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Brain monoamines during carrageenan-induced acute paw inflammation in rats

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Abstract—Paw inflammation was induced in rats by sub-plantar administration of carrageenan. Significant inflammatory oedema was observed 1 h later and the peak effect was noted between 3–4 h. The oedema was markedly reduced after 12–24 h. Steady state levels of whole brain and hypothalamic monoamines were estimated spectrofluorometrically during the course of the carrageenan-induced paw inflammation. In addition, the rate of accumulation of the brain 5-hydroxytryptamine (5-HT) and noradrenaline (NA) was assessed in clorgyline-pretreated rats during the inflammation. The whole brain and hypothalamic concentrations of 5-HT and NA were augmented during the early phase of the inflammation, but fell below control values when peak inflammation was achieved. Thereafter, the monoamine levels tended to normalize by 24 h when the inflammation had virtually subsided. On the contrary, whole brain and hypothalamic dopamine levels remained largely unaffected. The rate of accumulation of brain 5-HT and NA were enhanced during carrageenan inflammation, indicating that the turnover of these monoamines is augmented during the inflammatory process. The results suggest that acute peripheral inflammation may significantly affect central 5-HT and noradrenergic activity in rats.

The cascade of events which initiate, maintain and terminate peripheral inflammation is now fairly well elucidated (Bonta 1978). However, little is known about the role of the central nervous system (CNS), if any, in the putative modulation of peripheral inflammation (Bonta 1978). Schizophrenics have an unusually low incidence of rheumatoid arthritis and have shown attenuated inflammatory response to injury or infection (Horrobin 1977). Acute inflammatory oedema is inhibited by general anaesthetics (Griswold et al 1982; Bhattacharya et al 1987) and narcotic analgesics (Bonta 1978). In recent reports from this laboratory, carrageenan-induced acute paw oedema in rats was shown to be attenuated following the central administration of NA (Bhattacharya & Das 1986), 5-HT (Bhattacharya & Das 1985a), histamine (Bhattacharya & Das 1985b), prostaglandin (PG) $F_{2\alpha}$ (Bhattacharya & Das 1984a), GABA and glycine (Bhattacharya & Sarkar 1986). On the contrary, the central administration of acetylcholine (Das & Bhattacharya 1985), PGE_2 (Bhattacharya & Das 1984a) and glutamic acid (Bhattacharya & Sarkar 1986), augmented the inflammatory oedema. These findings indicate that central neurotransmitter systems can modulate acute peripheral inflammation.

It is known that acute inflammation is often self-limiting and it has been postulated that this is a consequence of automodulatory processes initiated by the inflammatory process (Bonta 1978). Since several neurotransmitter systems do appear to exert an anti-inflammatory effect, it is possible that at least part of this proposed automodulatory process results from activation of these central neuroregulators. However, no attempt appears to have been made to investigate the effect of acute peripheral inflammation on central neurotransmitter activity, apart from a report which shows that rat brain PGE_2 and $PGF_{2\alpha}$ levels are augmented during carrageenan-induced paw inflammation (Bhattacharya & Das 1984b). We now report the effect of carrageenan-induced inflammation on rat brain monoaminergic activity.

Materials and methods

The studies were conducted on male Wistar strain albino rats (120–180 g), 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 had free access to standard pellet chow and water. Experiments were conducted at this ambient temperature between 0900 and 1400 h.

Inflammation of the paw was induced by carrageenan (0·1 mL of 1% suspension in 0·9% NaCl), injected below the plantar aponeurosis of the hind paw (Winter et al 1962). The index of inflammation was the increase in the paw volume after the injection of the phlogistic agent. The paw volume, up to the ankle joint, was measured by a mercury plethysmograph, before and at hourly intervals for 4 h, and then at 8 h, 12 h and 24 h after carrageenan administration, and has been expressed in units, each unit representing 1 cm (volume = 0·075 mL) length of displaced mercury. Groups of rats were decapitated, 30 min, 1, 2, 3, 4, 8, 12 and 24 h after the induction of the inflammation, and the brains quickly removed for spectrophotofluorometric estimation of monoamines (Haubrich & Denzer 1975). In a separate

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paradigm, carrageenan oedema was induced as before, the rats were killed at the same times and the hypothalamus was dissected out as the region lying between the rostral borders of the optic chiasma and mammilary bodies, and medial to the area lateralis. Hypothalami of three rats were pooled for the assay of monoamines. The rate of accumulation of rat brain 5-HT and NA was assessed by the method of Neff & Tozer (1968), after the administration of clorgyline (2 mg kg^{-1} i.p.), which was administered just before the induction of the inflammation. The rats were killed at 30 min, 1, 2, 3 and 4 h for removal of the brains and assay of the monoamines. Student's *t*-test was used for statistical analysis of the data.

Results and discussion

Carrageenan induced significant paw oedema in rats which peaked between 3–4 h, declined by 8 h and was markedly reduced by 24 h (Fig. 1). Clorgyline pretreatment did not significantly affect the course of the carrageenan oedema, the values (in units ± s.e.) in this group at 1, 2, 3, 4 and 24 h being 1·96 ± 0·18, 3·04 ± 0·14, 3·82 ± 0·21, 3·44 ± 0·19 and 0·74 ± 0·12 (n = 5), respectively, as compared to the values of the untreated control group which were 1·82 ± 0·12, 2·99 ± 0·17, 3·74 ± 0·18, 3·21 ± 0·21 and 0·82 ± 0·1, at these times respectively. The steady state concentrations of brain 5-HT and NA showed qualitatively similar changes during carrageenan inflammation. Significant increase was noted 30 min and 1 h after carrageenan administration, the maximal increase being noted at the latter period. Thereafter, the amine levels tended to return to control values at 2 h and then steadily decreased below control values at 3 h, 4 h and 8 h. By 12 h there was a tendency for normalization of the monoamine concentrations which was achieved at 24 h (Fig. 1). Dopamine (DA) concentrations remained largely unaffected throughout the course of the inflammation, the values (in $\mu g\ g^{-1}$, mean ± s.e.) at 0, 0·5, 1, 2, 3, 4 and 24 h being 0·81 ± 0·05, 0·9 ± 0·08, 0·93 ± 0·09, 0·82 ± 0·06, 0·76 ± 0·08, 0·84 ± 0·06 and 0·86 ± 0·12, respectively. The hypothalamic 5-HT and NA concentrations followed a similar pattern during carrageenan inflammation, except that the augmented amine levels during the early phase of the inflammation appeared to be more sustained and were discernible at 2 h as well (Fig. 1). The hypothalamic DA levels remained virtually unchanged during the inflammation, the values at 0, 0·5, 1, 2, 3, 4 and 24 h being 0·72 ± 0·12, 0·86 ± 0·12, 0·94 ± 0·1, 0·82 ± 0·1, 0·69 ± 0·12, 0·74 ± 0·1 and 0·76 ± 0·14, respectively. The mean rate of accumulation of brain 5-HT and NA, assessed in clorgyline-treated rats, was augmented during the course of the carrageenan inflammation (Table 1).

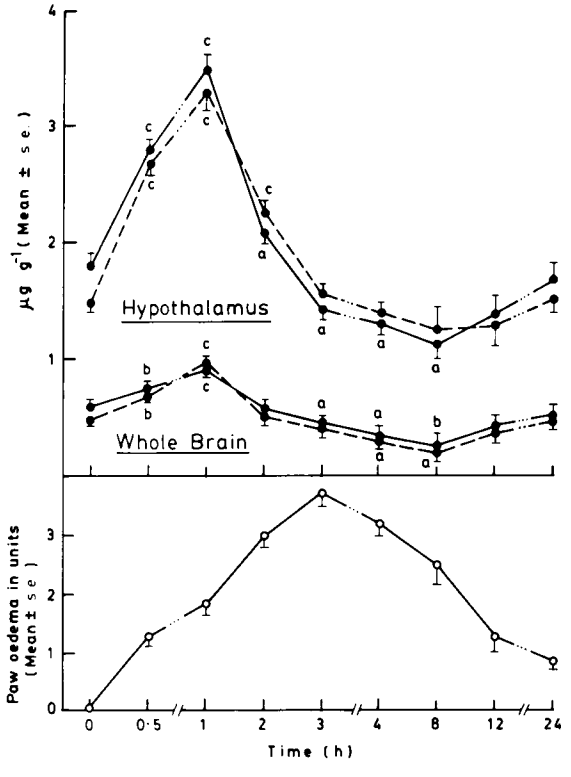

FIG. 1. Rat brain 5-HT (——) and noradrenaline (- - -) during carrageenan-induced paw oedema. The upper panel indicates the time course of whole brain and hypothalamic monoamine concentrations during the oedema. Each point represents the mean ± s.e.m. of 6 observations, except the whole brain monoamine levels at 0 h, where n is 15. The lower panel shows the time course of carrageenan-induced paw oedema. Each point represents the mean of 10 observations ± s.e.m. The symbols a, b and c indicate statistical significance in comparison to the control group (0 h) as $P < 0{\cdot}05$, $< 0{\cdot}01$ and $< 0{\cdot}001$, respectively. Points in the upper panel without these symbols indicate that the values are statistically non-significant in comparison with the values at 0 h.

Table 1. Effect of carrageenan-induced acute paw inflammation on the rate of accumulation ($\mu g\ g^{-1}\ h^{-1}$) of rat brain 5-HT and noradrenaline in clorgyline (2 mg kg^{-1} i.p.)-treated animals

	5-HT ($\mu g\ g^{-1}$ wet tissue)		Noradrenaline ($\mu g\ g^{-1}$ wet tissue)	
Time (h)	Clorgyline	Clorgyline + carrageenan	Clorgyline	Clorgyline + carrageenan
0	1·1 ± 0·12	1·3 ± 0·1	0·8 ± 0·06	1·0 ± 0·09
0·5	2·0 ± 0·16	2·8 ± 0·09[b]	1·7 ± 0·12	2·3 ± 0·1[c]
1	2·6 ± 0·11	3·3 ± 0·08[c]	2·3 ± 0·16	3·5 ± 0·12[b]
2	3·1 ± 0·14	3·9 ± 0·09[b]	2·7 ± 0·14	3·9 ± 0·11[c]
3	3·3 ± 0·19	4·3 ± 0·12[b]	3·1 ± 0·13	4·2 ± 0·12[c]
4	3·8 ± 0·16	4·6 ± 0·08[a]	3·4 ± 0·11	4·5 ± 0·13[c]
Mean rate of accumulation	1·42	1·91	1·41	2·12
Increase in rate of accumulation ($\mu g\ g^{-1}\ h^{-1}$)		0·49		0·71

Values represent mean ± s.e. (n = 5); [a], [b], [c] denote statistical significance in comparison to the respective clorgyline-treated group, as $P < 0{\cdot}05$, $< 0{\cdot}01$ and $< 0{\cdot}001$, respectively (*t*-test).

It has been suggested that there are two clearly delineated phases in the inflammatory response induced by carrageenan. The first is initiated within a few minutes and is completed by 1 h, while the second phase begins at 1 h and continues for at least 3 h (Van Arman 1979). However, inter-laboratory variations have been observed, sometimes even with the same sample of carrageenan and there are reports that peak paw inflammation in rats is induced 4–5 h after carrageenan administration and the oedema is minimal by 24–48 h (Van Arman 1979), whereas others have shown that inflammation and hyperalgesia persist 24–96 h after administration of the phlogistic agent (Guilbaud et al 1986). The time course of carrageenan-induced paw oedema in rats, observed by us, is in keeping with our earlier reports (Bhattacharya & Das 1984a, b, 1985a, b, 1986; Bhattacharya & Sarkar 1986; Bhattacharya et al 1987; Das & Bhattacharya 1985).

The rat brain concentrations of 5-HT and NA showed a biphasic alteration during the carrageenan-induced paw inflammation, with an initial rise during the early phase of the inflammation and subsequently a fall below control values when

the inflammatory process became marked. The amine levels returned to normal as the inflammation waned. The DA concentrations remained fairly constant throughout the inflammation. Qualitatively similar changes were observed with hypothalamic monoamines. It is worth recording that, unlike the anti-inflammatory effect of centrally administered 5-HT (Bhattacharya & Das 1985a) and NA (Bhattacharya & Das 1986), DA had little effect on the peripheral oedema induced by carrageenan (Bhattacharya & Das 1986). The increase in the rate of accumulation of brain 5-HT and NA after pretreatment with an irreversible and selective MAO-A inhibitor clorgyline, during carrageenan inflammation, indicates that the synthesis of these amines and hence their turnover (Neff & Tozer 1968) is augmented by the inflammatory process. 5-HT and NA are preferred substrates for MAO-A, and the dose of clorgyline used has been shown to inhibit the enzyme by 78–81% between 1–4 h of its administration (Bhattacharya et al 1986). It appears, therefore, that the central 5-HT and noradrenergic neurotransmitter systems are activated during the course of an acute peripheral inflammation, possibly in an attempt to limit the inflammation by augmenting central inhibitory modulatory influences over the inflammatory process. The marked fall in the levels of rat brain 5-HT and NA during peak inflammation may reflect the inability of the augmented amine synthesis to keep pace with their enhanced utilization.

Apart from oedema, sub-plantar carrageenan administration also induces hyperalgesia (Guilbaud et al 1986) which is known to activate, among other neurotransmitter systems, the central 5-HT and noradrenergic neurons, which form an integral part of an endogenous pain control system activated by hyperalgesia (Basbaum & Fields 1984). This physiological activation of an analgesia-inducing neural system by pain makes it likely that a similar inhibitory modulatory system involving the CNS may also exist for peripheral inflammation.

As far as could be ascertained, this appears to be the first report on the effect of acute inflammation on brain monoaminergic activity. However, the observed neurochemical changes may well be consequence of the stress induced by the nocisponsive stimuli of inflammation and pain induced by carrageenan. The neurochemical changes in the CNS during stress have been reviewed (Anisman et al 1985). Stress of moderate severity enhances the synthesis and steady state concentrations of central 5-HT and NA. However, with severe stress these amine values tend to fall below basal levels after an initial transient rise because the augmented utilization cannot be compensated for by increased synthesis of the amines. Similar changes are also induced by stress in the hypothalamus. Brain DA appears to be more resistant to stress-induced change compared with the other monoamines (Anisman et al 1985). Stress is also known to activate the hypothalamo-pituitary-adrenocortical axis and both 5-HT and NA have been implicated in stress-induced activation of adrenocorticotrophic hormone release (Anisman et al 1985). Hence, this might be yet another mechanism for automodulation of the inflammatory process. It would be interesting to know whether the changes induced by carrageenan hold true for other models of acute inflammation as well, and whether central monoaminergic activity is influenced by subacute or chronic inflammation. These studies are in progress.

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