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Inhibition of carrageenin-induced rat paw oedema by crotapotin, a polypeptide complexed with phospholipase A_2

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1 The effect of purified crotapotin, a non-toxic non-enzymatic chaperon protein normally complexed to a phospholipase A_2 (PLA_2) in South America rattlesnake venom, was studied in the acute inflammatory response induced by carrageenin (1 mg/paw), compound 48/80 (3 μg/paw) and 5-hydroxytryptamine (5-HT) (3 μg/paw) in the rat hind-paw. The effects of crotapotin on platelet aggregation, mast cell degranulation and eicosanoid release from guinea-pig isolated lung were also investigated.

2 Subplantar co-injection of crotapotin (1 and 10 μg/paw) with carrageenin or injection of crotapotin (10 μg/paw) into the contralateral paw significantly inhibited the carrageenin-induced oedema. This inhibition was also observed when crotapotin (10–30 μg/paw) was administered either intraperitoneally or orally. Subplantar injection of heated crotapotin (15 min at 60°C) failed to inhibit carrageenin-induced oedema. Subplantar injection of crotapotin (10 μg/paw) also significantly inhibited the rat paw oedema induced by compound 48/80, but it did not affect 5-HT-induced oedema.

3 In adrenalectomized animals, subplantar injection of crotapotin markedly inhibited the oedema induced by carrageenin. The inhibitory effect of crotapotin was also observed in rats depleted of histamine and 5-HT stores.

4 Crotapotin (30 μg/paw) had no effect on either the histamine release induced by compound 48/80 *in vitro* or on the platelet aggregation induced by both arachidonic acid (1 mM) and platelet activating factor (1 μM) in human platelet-rich plasma. The platelet aggregation and thromboxane B_2 (TXB_2) release induced by thrombin (100 mu ml^{-1}) in washed human platelets were also not affected by crotapotin. In addition, crotapotin (10 μg/paw) did not affect the release of 6-oxo-prostaglandin $F_{1\alpha}$ and TXB_2 induced by ovalbumin in sensitized guinea-pig isolated lungs.

5 Our results indicate that the anti-inflammatory activity of crotapotin is not due to endogenous corticosteroid release or inhibition of cyclo-oxygenase activity. It is possible that crotapotin may interact with extracellular PLA_2 generated during the inflammatory process thereby reducing its hydrolytic activity.

Keywords: Crotapotin; crotoxin; phospholipase A_2; acute inflammation; carrageenin oedema; histamine release; platelet aggregation

Introduction

Crotoxin, the main neurotoxic component of the venom of the South American rattlesnake *Crotalus durissus terrificus*, is a protein complex composed of a phospholipase A_2 (PLA_2) and a polypeptide named crotapotin (Slotta & Fraenkel-Conrat, 1938). The PLA_2 blocks neuromuscular transmission (Brazil, 1966) and is responsible for the neurotoxic and myotoxic activities observed *in vivo* (Gopalakrishnakone *et al.*, 1984). Crotapotin consists of three polypeptides linked by disulphide bridges and is thought to act as a chaperon protein for PLA_2 (Bon *et al.*, 1979). Although crotapotin has been reported to be enzymatically and pharmacologically inactive (Haberman & Breithaupt, 1978; Bon *et al.*, 1979; Verheij *et al.*, 1980; Gopalakrishnakone *et al.*, 1984), it does enhance the toxicity of PLA_2 (Bon, 1982).

PLA_2 is responsible for arachidonic acid mobilization from cell membranes and is believed to play a key role in the inflammatory process (Flower & Blackwell, 1976; Vadas & Pruzanski, 1986; Pruzanski *et al.*, 1993). Extracellular group II PLA_2 levels are elevated in glycogen-induced ascitic fluid in rabbits (Franson *et al.*, 1978) and in the serum of rabbits with experimental endotoxaemia (Vadas & Hay, 1983), of patients with septic shock and rheumathoid arthritis (Pruzanski *et al.*, 1985; Green *et al.*, 1991) and of healthy human volunteers following lipopolysaccharide (LPS) administration (Pruzanski *et al.*, 1992). Since crotapotin binds to PLA_2 *in vitro* (Rubsamen *et al.*, 1971), we have investigated the influence of the former protein on acute inflammatory responses where PLA_2 activation is clearly involved such as in carrageenin-induced rat hind paw oedema (Di Rosa *et al.*, 1971). The actions of crotapotin on mast cell degranulation, platelet aggregation and eicosanoid release (thromboxane A_2 and prostacyclin) from guinea-pig isolated lungs have also been investigated.

Methods

Rat paw oedema

Male Wistar rats (150–200 g) were used. Hind paw oedema was induced by a single subplantar injection of carrageenin (1 mg/paw), 5-hydroxytryptamine (5-HT) (3 μg/paw), compound 48/80 (3 μg/paw) or crotoxin complex (1–10 μg/paw) in the left paw of rats under light ether anaesthesia in a final volume of 0.1 ml. All drugs were dissolved in sterile saline (0.9%). Paw volume was measured immediately before the injection of the irritant and at selected time intervals thereafter with a hydroplethysmometer (model 7150, Ugo Basile, Italy). Crotapotin (1–10 μg/paw) was dissolved in saline (0.9%) and injected into the paw immediately before the irritant. In another set of experiments, crotapotin (10–30 μg kg^{-1}) was given either intraperitoneally or orally 30 min before the local administration of the irritant. Results were expressed as the increase in paw volume (ml) calculated by

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subtracting the basal (pre-injection) volume. In some cases, the area under the time-course curve (AUC) was calculated using a trapezoidal rule and the results expressed as ml min^{-1}.

Adrenalectomy procedure

Male Wistar rats (150–200 g) anaesthetized with ether were used. The dorsal part of the animals was shaved and a small incision of 1 cm performed. The adrenal glands were removed and the rats used one week after surgery. During the entire post-surgical period, the rats were allowed to drink only saline. The induction of the oedema induced by carrageenin injection was performed as described above. Sham-operated rats received water instead of saline after surgery.

Depletion of histamine and 5-HT stores

Rats were depleted of their stores of histamine and 5-HT by repeated injections of compound 48/80 (Spector & Willoughby, 1959; Di Rosa *et al.*, 1971). Briefly, a 0.1% (w/v) solution of compound 48/80 in saline was given i.p. morning and evening for eight doses, starting with an evening dose. The dose employed was 0.6 mg kg^{-1} for the first six injections and 1.2 mg kg^{-1} for the last two doses. Crotapotin and carrageenin were given 5–6 h after the last injection of compound 48/80. Histamine and 5-HT depletion was considered efficient since this treatment abolished the oedema induced by compound 48/80 (data not shown; $n = 10$).

Isolation and incubation of peritoneal mast cells

Male Wistar rats (200–300 g) were exsanguinated under ether anaesthesia and 10 ml of Krebs-Ringer phosphate solution (KRP, pH 7.4) were injected into the peritoneal cavity. The abdomen was carefully massaged, the fluid withdrawn and spun at 300 *g* for 5 min at 4°C. The resulting cell pellet (of which mast cells comprised 10%) was gently resuspended in a small volume of KRP. The viability of the mast cells (as assessed by 0.1% (w/v) trypan blue dye exclusion) was approximately 90%. Aliquots of the mast cell suspension (0.5 ml) were warmed to 37°C for 10 min. Compound 48/80 was added to the suspension (final volume of 1.0 ml) and the incubation carried out for a further 20 min. When required, crotapotin was incubated (10 min) with the mast cell suspension before adding compound 48/80. The reaction was stopped by placing the test tubes in ice-cold water. The cells were then centrifuged (300 *g*, 10 min) and the supernatant removed for histamine determination. Krebs-Ringer solution (1.0 ml) was added to the cell pellet which was then boiled at 100°C for 10 min to release residual histamine. Histamine concentrations were determined with a double antibody radioimmunoassay (Biomerica). Histamine release was expressed as a percentage of the total cellular content of the amine. All values (means ± s.e.mean) were corrected for the spontaneous histamine release occurring in the absence of stimulus. The composition of the KRP solution was (mM): NaCl 154, KCl 6.2, $NaHCO_3$ 11.9, NaH_2PO_4 0.3, $MgSO_4$ 1.5, glucose 5.6 and $CaCl_2$ 2.8.

Preparation of platelet-rich plasma and washed platelets: measurement of platelet aggregation and thromboxane B_2 (TXB_2) release

Blood from healthy volunteers who had not taken drugs for at least 15 days was collected by venepuncture into a plastic flask containing 3.8% sodium citrate. Platelet-rich plasma (PRP) was prepared by centrifugation at 200 *g* for 12 min at room temperature. Platelet-poor plasma (PPP) was obtained by centrifuging the remaining blood at 900 *g* for 8 min at room temperature. In some experiments, PRP was centrifuged (900 *g*, 8 min) in the presence of the prostacyclin analogue, iloprost (0.8 nM). The supernatant was removed and the platelet pellet was resuspended in 15 ml of calcium-free oxygenated (95% O_2/5% CO_2) Krebs buffer. Iloprost (0.8 nM) was added again and the platelets were centrifuged (900 *g*, 8 min) at room temperature. The supernatant was aspirated and the pellet resuspended in calcium-free Krebs solution (Radomski & Moncada, 1983). The platelet count was determined automatically (Coulter Counter model T 890, Hileah, Fla., U.S.A.) and adjusted to 1×10^8 cells ml^{-1}.

Calcium chloride (1 mM) was added to the final platelet suspension. A suspension of either PRP or washed platelets (500 μl) was incubated at 37°C for 1 min in a Payton dual-channel aggregometer (Born & Cross, 1963) with continuous stirring at 900 r.p.m. and then stimulated with arachidonic acid (1 mM), platelet-activating factor (PAF; 1 μM) and thrombin (200 mu ml^{-1}). Changes in optical density (OD) were recorded for 5 min after stimulation. For the measurement of TXB_2 release, washed platelet samples were exposed to thrombin (200 mu ml^{-1}) followed by centrifugation for 3 min at full speed in a Beckman microfuge and the supernatant removed and stored at −20°C until assayed. When required, platelets were pre-incubated with either indomethacin (10 μM) or crotapotin (100–500 μg ml^{-1}) before aggregation. The procedure for the determination of TXB_2 levels by radioimmunoassay and the specificity of the antiserum employed have been described elsewhere (Salmon, 1978). The composition of the Krebs solution was (mM): NaCl 137, KCl 2.7, $NaHCO_3$ 11.9, NaH_2PO_4 0.3, $MgSO_4$ 0.8, glucose 5.6 and $CaCl_2$ 1.0.

Guinea-pig isolated lungs

Male guinea-pigs (250–350 g) were actively sensitized by intraperitoneal injection of 50 mg of ovalbumin together with a further 50 mg given subcutaneously (each in 1 ml of 0.9% saline; Payne & De Nucci, 1987). Two weeks later, the animals were anaesthetized with pentobarbitone sodium (Sagatal, 60 mg kg^{-1}, i.p). Following mid-thoracotomy, the pulmonary artery was cannulated and perfused for 5 min with 25 ml of heparinized (10 u ml^{-1}) Krebs solution. The trachea was cannulated and the lungs were removed and suspended in a heated chamber. The lungs were perfused via the pulmonary artery with warmed (37°C) and oxygenated (95% O_2/5% CO_2) Krebs solution at 5 ml min^{-1} and left to stabilize for 20 min (Bakhle *et al.*, 1985). Crotapotin (10 μg ml^{-1}) was infused through the lungs for 30 min at 0.1 ml min^{-1}. Control lungs were infused (0.1 ml min^{-1}) with saline instead of crotapotin. Lung effluent was collected before challenge with ovalbumin and in 4 min fractions after challenge. 6-Oxo-prostaglandin $F_{1\alpha}$ and TXB_2 in the lung effluent were determined by specific radioimmunoassay (RIA) after suitable dilution in RIA buffer without prior extraction or purification.

Materials

λ Carrageenin, compound 48/80, arachidonic acid, 5-hydroxytryptamine, L-α-phosphatidylcholine β-acetyl-γ-O-alkyl (platelet-activating factor), ovalbumin and indomethacin were obtained from Sigma Chemical Co (U.S.A.). Iloprost was obtained from Schering (Germany). [^{3}H]-histamine radioimmunoassay kits was purchased from Biomerica (U.S.A.). 5,6,8,9,11,12,14,15 [^{3}H]-TXB_2 (specific activity 140 Ci $mmol^{-1}$) and 6-oxo-5,6,8,9,11,14,15(n)-[^{3}H]-$PGF_{1\alpha}$, (specific activity 150 Ci $mmol^{-1}$) were obtained from Amersham International (U.K.), respectively. The 6-oxo-$PGF_{1\alpha}$ and TXB_2 antisera were provided by Dr J. Salmon (Wellcome Research Laboratories, Beckenham, UK). All the salts were obtained from Merck (Darmstadt, Germany). *Crotalus durissus terrificus* venom was obtained from the Instituto Butantan (São Paulo, Brazil). Crotoxin and crotapotin were isolated and purified as previously described (Landucci *et al.*, 1994). Heated crotapotin was obtained by heating the protein for 15 min at 60°C.

Statistical analysis

Results are expressed as mean ± s.e.mean for n experiments. In some experiments of rat paw oedema, the area under the time course curve (AUC) was determined by using the trapezoidal rule. Statistical comparison was undertaken by means of Student's unpaired t test (two-tailed) or by analysis of variance (ANOVA) and application of the Bonferroni corrected P value for multiple comparisons. Values of $P<0.05$ were considered as significant.

Results

Effect of crotapotin and crotoxin on carrageenin-induced rat paw oedema

The subplantar injection of carrageenin (1 mg/paw) induced a paw oedema of slow onset (0.21 ± 0.03 ml by the 1st h) and prolonged duration (0.52 ± 0.05 ml by the 3rd h, $n = 20$). In contrast, crotoxin (10 μg/paw) induced a paw oedema of rapid onset (0.53 ± 0.03 ml at 0.5 h) and short duration (0.11 ± 0.02 ml at 2 h, $n = 15$). The co-injection of crotoxin with carrageenin in the rat paw caused a greater oedema (Figure 1). In contrast to crotoxin, crotapotin (3–100 μg/paw) did not cause oedema formation (data not shown; $n = 15$). However, co-injection of crotapotin (1–10 μg/paw; $n = 20$) produced a dose-dependent reduction of the carrageenin-induced oedema (Figure 2). Inhibition was also observed when crotapotin (10 μg/paw) was administered in the contralateral paw ($n = 10$; Figure 2). In addition, intraperitoneal administration of crotapotin significantly inhibited carrageenin-induced oedema (2.0 ± 0.3, 0.96 ± 0.24 and 0.76 ± 0.25 ml min^{-1}, AUC for control, crotapotin 10 and 30 μg kg^{-1}, respectively, $n = 10$, $P<0.05$). Oral administration of crotapotin also inhibited carrageenin-induced oedema (1.60 ± 0.26, 0.70 ± 0.06 and 0.46 ± 0.10 ml min^{-1}, AUC for control, crotapotin 10 and 30 μg kg^{-1}, respectively, $n = 10$, $P<0.05$). Subplantar injection of heated crotapotin (10 μg/paw) did not affect carrageenin-induced oedema (2.80 ± 0.30 and 2.70 ± 0.20 ml min^{-1}, AUC for carrageenin-induced oedema in the absence and in the presence of heated crotapotin, respectively, $n = 5$).

The carrageenin-induced oedema in adrenalectomized rats was significantly larger when compared to that observed in sham-operated animals. The subplantar injection of crotapotin (10 μg/paw) in adrenalectomized animals also caused a significant inhibition of carrageenin-induced oedema ($n = 10$; Figure 3). In rats depleted of histamine and 5-HT by chronic intraperitoneal injection of compound 48/80, the oedema induced by carrageenin was significantly reduced at 1–3 h after injection. In these animals, the co-injection of crotapotin (10 μg/paw) abolished carrageenin-induced oedema ($n = 15$; Figure 4).

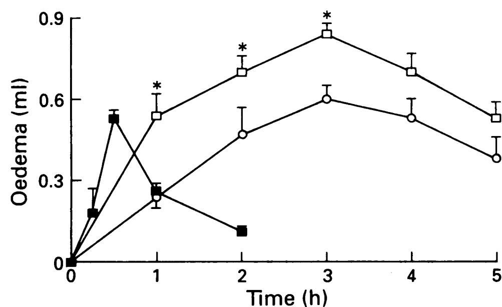

Figure 1 Effect of subplantar injection of crotoxin on carrageenin (1 mg/paw)-induced oedema. Crotoxin (10 μg/paw) was injected alone (■) or co-injected with carrageenin (□) in the rat paw. Control animals received carrageenin with saline (O) instead of crotoxin. The oedema is expressed as the increase in paw volume (ml) above its basal value. Each point represents the mean with s.e.mean from 15 rats. *$P<0.05$ compared to control animals receiving carrageenin with saline.

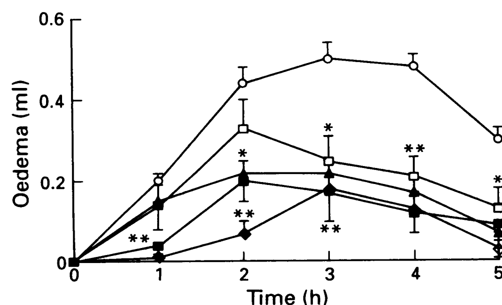

Figure 2 Subplantar injection of crotapotin either together with carrageenin or in the contralateral paw inhibits carrageenin (1 mg/paw)-induced rat paw oedema. Crotapotin was co-injected with carrageenin at doses of 1 (□), 3 (■) and 10 (◆) μg/paw. Crotapotin (10 μg/paw) was also injected in the contralateral paw (▲). Control animals (O) received carrageenin with saline instead of crotapotin. The oedema is expressed as the increase in paw volume (ml) above its basal value. Each point represents the mean with s.e.mean from 10–20 rats. *$P<0.05$, **$P<0.01$ as compared with control rats.

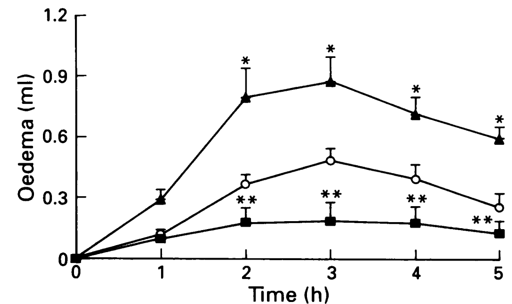

Figure 3 Crotapotin inhibits carrageenin (1 mg/paw)-induced paw oedema in adrenalectomized rats. The animals were adrenalectomized as stated in the Methods. Adrenalectomized rats (▲) showed greater oedema formation than sham-operated animals (O). Subplantar injection of crotapotin (10 μg/paw) in adrenalectomized rats (■) significantly inhibited carrageenin-induced oedema. The oedema is expressed as the increase in paw volume (ml) above its basal value. Each point represents the mean with s.e.mean from 10 rats. *$P<0.05$ compared with sham-operated rats. **$P<0.01$ compared with adrenalectomized rats.

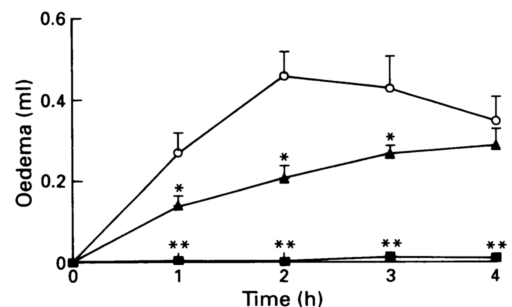

Figure 4 Crotapotin inhibits carrageenin (1 mg/paw)-induced rat paw oedema in rats chronically depleted of histamine and 5-HT. The depletion of these autacoids was performed as stated in the Methods. The oedema induced by carrageenin in the depleted animals (▲) was significantly reduced when compared to control animals (O). Subplantar injection of crotapotin (10 μg/paw) in the depleted rats (■) virtually abolished the oedema. The oedema is expressed as the increase in paw volume (ml) above its basal value. Each point represents the mean with s.e.mean from 15 rats. *$P<0.05$ compared with control rats. **$P<0.01$ compared with depleted animals.

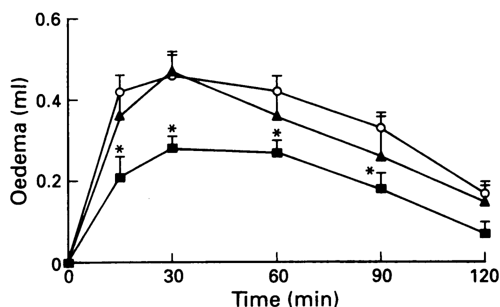

Figure 5 Crotapotin inhibits compound 48/80 (3 µg/paw)-induced paw oedema. Crotapotin was co-injected with compound 48/80 at doses of 3 (▲) and 10 (■) µg/paw. Control animals (○) were injected with compound 48/80 and saline. The oedema is expressed as the increase in paw volume (ml) above its basal value. Each point represents the mean with s.e.mean from 20 rats. $*P<0.05$ compared with control rats.

Effect of crotapotin on compound 48/80 and 5-HT-induced oedema

Subplantar injection of either compound 48/80 (3 µg/paw) or 5-HT (3 µg/paw) induced paw oedema that reached a maximum at 30 min and ended after 2 h. Subplantar injection of crotapotin (3 and 10 µg/paw) inhibited compound 48/80-induced oedema only at the higher dose ($n = 20$; Figure 5). In contrast, crotapotin (10 µg/paw) had no effect on 5-HT-induced oedema (3.90 ± 0.30 ml min $^{-1}$ and 3.20 ± 0.20 ml min^{-1}, AUC in the absence and in the presence of crotapotin, respectively, $n = 5$).

Effect of crotapotin on rat mast cell degranulation in vitro

Crotoxin (30 µg ml^{-1}) induced histamine release ($44.6 \pm 3.0\%$, $n = 5$) from rat peritoneal mast cells. In contrast, crotapotin (100 µg ml^{-1}) did not induce histamine release *per se* ($10.0 \pm 2.0\%$) nor did it affect the compound 48/80 (1.0 µg ml^{-1})-induced histamine release ($68.7 \pm 3.5\%$ and $68.1 \pm 3.0\%$, histamine release in the absence and in the presence of crotapotin, respectively, $n = 3$).

Effect of crotapotin on human platelet aggregation and on TXB_2 release

Crotapotin (50–100 µg ml^{-1}) did not affect the platelet aggregation induced by either PAF (1 µM, $n = 4$) or arachidonic acid (1 mM, $n = 4$) in PRP nor did it influence the platelet aggregation induced by thrombin (200 mu ml^{-1}) in washed platelets ($n = 4$; not shown). Crotapotin (100 and 500 µg ml^{-1}) did not induce TXB_2 release from washed platelets nor did it affect the TXB_2 release induced by thrombin (Table 1).

Effect of crotapotin on 6-oxo-$PGF_{1\alpha}$ and TXB_2 release from sensitized guinea-pig isolated lungs

Infusion of crotapotin (10 µg ml^{-1}) affected neither 6-oxo-$PGF_{1\alpha}$ (14.0 ± 2 and 14.1 ± 2 ng ml^{-1} for control and crotapotin-treated lungs, respectively, $n = 5$) nor TXB_2 (177.2 ± 37.8 and 176 ± 34.9 ng ml^{-1} for control and crotapotin-treated lungs, respectively, $n = 5$) release induced by ovalbumin (100 µg ml^{-1}) in the sensitized guinea-pig lungs.

Discussion

Our results show that crotapotin (but not the crotoxin complex) significantly inhibited carrageenin-induced rat paw oedema during the early and late phases of the response.

Table 1 Lack of effect of crotapotin on the release of thromboxane B_2 induced by thrombin in human washed platelets

Treatment	*Thromboxane B_2* (ng ml^{-1})
Basal	4.5 ± 0.3 ($n = 5$)
Thrombin (100 mu ml^{-1})	158.6 ± 18.4 ($n = 5$)*
Crotapotin (500 µg ml^{-1})	3.8 ± 0.2 ($n = 3$)
Crotapotin + Thrombin	177 ± 24.2 ($n = 5$)

$*P<0.01$ when compared to basal values. n represents the number of experiments

The most frequently encountered mechanism of action amongst anti-inflammatory drugs is the inhibition of prostaglandin synthesis (Vane, 1971; Smith & Willis, 1971). Indeed, carrageenin-induced oedema is mainly characterized by the pivotal role of prostaglandin release (Di Rosa *et al.*, 1971). However, the findings that crotapotin did not inhibit the release of either prostacyclin from guinea-pig lungs or TXB_2 from platelets as well as the aggregation induced by arachidonic acid indicate that this protein has no inhibitory activity on cyclo-oxygenase itself. Since inflamed tissues are known to express inducible cyclo-oxygenase (COX II; Lee *et al.*, 1992; Masferrer *et al.*, 1992), it is possible that crotapotin may interfere with the induction of this enzyme.

Adrenal corticosteroids are well known anti-inflammatory substances. Their anti-inflammatory effects are in part attributed to the synthesis of lipocortins, a family of glucocorticoid-induced proteins with anti-phospholipase activity (Flower, 1988). Lipocortins inhibit carrageenin-induced rat paw oedema presumably by preventing arachidonic acid mobilization from membrane phospholipids (Parente *et al.*, 1984; Flower *et al.*, 1986; Cirino *et al.*, 1989). Our results showing that crotapotin inhibited carrageenin-induced oedema in adrenalectomized rats to the same extent as in sham-operated rats, clearly indicate that the anti-oedematogenic effect of crotapotin is independent of the release of endogenous corticosteroids.

Mast cell degranulation followed by the release of both histamine and 5-HT is the first event in carrageenin-induced oedema (Di Rosa *et al.*, 1971). The finding that the oedema induced by 5-HT was not affected by crotapotin ruled out the possibility that this protein was acting as a 5-HT antagonist. The partial inhibition by crotapotin of compound 48/80-induced oedema suggests that the prevention of mast cell degranulation is a factor which may contribute to its anti-oedematogenic effect in the early stage of the inflammatory process. However, the marked inhibition caused by crotapotin on carrageenin-induced oedema cannot be explained solely by this action since other drugs which also prevent mast cell degranulation are less effective in this type of oedema (Di Rosa *et al.*, 1971). The finding that crotapotin had no effect on the *in vitro* histamine release induced by compound 48/80 may reflect the different mast cell population studied (i.e. paw vs peritoneal mast cells). In other species such as the mouse, bone marrow-derived mast cells have a different granule density, histamine content and histamine releasing capacity compared to peritoneal mast cells (Chiu & Burrall, 1990).

Crotapotin does not bind to membranes but may prevent non-specific binding of the PLA_2 component to them (Bon *et al.*, 1979). This raises the possibility that crotapotin could interact with extracellular PLA_2 generated during the inflammatory process thereby reducing the hydrolytic activity of the latter. Indeed, this type of interaction with other group II PLA_2 has already been shown by Choumet *et al.* (1993). These workers demonstrated the existence of a complex between crotapotin and the single chain PLA_2, agkistrodotoxin and that the formation of this complex enhanced the biological activity of the PLA_2. It is interesting to note that crotapotin is derived by post-translational maturation from a

precursor, proCA, homologous with secreted PLA_2 (Bouchier *et al.*, 1991). Thus, it is not unreasonable to suggest that crotapotin may in someway be able to interact with the secreted group II PLA_2 to reduce its hydrolytic activity. Alternatively, crotapotin may influence PLA_2 activity by interfering with on/off binding rates to membrane surfaces (Berg *et al.*, 1991; Jain *et al.*, 1991).

Crotapotin was effective when given orally. Since it is unlikely that a protein such as crotapotin could resist gastric proteolysis and be absorbed, it is probable that in this circumstance smaller peptide(s) is (are) responsible for the anti-oedematogenic activity observed. For instance, antiflammins are nonapeptides derived from regions of high similarity in uteroglobin and lipocortins and do retain their anti-inflammatory activity (Cirino & Flower, 1987; Cabré *et al.*, 1992). It is interesting to note that the crude venom of *Crotalus durissus terrificus* induces analgesia in mice when given orally (Giorgi *et al.*, 1993). The identification of small crotapotin-derived peptides which retain the anti-inflammatory activity of the parent protein and the delineation of their mechanism of action may widen the perspectives for the development of a new class of anti-inflammatory agents. Actually, *Crotalus durissus terrificus* venom was used clinically in the past for treatment of several diseases, including cancer, epilepsy and leprosy (Jenkins & Pendleton, 1914; Brazil, 1934).

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