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Intracerebroventricularly administered bradykinin augments carrageenan-induced paw oedema in rats

S. K. BHATTACHARYA, P. J. R. MOHAN RAO, NEETA DAS, G. DAS GUPTA, *Neuropharmacology Laboratory, Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India*

Abstract—Intracerebroventricular (i.c.v.) administered bradykinin (2·5 and 5·0 μg/rat) was found to augment carrageenan-induced acute paw oedema throughout the 4 h post-carrageenan observation period. The effect was statistically significant with the higher dose. The pro-inflammatory effect of i.c.v. bradykinin was antagonized following pretreatment with hemicholinium and atropine ethoiodide administered i.c.v., drugs that reduce central cholinergic activity. Similarly, central administration of drugs that inhibit the synthesis of eicosanoids, hydrocortisone, diclofenac and paracetamol, also attenuated the pro-inflammatory effect of bradykinin. The findings indicate that the inflammation-promoting effect of centrally administered bradykinin involves the central prostaglandin and cholinergic neurotransmitter systems.

Bradykinin is known to be released in the periphery and has been implicated in a variety of physio-pathological conditions, including inflammation (Colman & Wong 1979). It is now evident that the kinin exerts discernible effects on the mammalian central nervous system (CNS), leading to the postulate that it functions as a central neuromodulator (Clark 1979). The kinin has been identified in the CNS of several animal species, including rats, and enzyme systems capable of synthesizing and inactivating bradykinin and other kinins have been demonstrated in the mammalian brain and cerebrospinal fluid (Clark 1979; Shisheva et al 1983).

There are indications that the CNS may modulate peripheral inflammation. Schizophrenics have an unusually low incidence of rheumatoid arthritis and show reduced inflammatory response to injury and infection (Horrobin 1977). Experimentally induced acute inflammation is attenuated by general anaesthetics (Griswold et al 1982; Bhattacharya et al 1987), narcotic analgesics, spinal transection, and acute or chronic denervation (Brown et al 1968). Patients with thalamic or spinothalamic lesions show a substantially decreased flare response to histamine, indicating that the vasodilator component of inflammation is modulated by the CNS (Appenzeller & McAndrews 1966).

In recent reports from this laboratory, the central cholinergic (Das & Bhattacharya 1985), prostaglandin (PG) E_2 (Bhattacharya & Das 1984) and some excitatory amino acid (Bhattacharya & Sarkar 1986) neurotransmitter systems have been shown to augment carrageenan-induced paw oedema in rats. On the contrary, the central noradrenergic (Bhattacharya & Das 1986), 5-hydroxytryptaminergic (Bhattacharya & Das 1985a), histaminergic (Bhattacharya & Das 1985b), $PGF_2\alpha$ (Bhattacharya & Das 1984) and inhibitory amino acid (Bhattacharya & Sarkar 1986) neurotransmitter systems attenuate carrageenan-induced acute inflammation. Since it has been postulated that bradykinin functions as a neuromodulator (Clark 1979), the effect of i.c.v. administered bradykinin has been investigated on carrageenan-induced paw oedema, taken as the experimental model of acute inflammation, in rats.

Materials and methods

The studies were conducted on inbred Wistar strain albino rats (150–200 g) of both sexes. The rats were housed in colony cages at an ambient temperature of 25±2°C and 45–55% relative humidity, with a 12 h light-dark cycle. The rats were fed on standard pellet chow and given tap water through drinking bottles. 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 paw (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

Correspondence to: S. K. Bhattacharya, Neuropharmacology Laboratory, Dept of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

Table 1. Effect of i.c.v. administered bradykinin on carrageenin-induced paw inflammation in rats, and the effects of some pharmacological agents on the pro-inflammatory effect of bradykinin.

Groups	n	Increase in paw volume in units (mean ± s.e.m.) 1 h	2 h	3 h	4 h
Control (vehicle)	10	1·09 ± 0·14	1·87 ± 0·22	2·27 ± 0·14	2·20 ± 0·22
Bradykinin (2·5 μg)	7	1·46 ± 0·16	2·17 ± 0·11	2·47 ± 0·20	2·29 ± 0·25
Bradykinin (5 μg)	20	1·55 ± 0·11*	2·82 ± 0·16*	3·27 ± 0·25**	3·22 ± 0·32*
Hemicholinium	5	1·11 ± 0·12	1·78 ± 0·14	2·16 ± 0·19	2·00 ± 0·18
Hemicholinium + bradykinin (5 μg)	5	0·72 ± 0·28[a]	0·42 ± 0·14[c]***	0·82 ± 0·25[c]***	1·72 ± 0·15[c]
Atropine	5	1·02 ± 0·16	0·92 ± 0·12	2·43 ± 0·18	2·36 ± 0·21
Atropine + bradykinin (5 μg)	6	1·20 ± 0·18	0·98 ± 0·12[c]**	1·44 ± 0·19[c]**	1·41 ± 0·16[c]*
Hydrocortisone	5	1·02 ± 0·18	1·66 ± 0·12	2·08 ± 0·16	1·98 ± 0·19
Hydrocortisone + bradykinin (5 μg)	6	1·20 ± 0·12[a]	1·62 ± 0·25[a]	2·13 ± 0·36[a]	2·12 ± 0·38[a]
Paracetamol	5	0·86 ± 0·32	1·42 ± 0·29	1·98 ± 0·32	2·00 ± 0·19
Paracetamol + bradykinin (5 μg)	10	0·47 ± 0·14[b]**	0·59 ± 0·16[b]***	1·12 ± 0·22[b]***	1·48 ± 0·30[b]
Diclofenac	5	0·80 ± 0·24	1·29 ± 0·20	1·82 ± 0·33	1·79 ± 0·21
Diclofenac + bradykinin (5 μg)	8	0·52 ± 0·12[b]**	0·84 ± 0·18[a]***	1·26 ± 0·28[b]**	1·38 ± 0·24[b]*

*, ** and *** indicate statistical significance (P) at $<0·05$, $<0·01$ and $<0·001$, respectively, in comparison with the vehicle-treated group.
[a], [b] and [c] indicate statistical significance at $<0·05$, $<0·01$ and $<0·001$, respectively, in comparison with the bradykinin (5 μg)-treated group.
Figures without superscripts indicate that the values are statistically insignificant when compared with respective control or bradykinin-treated group.
The Student's t-test was used for statistical analysis.

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

The following drugs, with doses and pretreatment times given in parentheses, were used: bradykinin triacetate (2·5 and 5 μg/rat, immediately after carrageenan), hemicholinium-3 (20 μg/rat, 30 min) atropine ethoiodide (10 μg/rat, 30 min), hydrocortisone sodium succinate (25 μg/rat, 30 min), diclofenac sodium (10 μg/rat, 30 min) and paracetamol (50 μg/rat, 30 min). The doses mentioned refer to the respective salts and the pretreatment time to the period before bradykinin administration. The doses and pretreatment times are based on data available from earlier studies from this laboratory (Bhattacharya & Das 1984; Bhattacharya et al 1986a; Das & Bhattacharya 1985).

Results

The results are summarized in Table 1. None of the pharmacological agents used to investigate the effect of bradykinin, namely, hemicholinium, atropine ethoiodide, hydrocortisone, diclofenac and paracetamol, had any significant effect on the inflammation induced by carrageenan, after i.c.v. administration and in the doses used. Bradykinin (2·5 and 5 μg) augmented the inflammatory oedema, the effect being statistically significant only with the latter dose when compared with i.c.v. artificial CSF-administered controls. The pro-inflammatory effect of bradykinin (5 μg) was evident throughout the 4 h period of observation, with a progressive increase of paw volume from 1 to 3 h when a maximum was reached (Table 1). Pretreatment of the rats with hemicholinium, an inhibitor of acetylcholine biosynthesis, atropine ethoiodide, a quaternary cholinergic muscarinic receptor antagonist, and the eicosanoid synthesis inhibitors, hydrocortisone, paracetamol and diclofenac, significantly inhibited the pro-inflammatory effect of bradykinin (5 μg) (Table 1). The increase in the inflammatory oedema, following carrageenan administration, was significantly less than that observed in the vehicle-treated control animals, in the hemicholinium-(2 and 3 h), atropine-(2,3 and 4 h), paracetamol-(1,2 and 3 h) and diclofenac-(1,2,3 and 4 h) treated bradykinin (5 μg)-administered rats (Table 1).

Discussion

A wide spectrum of interactions between bradykinin and PGs in the periphery, has led to the speculation that PGs function as mediators of peripheral kinin action (McGiff et al 1975). Some recent studies indicate that this speculation may also hold true for the CNS. Bradykinin-induced hyperthermia in rabbits (Almeida e Silva & Pela 1978) and rats (Mohan Rao & Bhattacharya 1987a), pressor response (Kariya et al 1982) and catalepsy (Bhattacharya et al 1986a) in rats, have been shown to be PG-mediated effects. Centrally administered bradykinin has been reported to increase selectively rat brain PGE_2 levels, leaving $PGF_{2\alpha}$ concentrations virtually unaffected (Bhattacharya et al 1986b). Bradykinin is known to activate phospholipase A_2, the enzyme which liberates arachidonic acid, the eicosanoid precursor, from phosphatidylcholine and phosphatidylethanolamine, the two membrane-bound phospholipids containing bulk of the esterified arachidonic acid in cells (Juan 1977). Since activation of phospholipase A_2 and the release of free arachidonic acid is believed to be the rate-limiting step in the synthesis of eicosanoids (Irvine 1982), the observed inflammation-promoting effect of the kinin could, therefore, be due to increased central PGE_2 activity, the levels of which are selectively raised in the rat brain by bradykinin (Bhattacharya et al 1986b) and the central administration of which has been reported to augment carrageenan inflammation in this species (Bhattacharya & Das 1984). Hydrocortisone, which inhibits phospholipase A_2 activity (Hirata 1983), and the cyclo-oxygenase inhibitors, diclofenac and paracetamol, the latter being a selective inhibitor of central PG synthesis (Flower 1974), attenuated the inflammation-promoting effect of i.c.v. bradyki-

nin, indicating that the effect is likely to be PG-mediated. Centrally administered hydrocortisone has been shown to inhibit bradykinin-induced hyperthermia in rats, in doses themselves bereft of any effect on rectal temperature (Mohan Rao & Bhattacharya 1987a).

Bradykinin has been reported to augment central cholinergic activity (Wisniewski & Bodzenta 1975) and centrally administered acetylcholine has been shown to enhance carrageenan-induced paw oedema in rats (Das & Bhattacharya 1985). The inflammation-promoting effect of centrally administered acetylcholine was found to be primarily attributable to the resultant augmented peripheral cholinergic activity (Das & Bhattacharya 1985). However, PGEs are known to facilitate rat brain cholinergic activity (Perez-Cruet et al 1971) and it is possible that the effect of bradykinin is a consequence of PGE-modulated increase in the central cholinergic activity.

An interesting feature of the study was the significant reduction in the paw oedema induced by bradykinin (5 μg) in hemicholinium-, atropine-, paracetamol- and diclofenac-pretreated rats. It appears that bradykinin can actually attenuate carrageenan-induced oedema, once the pro-inflammatory effect is pharmacologically attenuated. Bradykinin is known to release a number of vasoactive substances in the periphery, including 5-hydroxytryptamine (5-HT) and histamine (Garcia Leme 1978). Whether a similar event takes place in the CNS is a matter of conjecture, although i.c.v. bradykinin has been reported to enhance rat brain 5-HT activity (Bhattacharya et al 1986b), and the antinociceptive action of centrally administered bradykinin has been reported to be 5-HT-mediated (Mohan Rao & Bhattacharya 1987b). Both 5-HT (Bhattacharya & Das 1985a) and histamine (Bhattacharya & Das 1985b) have been shown to inhibit carrageenan-induced paw oedema in rats after i.c.v. administration. It is possible that centrally administered bradykinin has a dual effect on peripheral inflammation. The predominant pro-inflammatory effect is replaced by an anti-inflammatory action with the blockade of the eicosanoid-cholinergic system in the CNS.

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