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# Pharmacological modulation of 2-methyl-carbamate-PAF induced rat paw oedema

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**Abstract**—Intraplantar injections of 2-methyl-carbamate-PAF (2-MC) (0·125–16·0 μg/paw) into the rat paw were followed by a bell-shaped dose response curve for inflammatory oedema, with an ascending phase at 0·125–2·0 μg/paw, and a descending phase at 4·0–16·0 μg/paw. The inflammatory response to 2-MC was partially inhibited by pre-treatment with aspirin (200 mg $kg^{-1}$), NDGA (100 mg $kg^{-1}$), dexamethasone (0·1 mg $kg^{-1}$), verapamil (50 mg $kg^{-1}$) and by a specific PAF antagonist BN 52021 (5–10 mg $kg^{-1}$). The cyclo-oxygenase inhibitors indomethacin (2 mg $kg^{-1}$) and piroxicam (1·8 mg $kg^{-1}$) as well as antihistamine meclizine (40 mg $kg^{-1}$) and ranitidine (50 mg $kg^{-1}$) failed to block the oedematogenic response to 2-MC. Our data suggest that 2-MC induced rat paw oedema is mediated by PAF-acether receptors and is partially dependent on arachidonate lipoxygenase pathway and extracellular $Ca^{2+}$.

2-Methyl-carbamate-PAF (2-MC), a synthetic analogue of PAF-acether, is equi-effective to the latter in inducing platelet aggregation and bronchoconstriction (Touqui et al 1984). Injections of PAF-acether or 2-MC into the rat hind paw trigger an inflammatory response characterized by an acute oedema (Bonnet et al 1981; Cordeiro et al 1986a). Pharmacological evaluation of PAF-acether-induced oedema showed there was a partial dependence on lipoxygenase derivatives and extracellular $Ca^{2+}$, under conditions where cyclo-oxygenase inhibitors were ineffective (Goldenberg & Meurer 1984; Cordeiro et al 1986b). Further studies indicated a cross-desensitization between the oedema induced by PAF-acether and that by 2-MC, suggesting that these substances induce oedema via interaction with the same receptor (Cordeiro et al 1986a). The aim of the present study was to analyse the pharmacological modulation of 2-MC-induced oedema, in order to investigate its mechanism of action.

## Materials and methods

Wistar rats of either sex, 150–200g, were used. Paw oedema was produced by intraplantar injections of 0·1 mL of 2-MC or PAF-acether into one hindpaw, the other being injected with the same volume of the vehicle. The oedema was plethysmographically (Ferreira 1979) measured 2 h after the intraplantar injection. Indomethacin (Sigma) (2 mg $kg^{-1}$) aspirin (Aspegic, Egic-Joulie, France) 200 mg$kg^{-1}$) and ranitidine (Antak, Glaxo) (50 mg $kg^{-1}$) were diluted with sterile 0·9% NaCl (saline). Piroxicam (Pfizer) (1·8 mg $kg^{-1}$), meclizine (Pfizer) (40 mg $kg^{-1}$), nordihydroguaiaretic acid (NDGA; Sigma) (100 mg $kg^{-1}$) and verapamil (50 mg $kg^{-1}$) were dissolved in Tween 80 and made up to the final volume with saline. These drugs were injected i.p. 1 h before the induction of oedema. Dexamethasone (Decadron R, Merck, Sharp and Dohme) (0·1 mg $kg^{-1}$) was administered i.p. 12 and 1 h before the 2-MC injection. BN 52021 (3-(1,1-dimethyl-ethyl)hexahydro-1,4,7b-trihydroxy-8-α-methyl-9H-1,7-α-(epoxy methanol)-1H,6H-cyclopenta(c)furo(2,3-b)furo(3,12:3,4)cyclopenta(1,2-d)furan-5,9,12 (4H)-trione) was kindly provided by Dr P. Braquet (IHB-IPSEN-Institute for Therapeutic Research, Le Plessis Robinson, France) and 2-MC by Hoffmann-La Roche. The data were statistically analysed by means of Student's *t*-test for unpaired samples. *P* values of 0·05 or less were considered significant.

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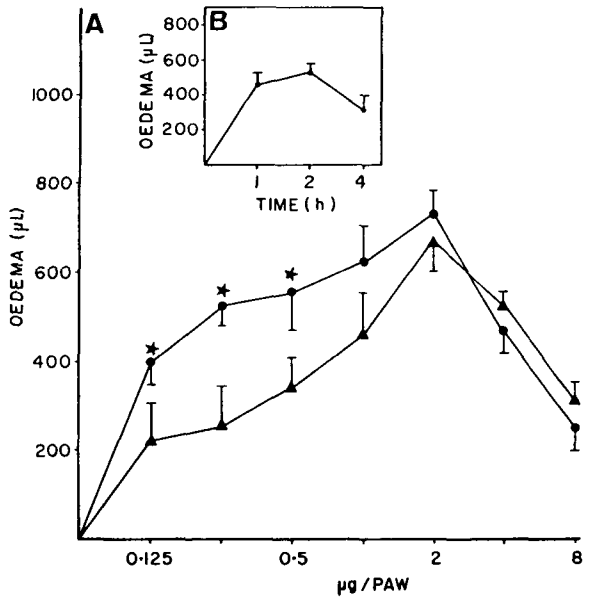


FIG. 1. A. Dose-response curve for both PAF-acether (▲) and 2-MC (●). The oedema was measured 2 h after the intraplantar injection of the agonists. Each point represents the mean ± standard deviation from at least 5 animals. Statistically significant differences are indicated by an asterisk. B. Time course of the oedema induced by intraplantar injection of 2-MC (0·25 μg/paw). Each point represents the mean ± standard deviation from at least 5 animals.

## Results and discussion

As previously described for PAF-acether (Martins et al 1987), intraplantar injections of 2-MC, over the dose range of 0·025–16·0 μg/paw, yielded a bell-shaped dose response curve for oedema. An ascending phase was obtained at 0·025–2·0 μg/paw, whereas a decreased oedema was observed at 4·0–16·0 μg/paw (Fig. 1 A). At low doses (0·025–1 μg/paw), 2-MC was more effective than PAF-acether itself as a pro-inflammatory agent (Fig. 1 A). This phenomenon could be associated with the rapid plasmatic degradation of PAF-acether (B. B. Vargaftig, personal communication), suggesting that the effectiveness of 2-MC is related to its resistance to metabolic inactivation. The time course of 2-MC-induced oedema is shown in Fig. 1 B, the injection of 2-MC (0·25 μg) into the paw induced a significant oedema that peaked at 2 h reducing thereafter so that 24 h later no oedema was observed (not shown).

In most cases, PAF-acether-induced effects are receptor mediated phenomena (Braquet et al 1987). To test the hypothesis that 2-MC interacts with PAF-acether receptors, we examined the effect of BN 52021, a specific PAF-acether antagonist, on 2-MC-induced oedema. It was observed that BN 52021 dose-dependently inhibited 2-MC-induced oedema (Table 1). These

Table 1. Effect of BN 52021 on 2-MC-induced paw oedema.

| Treatment | Dose (mg kg$^{-1}$) | Oedema ($\mu$L) | Inhibition % |
|---|---|---|---|
| None | | 563·5 ± 83·2 | — |
| BN 52021 | 5 | 223·1 ± 19·7 | 60·4* |
| | 10 | 134·6 ± 78·6 | 76·1* |

BN 52021 was administered intraperitoneally 1 h before the intraplantar injection of 0·25 $\mu$g of 2-MC. Oedema values represent the mean ± standard deviation from at least 5 animals. Statistically significant differences are indicated by an asterisk.

Table 2. Effect of anti-inflammatory drugs on 2-MC-induced oedema.

| Treatment | Dose (mg kg$^{-1}$) | n | Inhibition % |
|---|---|---|---|
| Dexamethasone | 0·1 | 10 | 40·5* |
| NDGA | 100 | 8 | 48·5* |
| Aspirin | 200 | 9 | 44·2* |
| Indomethacin | 2 | 5 | 17·4 |
| Piroxicam | 1·8 | 9 | 11·4 |
| Meclizine | 40 | 5 | 10·5 |
| Ranitidine | 50 | 5 | — |
| Verapamil | 50 | 5 | 64·6* |

All drugs, except dexamethasone, were administered intraperitoneally 1 h before the injection of 2-MC (0·25 $\mu$g) into the rat paw. The corticosteroid was administered 12 and 1 h before the lipid. Statistically significant differences are indicated by an asterisk.

data are in accordance with those of Cordeiro et al (1986a), who demonstrated the existence of cross-desensitization between the oedema induced by PAF-acether and 2-MC. Taken together, these data strongly suggest that 2-MC interacts with the PAF-acether receptor to induce oedema formation. It should be noted that BN 52021 at 10 mg kg$^{-1}$ failed to totally prevent the 2-MC-induced oedema. This result could be explained assuming that 10 mg kg$^{-1}$ of BN 52021 is not sufficient to completely block PAF-acether receptors. Nevertheless, Cordeiro et al (1986b) also failed to suppress PAF-acether-induced rat paw oedema even with large amounts of BN 52021, suggesting that PAF-acether and, possibly, its analogue 2-MC may also act in sites different from those blocked by BN 52021 treatment.

PAF-acether induces the generation of inflammatory mediators, such as prostaglandins and leukotrienes (Tarayre et al 1986; Del Monte & Subissi 1988); thus we decided to investigate in this model the effects of drugs which interfere with arachidonate metabolism. It was observed that dexamethasone (0·1 mg kg$^{-1}$) significantly inhibited 2-MC-induced oedema, under conditions where cyclo-oxygenase inhibitors such as indomethacin (2 mg kg$^{-1}$) and piroxicam (1·8 mg kg$^{-1}$) were ineffective. These data suggest that prostaglandins are not important for 2-MC inflammatory action (Table 2). Likewise, Goldenberg & Meurer (1984) and Cordeiro et al (1986b) were also unable to inhibit PAF-acether-induced oedema with indomethacin and piroxicam. In contrast, aspirin (200 mg kg$^{-1}$) markedly inhibited 2-MC-induced oedema. In view of the lack of effectiveness of indomethacin and piroxicam in our model, cyclo-oxygenase inhibition probably does not account for the protective effect observed for aspirin in this case. The role of lipoxygenase products was assayed by treating the animals with NDGA, a dual cyclo- and lipoxygenase blocker. The ability of NDGA (50 mg kg$^{-1}$) to inhibit 2-MC-induced oedema, together with the data stated above, indicate that lipoxygenase metabolites are probably involved in the inflammatory reaction triggered by 2-MC. Similarly, lipoxygenase derivatives seem to play a role in several effects of PAF-acether, e.g. paw oedema (Cordeiro et al 1986b), pleurisy (Martins et al 1989), its toxicity to mice (Myers et al 1983), and pulmonary oedema (Voelkel et al 1983).

Also, verapamil (50 mg kg$^{-1}$), a calcium channel blocker, reduced 2-MC-induced oedema (Table 2), suggesting that mobilization of extracellular calcium is required for the development of the phenomenon. We further investigated the involvement of histamine in the 2-MC induced oedema. Both, $H_1$ and $H_2$-histamine antagonists such as meclizine (40 mg kg$^{-1}$) and ranitidine (50 mg kg$^{-1}$), respectively, failed to inhibit the oedema triggered by 2-MC (Table 2), demonstrating that histamine is not involved in this phenomenon. Nevertheless, a different result was obtained with PAF-acether-induced paw oedema which was sensitive to $H_2$-histamine antagonists (Cordeiro et al 1985).

In conclusion, this study suggests that the inflammatory reaction triggered by 2-MC depends on the interaction with PAF-acether receptors and is partially mediated by metabolites of the arachidonate lipoxygenase pathway and extracellular $Ca^{2+}$.

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