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Effect of some centrally administered putative amino acid neurotransmitters on carrageenan-induced paw oedema in rats

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The possible modulatory effect of central amino acid neurotransmitters on carrageenan-induced paw inflammation has been investigated in rats. Eight putative amino acid neurotransmitters were administered intracerebroventricularly and their effect on the peripheral inflammation was noted. The inhibitory amino acid transmitters, GABA, glycine and taurine attenuated the peripheral oedema, while the excitatory amino acid transmitters, glutamic acid and aspartic acid had a pro-inflammatory effect. However, the other putative amino acid neurotransmitters, proline and alanine (inhibitory) and cysteic acid (excitatory) did not affect carrageenan-induced oedema.

The cascade of events, mediated by various autacoids, culminating in inflammation and the different factors which serve to initiate, sustain or limit the inflammatory response are now fairly well defined (Bonta 1978). Unlike the peripheral influences modulating inflammation, little is known about the role of the central nervous system (CNS), if any, in peripheral inflammation (Bonta 1978). Schizophrenics are known to have an unusually low incidence of rheumatoid arthritis and to show reduced inflammatory response to injury or infection, and to exhibit minimal wheal-flare response to intradermal histamine (Horrobin 1977). General anaesthetics and narcotic analgesics have been reported to have anti-inflammatory effects in rats (Fearn et al 1965). There are no studies on the possible modulatory effect of central neurotransmitters on peripheral inflammation apart from some recent communications from this laboratory indicating that central prostaglandins (Bhattacharya & Das 1984) and acetylcholine (Das & Bhattacharya 1985) exert a pro-inflammatory effect on carrageenan-induced paw oedema in rats. Conversely, 5-HT (Bhattacharya & Das 1985a) and histamine (Bhattacharya & Das 1985b), administered centrally, attenuate the oedema.

Biochemical, electrophysiological and pharmacological studies indicate that certain amino acids may serve as neurotransmitters or neuromodulators in the mammalian CNS. These amino acid neurotransmitter candidates have been classified as inhibitory or excitatory, depending upon the electrophysiological response to the agent. Inhibitory transmitters cause hyperpolarization or partial depolarization of nerve cells, inhibiting cell firing, whereas excitatory transmitters cause depolarization sufficient to generate an action potential (Enna 1979).

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Since our studies suggest that central neurotransmitters may modulate peripheral inflammation, we now report the effect of centrally administered putative amino acid neurotransmitters on carrageenan-induced paw inflammation.

Materials and methods

The studies were conducted on inbred Wistar strain albino rats (120–180 g) of either sex. The rats were housed in colony cages at an ambient temperature of 25 ± 2 °C and fed on standard pellet chow. Experiments were conducted at this ambient temperature between 0900 and 1400 h. Paw inflammation was induced by carrageenan (0·1 ml of 1% suspension in 0·9% saline) injected below the plantar aponeurosis of the hind paws (Winter et al 1962). The paw volume, up to the ankle joint, was measured before and at hourly intervals for 4 h after carrageenan administration by means of a mercury plethysmograph. The increase in paw volume has been expressed in units, each unit representing 1 cm (volume = 0·075 ml) length of the displaced mercury column.

Intracerebroventricular (i.c.v.) cannulation of the right lateral ventricle was performed in pentobarbitone sodium (40 mg kg^{-1} i.p.) anaesthetized rats and an indwelling cannula was stereotaxically inserted (Feldberg & Lotti 1967). The rats were used a week after the cannulation. All the drugs used were administered i.c.v. dissolved in 10 μl of artifical cerebrospinal fluid (CSF). Control animals received an equivalent volume of artificial CSF via the same route.

The following amino acids were used: γ-aminobutyric acid (GABA), glycine, taurine, L-proline, L-alanine, L-glutamic acid, L-aspartic acid and L-cysteic acid. Graded doses of these amino acids were administered i.c.v., 30 min before the administration of carrageenan. In a separate experiment, the same doses were administered i.p. 30 min before the induction of inflammation, so as to note their peripheral effects, if any.

Statistical significance of the data was evaluated by use of the Student's two tailed *t*-test.

Results and discussion

The results are summarized in Table 1. GABA (10, 20 and 50 μg), glycine (10, 20 and 50 μg) and taurine (20 and 50 μg), administered i.c.v., produced a dose-related attenuation of carrageenan-induced oedema. However, the anti-inflammatory effect was statistically significant with the last two doses of GABA and glycine, and the

Table 1. Effects of some i.c.v. administered putative amino acid neurotransmitters on carrageenan-induced paw inflammation in rats.

Groups		n	Increase in paw volume in units (mean ± s.e.m.) 1 h	2 h	3 h	4 h
Control (vehicle)		10	1·76±0·14	2·82±0·19	3·49±0·21	3·04±0·16
GABA	10 μg	6	1·51±0·16	2·50±0·12	3·26±0·18	2·62±0·14
	20 μg	6	1·24±0·12^{a}	2·08±0·11^{b}	2·66±0·19^{c}	2·19±0·1^{c}
	30 μg	6	0 89±0·09^{c}	1·68±0·1^{c}	2·06±0·11^{b}	1·72±0·12^{c}
Glycine	10 μg	6	1·59±0·14	2·61±0·12	3·32±0·22	2·84±0·19
	20 μg	6	1·39±0·11	2·26±0·09^{a}	2·79±0·16^{a}	2·42±0·12^{b}
	50 μg	6	1·12±0·09^{b}	1·89±0·11^{c}	2·22±0·12^{c}	2·12±0·13^{c}
Taurine	20 μg	6	1·48±0·16	2·56±0·16	3·19±0·23	2·72±0·18
	50 μg	6	1·26±0·14^{a}	2·19±0·13^{a}	2·52±0·12^{b}	2·38±0·13^{b}
Proline	20 μg	5	1·64±0·18	2·76±0·16	3·21±0·22	2·98±0·19
	50 μg	5	1·54±0·14	2·62±0·15	3·10±0·17	2·81±0·16
Alanine	20 μg	5	1·81±0·12	2·79±0·12	3·42±0·19	3·02±0·16
	50 μg	5	1·68±0·13	2·68±0·14	3·29±0·16	2·96±0·12
Glutamic acid	10 μg	6	1·86±0·11	2·94±0·16	3·59±0·19	3·16±0·16
	20 μg	6	2·34±0·1^{b}	3·38±0·12^{a}	3·97±0·11	3·56±0·12^{a}
	50 μg	6	2·58±0·12^{c}	3·96±0·11^{c}	4·32±0·19^{b}	3·88±0·14^{b}
Aspartic acid	10 μg	5	1·72±0·18	2·94±0·16	3·56±0·17	3·19±0·13
	20 μg	6	2·09±0·1	3·21±0·15	3·82±0·12	3·48±0·13
	50 μg	6	2 39±0·11	3·62±0·13^{b}	4·14±0·12^{a}	3·66±0·12^{b}
Cysteic acid	20 μg	5	1·80±0·17	2·86±0·14	3·46±0·19	3·12±0·19
	50 μg	5	1·92±0·18	2·94±0·13	3·66±0·15	3·19±0·12

a, b and c indicate statistical significance (P) at $<0{\cdot}05$, $<0{\cdot}01$ and $<0{\cdot}001$, respectively, in comparison to the respective vehicle-treated control group. Figures without superscripts indicate that the values are statistically insignificant when compared to the respective vehicle-treated control group.

latter dose of taurine. The other two inhibitory amino acid transmitter candidates, proline and alanine, failed to affect carrageenan oedema at doses of 20 and 50 μg. There was no significant difference in the anti-inflammatory effects of GABA, glycine and taurine. Conversely, glutamic acid (10, 20 and 50 μg) and aspartic acid (10, 20 and 50 μg) induced a dose-related increase in the inflammatory response of carrageenan. However, this pro-inflammatory effect was statistically significant with the last two doses of glutamic acid and the last dose of aspartic acid only. The third excitatory amino acid transmitter candidate used, cysteic acid (20 and 50 μg), did not affect carrageenan-induced paw oedema.

The carrageenan model of acute inflammation was chosen for this study because of its wide use as an experimental model for the assay of anti-inflammatory agents. Furthermore, an excellent correlation exists between the anti-inflammatory effects of a number of drugs, when tested by this method, and their clinical efficacy in rheumatoid arthritis (Lombardino et al 1975).

Substantial evidence has now accumulated suggesting that certain amino acids may serve as neurotransmitters in the mammalian CNS. However, apart from GABA, little is yet known about the functional importance of these amino acids in the brain (Enna 1979). Several practical constraints, including the lack of specific pharmacological tools affecting these amino acid systems and their structural and electrophysiological similarities, serve as hindrances. Of the large number of amino acids which have been isolated and identified from the mammalian CNS, five, namely GABA, glycine, taurine, glutamic acid and aspartic acid, fulfil several of the criteria required of a putative central neurotransmitter (Enna 1979). Of the eight amino acids used in the present study, these were the ones which showed significant anti- or pro-inflammatory effects on carrageenan-induced paw oedema. It may not be entirely serendipitous that the inhibitory amino acids, GABA, glycine and taurine, attenuate the peripheral inflammation when they are administered centrally, while the excitatory amino acids, glutamic acid and aspartic acid, accentuate the oedema. It is not clear why the other amino acids, proline and alanine (inhibitory) and cysteic acid (excitatory), failed to affect carrageenan oedema. However, there is little evidence as yet to suggest that these amino acids function as central neurotransmitters (Enna 1979).

None of the amino acids used in this study affected carrageenan-induced inflammation when they were administered peripherally in the doses used for central administration. This suggests that the observed anti- or pro-inflammatory effects of the amino acids were not due to peripheral leakage. The present findings are too preliminary to hazard a guess on the possible mechanism(s) of the observed effects of the amino acids on carrageenan oedema. Except for glycine, which has the highest distribution in the medulla and spinal cord, the other amino acids GABA, taurine, glutamic acid and aspartic acid are found in reasonably high concentrations throughout the mammalian CNS (Enna 1979). As such, one might expect that they impinge upon and receive inputs from neurons using most, if not all, of the other transmitters involved in CNS transmission. GABA is known to interact with the central cholinergic, catecholaminergic and 5-HT systems, though the reports are equivocal (Iversen 1978; Pradhan & Bose 1978). It is not clear whether the other amino acids also show a similar interaction. Earlier studies from this laboratory have indicated that central 5-HT (Bhattacharya & Das 1985a) noradrenaline (Das & Bhattacharya 1983) and histamine (Bhattacharya & Das 1985b) exert a modulatory inhibitory influence over peripheral inflammation, while central prostaglandins (Bhattacharya & Das 1984) and acetylcholine (Das & Bhattacharya 1985) exert a modulatory pro-inflammatory effect. It may be possible that the observed effects induced by the centrally administered amino acid transmitter candidates, are through one or more of these neuro-transmitter systems rather than direct effects of their own. Further studies are required to resolve this question. It has been stressed that these amino acids appear to act as transmitters only in the CNS (Enna 1979), making the possibility of central modulation of peripheral inflammation decidedly attractive enough to warrant further studies in this direction.

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Effect of some vasodilators on cat femoral arteries

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Adenosine, cyclic-AMP (cAMP), papaverine and 1-methyl-3-isobutylxanthine (MIX) evoked dose-dependent vasodilatation in cat femoral arteries precontracted with 75 mM K^+. The vasodilator response induced at maximal concentration used was: papaverine > MIX > adenosine = cAMP. With regard to the potency of relaxant effects (IC10) the order was MIX = cAMP = papaverine > adenosine. The dilatation elicited by papaverine, adenosine and cAMP was increased by MIX. Preincubation with adenosine enhanced the relaxation induced by MIX and reduced that produced by cAMP. These results indicate that the effects of adenosine and cAMP seem not to be mediated by specific surface receptors but by a cAMP-dependent mechanism. The interference between adenosine and cAMP could be due to competition for a similar site and/or mechanism.

It has been suggested that the intracellular increase in cyclic-AMP (cAMP) is the mechanism by which some drugs produce relaxation of smooth muscle (Triner et al 1971; Andersson 1972; Gagnon et al 1980b). This compound seems to interfere with Ca^{2+} movements and free Ca^{2+} levels in the cells (Gagnon et al 1980b). There are several agents that increase the amount of cAMP by phosphodiesterase inhibition or activation of adenylate cyclase. In the former group are papaverine and xanthines (Triner et al 1971; Wells et al 1975) and the latter could include adenosine and related compounds (Kukovetz et al 1978; Collis & Brown 1983; Edvinsson & Fredholm 1983). Furthermore, the xanthines also have the ability to block the purinoreceptors located on the cell surfaces, antagonizing the effects of adenosine and analogous compounds (Gagnon et al 1980b; Toda et al 1982; Edvinsson & Fredholm 1983).

Papaverine (Toda 1974; Gagnon et al 1980b), xanthines (Gagnon et al 1980b) and adenosine and related compounds (Napoli et al 1980; Gagnon et al 1980a,b; Toda et al 1982) induce vasodilator responses in different vessels, probably as a consequence of the mechanisms of action described above.

The aim of the present study was to analyse the probable vasodilator effects of adenosine, cAMP, papaverine and 1-methyl-3-isobutylxanthine (MIX) as well as the possible interaction between them in cat femoral arteries.

Materials and methods

Cats (1·5–3 kg) were anaesthetized by i.p. administration of 35 mg kg^{-1} of sodium pentobarbitone and killed by bleeding. The femoral arteries were removed and dissected into cylindrical segments 4 mm in length. Each arterial cylinder was set up for isometric recording in an organ bath, as described by Nielsen & Owman (1971), containing 6 ml of Krebs-Henseleit solution (KHS) at 37 °C continuously bubbled with a 95% O_2–5% CO_2 mixture, which gave a pH of 7·4–7·5. Two stainless steel pins were introduced through the lumen of the arterial segments. One pin was fixed to the organ bath wall, while the other one was connected to a strain gauge for isometric tension recording. The latter pin was parallel with the former and was movable, thus permitting the application of resting tension in a perpendicular plane to the long axis of the vascular cylinder. The isometric contraction was recorded through a force-displacement transducer (Grass FTO3C) connected to a Grass Model 7D Polygraph. A resting tension of 1 g was applied to the segments which were readjusted every 15 min during a 90–120 min equilibration period before cumulative dose–response curves for different drugs were performed. The composition of the KHS was (mM): NaCl 115; KCl 4·6; $CaCl_2$ 2·5; KH_2PO_4 1·2; $MgSO_4.7H_2O$ 1·2; $NaHCO_3$ 25; glucose 11·1 and the disodium salt of ethylenediaminetetraacetic acid (Na_2EDTA) 0·03.

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