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# Effect of pre-existing inflammation on carrageenan-induced paw oedema in rats

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

Twenty-four hours after injection of carrageenan into one hind paw, injection of the same amount into the contralateral paw produced a significantly attenuated inflammatory response. However, when the second injection was given 7 days later, the inflammation induced in the contralateral paw was comparable with the initial response to carrageenan. A time-course study of carrageenan-induced inflammation in rats showed that significant oedema persisted 24 h after carrageenan administration and complete recovery was achieved in 7 days. The attenuated inflammatory response in the contralateral paw after 24 h was antagonized by bilateral adrenalectomy and chemical sympathectomy induced by 6-hydroxydopamine. Carrageenan-induced paw oedema was also significantly less in rats with subacute inflammation induced by the croton oil granuloma pouch technique. This attenuated response was antagonized by pretreatment of the rats with metyrapone, an inhibitor of adrenocorticoid synthesis, and by 6-hydroxydopamine. It is likely that the pre-existing acute or subacute inflammation attenuates the inflammatory response of carrageenan, by acting as a stressor, inducing activation of the sympatho-adrenal system.

Experimental stress has been reported to exert an attenuating effect on inflammation. Overcrowding- (Sofia 1980) and cold- (Glenn & Gray 1965) induced stress have been shown to inhibit adjuvant-induced inflammation, while surgical stress reduces the inflammatory response of phlogistic agents (Brown et al 1968). We have recently shown (Bhattacharya et al 1987) that immobilization-induced stress in rats induces a time-related reduction in the intensity of carrageenan oedema. A second injection of carrageenan into the contralateral paw after an interval of 2 h, has been reported to elicit faster hyperalgesia even though the oedematous response was much reduced (Ferreira et al 1978). It was suggested that the latter effect was due to the counter-irritant action of the first contralateral injection of the phlogistic agent. However, it is possible that the inflammation induced by the initial injection of carrageenan acts as the nocisponsive stressor to activate the sympatho-adrenal system, thereby attenuating subsequent inflammation. This possibility has now been investigated.

## *Materials and methods*

The study was conducted on male Wistar strain albino rats (120–180 g), housed individually at an ambient temperature of 25 ± 2 °C and 45–55% relative humidity, with a 12 h light–dark cycle. The rats were fed Hind Lever pellet chow with water freely available. The experiments were made between 0900 and 1400h, during the light phase.

Paw inflammation was induced by carrageenan (0·1 mL of 1% suspension in 0·9% saline) injected below the plantar aponeurosis of the left hind paw (Winter et al 1962). A second injection of carrageenan was given into the right hind paw of the same rats 24 h later. Paw volumes, up to the ankle joints were measured before and at hourly intervals for 4 h, and after 24 h, and in some instances 7 days, following carrageenan administration, by means of a mercury plethysmograph. The increase in the paw volume was expressed in units representing 1 cm (volume = 0·075 mL) length of the displaced mercury.

In another group of rats, subacute inflammation was induced by the croton oil (2% in arachis oil) granuloma pouch technique (Selye 1953). An air pouch was created by injecting 25 mL air into the loose connective tissue between the shoulder blades of the rat and 0·5 mL croton oil was then injected into the pouch. On the 3rd day the pouch was compressed manually to prevent adhesions. The volume of the inflammatory exudate within the pouch on the 6th day has been found to be 1·07 ± 0·11 mL (n = 21) in pilot experiments (Das 1983). Carrageenan inflammation was induced in this group in the left hind paw on the 6th day after croton oil injection, and the paw volume was recorded before and at hourly intervals for 4 h after carrageenan.

Bilateral adrenalectomy was performed under ether anaesthesia 48 h before the experiments. The rats were given saline (0·9%) instead of water in the post-operative period. Sham adrenalectomy was done in one group.

6-Hydroxydopamine (100 mg $kg^{-1}$ i.p.) was injected 72 h before the induction of acute inflammation by carrageenan, and metyrapone (20 mg $kg^{-1}$ i.p.) was administered once daily from day 1 to day 6 in the group in which subacute inflammation was induced by croton oil.

The differences in the means between the appropriate experimental groups was first analysed by analysis of variance (ANOVA). When the overall ANOVA was significant ($P < 0·05$), the data was further subjected to statistical analysis by the Student's *t*-test.

*Results and discussion*

Paw oedema was evident 1 h after carrageenan administration and peaked between 3–4 h (Table 1). Thereafter, there was a steady decline in the inflammation though significant oedema was discernible even at 24 h (2·15 ± 0·23 units, n = 11). However, on day 7 the oedema subsided and was barely detectable (0·65 ± 0·13 units, n = 11). When carrageenan was injected into the contralateral (right) paw, 24 h after the first induction of oedema, the intensity of this inflammation was significantly less compared with the initial oedema throughout the 4 h period of observation (Table 1). However, when the second oedema was induced in the contralateral (right) paw on day 7, there was no difference in the intensity of the inflammation compared with the initial oedema (Table 1). In bilaterally adrenalectomized rats and in rats pretreated with 6-hydroxydopamine (6-OHD), the oedema induced by carrageenan in the left paw was significantly higher as compared with that in the control group. However, the second oedema, induced by carrageenan administration into the contralateral (right) paw after 24 h, was not significantly different from the first oedema (Table 1).

Table 1. Carrageenan-induced paw oedema (CO) in rats in the presence of pre-existing acute carrageenan inflammation. Effect of adrenalectomy and 6-hydroxydopamine (6-OHD).

| Groups | n | Increase in paw volume in units (mean ± s.e.m.) 1 h | 2 h | 3 h | 4 h |
|---|---|---|---|---|---|
| Control (carrageenan left paw—day 1) | 17 | 1·75 ±0·12 | 3·13 ±0·18 | 3·39 ±0·21 | 3·37 ±0·19 |
| Normal rats | | | | | |
| CO—right paw: day 2 | 11 | 1·37 ±0·15[a] | 2·5 ±0·26[a] | 2·63 ±0·24[a] | 2·56 ±0·24[b] |
| CO—right paw: day 7 | 6 | 1·62 ±0·16 | 2·98 ±0·23 | 3·22 ±0·19 | 3·14 ±0·16 |
| Adrenalectomy group | | | | | |
| Sham adrenalectomy + CO—left paw: day 1 | 6 | 1·82 ±0·21 | 3·16 ±0·16 | 3·46 ±0·28 | 3·28 ±0·21 |
| Adrenalectomy + CO —left paw: day 1 | 7 | 1·98 ±0·12[a] | 3·46 ±0·14[a] | 3·88 ±0·14[a] | 3·64 ±0·12[a] |
| Adrenalectomy + CO —right paw: day 2 | 7 | 1·84 ±0·18 | 3·07 ±0·27 | 3·68 ±0·35 | 3·66 ±0·31 |
| 6-OHD-treated group | | | | | |
| 6-OHD + CO—left paw: day 1 | 6 | 1·96 ±0·14[a] | 3·58 ±0·18[b] | 3·96 ±0·16[a] | 3·74 ±0·12[a] |
| 6-OHD + CO—right paw: day 2 | 6 | 1·8 ±0·17 | 3·26 ±0·19 | 3·74 ±0·18 | 3·42 ±0·2 |

[a] and [b] indicate statistical signficance in comparison with the control group as $P < 0·05$ and $<0·01$, respectively (*t*-test). Values without superscripts are statistically insignificant compared with the control group.

Table 2. Carrageenan-induced paw oedema (CO) in rats in the presence of pre-existing subacute inflammation induced by croton oil (CR). Effect of metyrapone and 6-hydroxydopamine (6-OHD).

| Groups | n | Increase in paw volume in units (mean ± s.e.m.) 1 h | 2 h | 3 h | 4 h |
|---|---|---|---|---|---|
| Control (CO) | 17 | 1·75 ±0·12 | 3·13 ±0·18 | 3·39 ±0·21 | 3·37 ±0·19 |
| CR : CO (day 6) | 6 | 0·75 ±0·13[c] | 1·25 ±0·13[c] | 1·35 ±0·18[c] | 1·25 ±0·11[c] |
| Metyrapone : CO | 5 | 1·98 ±0·16 | 3·68 ±0·12[a] | 3·96 ±0·14[a] | 3·82 ±0·12[a] |
| 6-HD : CO | 6 | 1·96 ±0·14[a] | 3·58 ±0·18[b] | 3·96 ±0·16[a] | 3·74 ±0·12[a] |
| Metyrapone + CR : CO | 6 | 1·36 ±0·28 | 2·64 ±0·34 | 2·89 ±0·38 | 2·68 ±0·39 |
| 6-HD + CR : CO | 6 | 1·46 ±0·22 | 2·68 ±0·39 | 2·96 ±0·36 | 2·79 ±0·4 |

[a,b] and [c] indicate statistical significance in comparison with the control group as $P < 0·05$, $<0·01$ and $<0·001$, respectively (*t*-test). Values without superscripts are statistically insignificant compared with the control group.

Paw oedema induced by carrageenan on day 6 of croton oil-induced subacute inflammation was significantly less compared with that in controls. However, in rats pretreated with metyrapone or 6-OHD the paw inflammation induced by carrageenan was not significantly different from that in controls (Table 2). Earlier studies from this laboratory (Das 1983) have shown that the volume of the inflammatory exudate (in mL) extracted from the croton oil pouch in control, metyrapone-treated and 6-OHD-treated groups were 1·68 ± 0·16 (n = 21), 1·92 ± 0·19 (n = 5) and 2·02 ± 0·18 (n = 5), respectively, indicating that metyrapone or 6-OHD do not have an anti-inflammatory effect but exert pro-inflammatory effects on the experimental parameter.

The findings of the present study indicate that carrageenan-induced inflammation is attenuated in the presence of pre-existing acute or subacute inflammation. This is best exemplified by the absence of any effect of the primary carrageenan inflammation on the subsequent paw oedema when the latter was induced in the contralateral limb on day 7, when the first inflammatory episode had waned. Ferreira et al (1978) have reported that a second injection of carrageenan into the contralateral paw, after an interval of 2 h, elicited a potentiated algesic but an attenuated inflammatory response. It was speculated that those effects were due to the counter-irritant action of the first phlogistic injection. However, it is equally likely that the first inflammation, acting as a nocisponsive stressor, attenuates subsequent inflammation. As mentioned earlier, stress induced by overcrowding, cold and surgical

manipulations have been known to inhibit experimental inflammation (Glenn & Gray 1965; Brown et al 1968; Sofia 1980). In a recent comprehensive study on the subject (Bhattacharya et al 1987), immobilization-induced stress has been shown to inhibit carrageenan paw oedema in rats, the inhibition being related to the duration of the immobilization. Carrageenan-induced paw oedema has been shown to be significantly less in intracerebroventricularly cannulated rats compared with uncannulated animals (Bhattacharya & Das 1985, 1986). Since the rats were used one week after the cannulation, it was maintained that the attenuated inflammation was not due to surgical stress but that the physical presence of the cannula in the right lateral ventricle, and the administration of artificial cerebrospinal fluid, acted as the stressors.

There is conclusive evidence that the hypothalamo-pituitary-adrenocortical (HPA) and the sympathetic-adrenal medullary (SA) systems are activated by conditions which are aversive and nocisponsive in nature, like inflammation (Anisman et al 1985). The relative importance of these two systems in the response to a stressor has not been elucidated, nor has there been a resolution of the role of adrenal corticoids and catecholamines in the reaction to stress (Anisman et al 1985). However, immobilization stress-induced attenuation of acute inflammation has been conclusively shown to be the consequence of augmented central noradrenergic and peripheral catecholaminergic activity; activation of endogenous corticoid release was found to be of secondary importance (Bhattacharya et al 1987).

Bilateral adrenalectomy and pretreatment of the rats with 6-OHD, which is known to induce near complete sympatholytic activity consequent to degeneration of catecholaminergic neurons (Thoenen & Tranzer 1973), potentiated carrageenan oedema and antagonized the attenuation of the second inflammatory episode. This indicates that the reduced inflammatory response in the contralateral paw is likely to be due to the release of adrenal corticoids and catecholamines induced by the primary oedema.

Carrageenan-induced paw oedema was also significantly less in the presence of subacute inflammation induced by croton oil. Adaptive changes, particularly in the presence of stress-induced neurochemical and hormonal changes (Anisman et al 1985; Solomon et al 1985), are known to occur when the organism is exposed to continued aversive stimulation. However, inhibition of carrageenan-induced paw oedema by the subacute inflammation was comparable with that induced by the acute inflammatory response. The inhibition of carrageenan-induced inflammation by croton oil-induced subacute inflammation was antagonized by pretreatment with 6-OHD and by metyrapone, an inhibitor of adrenocorticoidogenesis (Temple & Liddle 1970), indicating that endogenous catecholamines and adrenocorticoids are responsible for the attenuation. Metyrapone was used instead of bilateral adrenalectomy because the death-rate among bilaterally adrenalectomized rats subjected to croton oil inflammation was unacceptably high as was also reported by Bhattacharya (1982) with rats subjected to immobilization-induced stress. The anti-inflammatory action of adrenal corticoids is well documented and it has been proposed that the peripheral catecholamines function as endogenous anti-inflammatory agents acting mainly by modulating the vascular changes initiating the inflammatory process (Brown & West 1965).

It is concluded that pre-existing acute or subacute inflammation can alter the course of a subsequent acute inflammatory response by activating the release of adrenal corticoids and peripheral catecholamines, presumably from the sympathetic nerve terminals and the adrenal medulla.

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