. Experiments were conducted at an ambient temperature of 25±2°C between 9.0 and 16.0 hrs. Intracerebroventricular (ICV) cannulation was performed in pentobarbitone sodium (40 mg/kg, i.p.), anaesthetized rats. Polyethylene cannula was inserted into the right lateral ventricle stereotaxically (8). The cannulated rats were divided into groups of six each for control and for different pharmacological agents.

The following drugs, with the dose and pretreatment time (period before administration of phlogistic agent) given in parenthesis, were administered centrally: Noradrenaline (NA, Sigma) (50 µg, 15 min), L-phenylephrine (Sigma) (50 µg, 15 min), clonidine (Sigma) (50 µg, 15 min), phenoxybenzamine (SKF) (50 µg, 15 min), propranolol (John Baker Inc.) (50 µg, 15 min), 6-hydroxydopamine (6-OHDA, Sigma) 375 µg, 48 h), Acetylcholine (ACh, Sigma) (50 µg, 15 min), scopolamine (Sigma) (10 µg, 15 min), and hemicholinium-3 (HC, Sigma) (20 µg, 45 min). All the drugs were dissolved in sterile artificial cerebrospinal fluid (Sodium chloride, 138.5 mM; Potassium chloride, 3.35 mM; Calcium chloride, 1.26 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 triple glass distilled water, pH 7.3, temperature: 37°C) and a constant volume of 10 µL of drug solutions were administered by a single icv injection. Control animals received an equivalent amount of artificial cerebrospinal fluid (CSF) through the same route.

Pedal inflammation was induced by injecting formalin (0.1 ml of 4% solution in 0.9% saline) below the planter aponeurosis of the hind paw (3). The increase in paw volume, after injection of phlogistic agent, was taken as the index of inflammation (expressed in ml). The paw volume of the rats upto the ankle joint was recorded by a standard volumetric technique, using calibrated plethysmometer (Ugo Basile, Varese, Italy), immediately prior to formalin injection (0 min, basal volume), then at 15 min, 30 min, 1 h and thereafter every one h upto 5 h. The change in paw volume in test groups was compared with that of untreated control group. The results are expressed as mean increase over basal ± S.E. The pressure in g in the formalin injected paw was recorded as pain threshold by Randall Selitto Assay (9), immediately prior to and 1, 3 and 5 h post-formalin injection. In brief, pain threshold was measured by applying pressure to inflammed paw at steadily increasing rate by means of pedal switch of Randall-Selitto apparatus (Ugo Basile, Varese, Italy). The end-point or "pain threshold" is defined as pressure necessary to cause animals to struggle. The change in pain threshold in test groups was compared with that of untreated control group.

After termination of the experiments, 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 cannula 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 (10).

RESULTS

The results of icv administered drugs on formaldehyde-induced pedal oedema are summarised in Table I. NA, and L-phenylephrine suppressed pedal oedema throughout the observation period, whereas clonidine and propranolol significantly decreased oedema volume at the later stage of observation (between 3 and 5 h). Conversely, phenoxybenzamine and 6-OHDA produced significant increase in oedema volume throughout the 5 h observation period.

ACh produced a significant increase in pedal oedema, having relatively more effect at the initial stage of inflammation. Scopolamine and

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

Drugs	*Oedema volume in ml (Mean ± S.E.)*						
	15 min	*30 min*	*1 h*	*2 h*	*3 h*	*4 h*	*5 h*
Noradrenergic system							
Control	0.33 ± 0.04	0.37 ± 0.03	0.50± 0.03	0.58 ± 0.04	0.74 ± 0.03	0.85 ± 0.03	1.01 ± 0.03
Noradrenaline	0.14 ± 0.02**	0.23 ± 0.03**	0.36 ± 0.02**	0.42 ± 0.03**	0.57 ± 0.03**	0.68 ± 0.03**	0.86 ± 0.03**
L-phenylephrine	0.14 ± 0.02**	0.22 ± 0.02**	0.38 ± 0.02*	0.46 ± 0.02*	0.62 ± 0.03*	0.75 ± 0.04	1.03 ± 0.06
Clonidine	0.25 ± 0.04	0.32 ± 0.03	0.46 ± 0.06	0.56 ± 0.05	0.64 ± 0.02*	0.72 ± 0.03*	0.83 ± 0.05**
Phenoxybenzamine	0.45 ± 0.03*	0.49 ± 0.02*	0.61 ± 0.02*	0.69 ± 0.02*	0.85 ± 0.03*	0.96 ± 0.03*	1.14 ± 0.04*
Propranolol	0.28 ± 0.03	0.37 ± 0.02	0.47 ± 0.02	0.54 ± 0.02	0.65 ± 0.02*	0.74 ± 0.02*	0.92 ± 0.02*
6-OHDA	0.43 ± 0.02*	0.49 ± 0.03*	0.60 ± 0.03*	0.73 ± 0.04*	0.90 ± 0.03*	1.02 ± 0.03**	1.19 ± 0.03***
Cholinergic system							
Control	0.44 ± 0.02	0.50 ± 0.03	0.57 ± 0.03	0.64 ± 0.02	0.70 ± 0.03	0.79 ± 0.03	0.89 ± 0.03
Acetylcholine	0.68 ± 0.03***	0.71 ± 0.03***	0.76 ± 0.02***	0.81 ± 0.03**	0.90 ± 0.03**	0.92 ± 0.03*	0.99 ± 0.93*
Scopolamine	0.14 ± 0.03***	0.17 ± 0.01***	0.23 ± 0.02***	0.49 ± 0.04**	0.53 ± 0.06*	0.64 ± 0.04*	0.71 ± 0.07*
Hemicholinium-3	0.23 ± 0.02***	0.22 ± 0.03***	0.26 ± 0.04***	0.50 ± 0.04**	0.62 ± 0.04	0.78 ± 0.05	0.84 ± 0.03

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

TABLE II : Effect of centrally administered drugs influencing noradrenergic and cholinergic systems on formaldehyde-induced pedal oedema in rats .

Drugs	*Pain threshold in g (Mean ± SE)*			
	0 h	*1 h*	*3 h*	*5 h*
Noradrenergic system				
Control	98.33 ± 6.41	63.33 ± 5.27	56.67 ± 4.97	46.67 ± 3.33
Noradrenaline	89.17 ± 3.00	86.67 ± 3.07**	71.67 ± 3.80*	60.00 ± 4.08*
L-phenylephrine	102.50 ± 8.04	86.67 ± 3.33**	70.00 ± 2.88*	60.83 ± 7.24
Clonidine	89.17 ± 5.83	76.66 ± 7.03	70.83 ± 3.52*	62.50 ± 3.67**
Phenoxybenzamine	100.83 ± 7.00	46.67 ± 3.67*	40.00 ± 4.08*	33.33 ± 3.57*
Propranolol	91.67 ± 7.03	77.50 ± 5.12	70.83 ± 3.00*	67.50 ± 6.68*
6-OHDA	97.50 ± 9.29	38.33 ± 7.38*	35.83 ± 3.75**	24.16 ± 4.36**
Cholinergic system				
Control	98.33 ± 6.91	61.67 ± 3.07	52.50 ± 3.59	46.67 ± 3.57
Acetylcholine	92.50 ± 8.92	36.67 ± 3.80***	27.50 ± 4.23**	25.00 ± 6.83*
Scopolamine	105.00 ± 8.16	78.33 ± 3.33**	72.50 ± 5.88*	65.00 ± 5.47*
Hemicholinium-3	96.67 ± 9.80	79.17 ± 3.00**	68.33 ± 8.57*	52.50 ± 5.88

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

HC showed inhibitory effects. Whereas, HC attenuated oedema volume only upto 2 h post-formalin injection, the action of the scopolamine was evident throughout the period with greater activity, initially.

Central effects of drugs influencing nor-adrenergic and cholinergic systems on formaldehyde-induced pain are summarised in Table II. NA, L-phenylephrine, clonidine and propranolol, significantly increased the pain threshold. Though the effect of NA was discernible throughout the period of observation, L-phenylephrine inhibited the pain perception at 1 and 3 h while clonidine and propranolol, decreased nociception at the later part of the observation. On the other hand, phenoxy-benzamine and 6-OHDA decreased pain threshold throughout the observation period.

Centrally given ACh significantly accentuated pedal pain throughout the observation period. Conversely, scopolamine and HC produced significant attenuation in pain. Scopolamine showed its effect for full term of 5 h while, the action of HC was evident only upto 3 h.

DISCUSSION

The formalin model of inducing pedal inflammation was chosen for this study as it is widely used as a noxious stimulus for inducing pain and in the development of oedema (11). Further, central neuronal changes are known to occur following subcutaneous injection of formalin (4) and supra-spinal regulation of formalin-induced inflammation has also been suggested (11). In the present study, injection of formalin into hind paw of rats produced localized inflammation and pain. Formalin showed a mean increase in the oedema volume and hyperalgesia of an increasing order upto the observation period of 5 h (Table I).

NA has gained wide acceptance as a neurotransmitter in mammalian CNS (12). In the present study, NA significantly inhibited paw oedema and hyperalgesia produced by formaldehyde. NA is reported to have either little (13) or no role (14) in increased plasma extravasation. The involvement of central NA in mitigating peripheral inflammation is further substantiated by a similar response to L-phenylephrine, a selective α-1 adrenoceptor, agonist. Probably, NA might bring about the anti-inflammatory and anti-nociceptive effect through α-1 adrenoceptors as evidenced in the present study, and by the enhancement of the oedema response and nociception to chemical denervation by 6-OHDA (13) and by increased rat brain NA level during early phase of carrageenin-induced inflammation (15). The involvement of α-adrenoceptor is supported by augmentation of oedema as well as nociception by central administration of non-selective adrenoceptor antagonist, phenoxybenzamine (13). Interestingly, however, clonidine, an α-2 adrenoceptor agonist which suppresses the sympathetic outflow in brain (16) significantly reduced the oedema volume as well as the pain response. This finding is in accordance with the observation of Tasker and Medzack (17) who suggested the involvement of α-1 adrenoceptors in clonidine-induced analgesia in formalin test, since the clonidine effect was antagonised by α-1 antagonist, prazosin but not by yohimbine.

With a view to assessing the role of β-adrenoceptors, a nonselective β-adrenoceptor blocker, propranolol was used which was found to decrease oedema volume and pain response significantly at 3-5 h. A similar anti-inflammatory response to carrageenin-induced oedema by propranolol has been reported earlier (18). However, this effect was not related to its β-adrenoceptor blockade but to its other activities such as anti-prostaglandin effect since another β-adrenoceptor blocker timolol and β-2 agonist, terbutaline were also found to inhibit the oedema response to carrageenin (18). The present observations, thus suggest the involvement of NA in peripheral inflammation.

ACh plays an important role as a neuro-transmitter in the CNS (12). Centrally administered ACh significantly increased formaldehyde-induced pedal oedema of rats alongwith a decrease in pain threshold.

Increased central cholinergic activity is known to be reflected by enhanced peripheral cholinergic activity which induced vasodilation (19). Further, cholinergic activation has been shown to elevate intracellular cyclic GMP which is pro-inflammatory (20). This could be possible mechanism underlying inflammation augmenting effect of ACh. The involvement of ACh is further substantiated on icv administration of scopolamine, a muscarinic cholinergic antagonist and HC, an ACh synthesis inhibitor which produced significant inhibitory effects on rat paw oedema and pain. These results indicate that the central cholinergic system in rats exerts a pro-inflammatory effect on peripheral inflammation. The observed effects of centrally administered ACh, scopolamine and HC on peripheral inflammation do not appear to be due to any peripheral leakage effect because ACh, HC and atropine were not found to produce any such effect when administered intraperitoneally (21).

The central neuro-transmitter systems do not function in isolation but intensive interconnections are known to exist between nor-adrenergic and cholinergic neurons in rat brain (22). Presence of cholinergically activated nor-adrenergic inhibitory systems has been suggested in rat cortex (22). Further, HC has been reported to enhance central nor-adrenergic activity (23) and the mechanism involved in the anti-inflammatory effect of HC, may also be attributed to this action.

In conclusion, the present study demonstrates that central nor-adrenergic system exerts anti-inflammatory and antinociceptive effects whereas, central cholinergic system shows a pro-inflammatory and pro-nociceptive action on formaldehyde-induced peripheral inflammation in rats. The study further reveals conclusively that the CNS is capable of modulating peripheral inflammation.

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