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ENTEROLOBIN INDUCES RAT PAW OEDEMA INDEPENDENTLY OF PAF-ACETHER

H. C. CASTRO-FARIA-NETO; R. S. B. CORDEIRO/+; M. A. MARTINS;
A. C. V. CORREIA-DA-SILVA; P. T. BOZZA; M. V. SOUSA* & L. MORHY*

Instituto Oswaldo Cruz, Departamento de Fisiologia e Farmacodinâmica, Caixa Postal 926, 20001 Rio de Janeiro, RJ, Brasil *Laboratório de Química de Proteínas, Depto. de Biologia Celular, Universidade de Brasília, 70910 DF, Brasil

The potential participation of PAF-acether (PAF) on the paw oedema triggered by enterolobin was investigated. Intraplantar injections of enterolobin (5-20 μg/paw) yielded a dose response curve for oedema which appeared after 30 min, peaked in the interval between 2-4 h and faded after 24 h. The pre-treatment with BN 52021, but not with other PAF antagonists such as PCA 4248 or WEB 2086, significantly blocked enterolobin-induced oedema. To clarify better the discrepant results obtained with the PAF antagonists, desensitization to PAF was performed. The oedema triggered by enterolobin was not modified in PAF desensitized animals. It was concluded that the paw inflammation induced by enterolobin does not require PAF mechanism.

Key words: toxic proteins – PAF – paw oedema

PAF-acether (PAF) is a lipid mediator implicated in several physiophatological conditions, including inflammatory reactions (for review see Braquet et al., 1987). In fact, PAF can produce increase in vascular permeability, leukocyte infiltration, pain and oedema formation (for reviews see Braquet et al., 1987; Pinckard et al., 1988).

We have previously proposed that in addition to PAF antagonist, the induction of specific desensitization to this lipid is a valuable tool to infer its role on acute inflammatory process (Cordeiro et al., 1986). Moreover, PAF selective desensitization seems to implicate a receptor dependent mechanism (Martins et al., 1989).

We recently demonstrated that a haemolitic protein isolated from the seeds of a Brazilian tree (*Enterolobium contortisiliquum*) possess a remarkable pro-inflammatory activity (Cordeiro et al., 1990). This protein was characterized as a 55 Kd, Asx, Glx, Ser and Thr rich protein, and was named enterolobin (Souza & Morhy, 1990).

The aim of this study was to investigate, by means of PAF receptor antagonists treatment and specific desensitization, the role of PAF in enterolobin-induced rat paw oedema. It was demonstrated that this protein does not require PAF mechanisms to induce paw oedema.

MATERIALS AND METHODS

Male Wistar rats (150-200 g) were used throughout this study. Enterolobin was obtained by extraction with 0.15 M Na Cl, precipitation by amonium sulphate from 0 to 33% of saturation and batch separation in DEAE-cellulose (Souza & Morhy, 1990). BN 52021, WEB 2086 and PCA 4248 (20 mg/kg) were administered intraperitoneally (i.p.) 1 h before enterolobin. Paw oedema was produced by a intraplantar injection of enterolobin (5-20 μg/paw) into one hindpaw, the other being injected with the same volume of the vehicle (saline 0.9%). The oedema was measured pletysmographically according to Ferreira (1979). Desensitization to PAF was performed as previously described (Cordeiro et al., 1986). BN 52021 (3-(1,1-dimethyl-ethyl) hexahydro-1,4,7b-trihydroxy-8-α-methyl-9H-1,7-α- (epoxymathanol)-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 – Le Plessis Robinson, France); WEB 2086

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+ Corresponding author.

(3-4- (2-chlorphenyl) -9-methyl-6H-thieno 3,2-f 1,2,4 triazolo-4,3-a 1,4 diazepin-2-yl-1-(4-morpholinyl)-1-propanone was a gift from Dr H. Heuer (Boehringer Ingelheim, Federal Republic of Germany); PCA 4248 (2- (Fenyl-tio) ethyl 5-methoxycarbonil-2, 4,6-trimethyl-1, 4-dihydropyridine-3-carboxylate) was a gift from Dr C. Sunkel (Alter, Madrid, Spain). The data were analyzed statistically by means of Student's T test for unpaired samples. P values of 0.05 or less were considered significant.

RESULTS AND DISCUSSION

According to our previous results (Cordeiro et al., 1990) the intraplantar injection of enterolobin, a haemolitic protein isolated from *Enterolobium contortisiliquum* seeds, in doses ranging from 5 to 20 μg/paw induced a dose response curve for inflammatory paw oedema. This oedema was appearent after 30 min, maximum between 2-4 h, decreasing thereafter (Fig. 1).

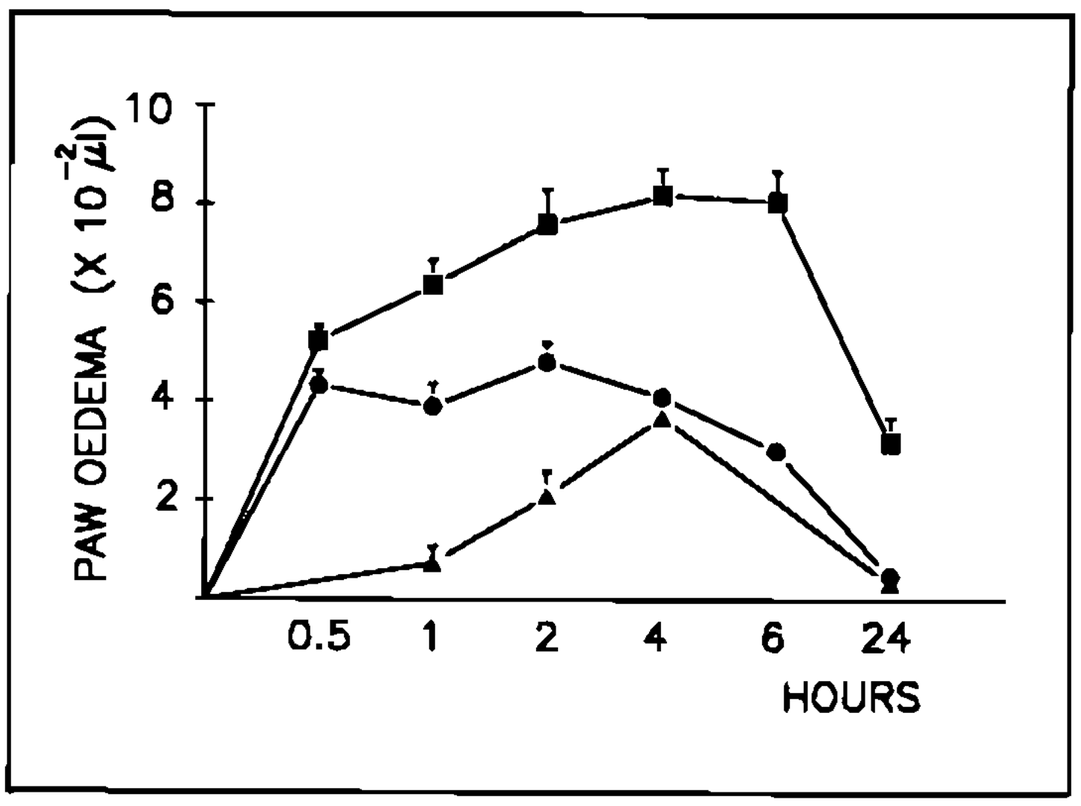

Fig. 1: dose-response curves and kinetics of enterolobin-induced paw oedema. The doses of enterolobin used were 5 μg/paw (▲), 10 μg/paw (●) and 20 μg/paw (■). Each point is the mean from at least 5 animals. Vertical lines represent SEM.

In this study, PAF-induced auto-desensitization and receptor antagonists were used, as tools, to investigate the potential involvement of this lipid in the paw oedema triggered by enterolobin. As shown in the Table, the pre-treatment with two unrelated PAF antagonists, namely PCA 4248 and WEB 2086, failed to block enterolobin-induced oedema, in conditions where PAF-induced inflammation was supressed (not shown). By contrast, BN 52021, a PAF antagonist obtained from the leaves of a millenary chinese tree, *Ginkgo biloba* (Braquet, 1988) showed a significant inhibitory activity on enterolobin-induced oedema. To clarify better the discrepant results obtained with the PAF antagonists, auto-desensitization to PAF was performed as proposed by Cordeiro (1986). The Table shows that, as well as PCA 4248 and WEB 2086, previous desensitization to PAF did not interfer with enterolobin-induced paw oedema, reinforcing the lack of involvement of PAF in this phenomenon. In addition, by the use of the rabbit platelet aggregation bioassay, no PAF-like activity could be detected on pleural exudates collected from enterolobin-injected rats (data not shown).

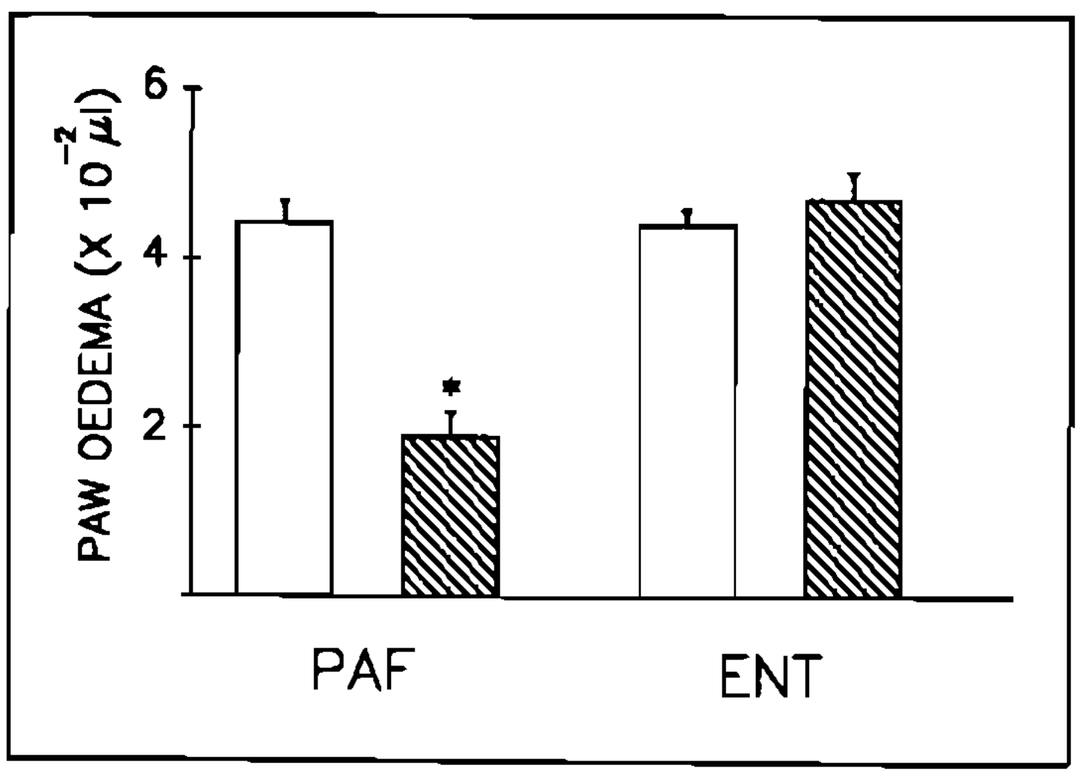

Fig. 2: effect of the intraplantar injection of PAF 1 μg/paw (PAF) or enterolobin 20 μg/paw (ENT) on animals which were previously desensitized to PAF (hatched columns). White columns represent control values for the oedema induced by PAF or enterolobin. Each bar is the mean, with SEM indicated by vertical lines, from 6 animals. Statistically significant differences are indicated by an asterisk.

TABLE

Effect of the pre-treatment with PAF antagonists on enterolobin-induced paw oedema

Treatment	Dose (mg/kg)	Paw oedema (μl)	% of inhibition
None	–	519 ± 26.0	–
WEB 2086	20	473 ± 30.1	8.9
PCA 4248	20	494 ± 72.8	4.8
BN 52021	20	382 ± 14.6	26.7*

The oedema was measured 1 h after the intraplantar injection of enterolobin (20 μg/paw). Data are represented as mean ± SEM from at least 5 animals. Statistically significant differences are indicated by an asterisk.

The inhibition presented by BN 52021 on enterolobin-induced oedema can not be explained, in this particular case, by it's ability in inhibiting PAF receptors. Accordingly, Cordeiro et al. (1988) presented evidence for an unespecific anti-inflammatory action of BN 52021 on carrageenin-induced mice pleurisy.

In conclusion, we demonstrated that enterolobin triggers paw inflammation throught PAF independent mechanisms.

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