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Mast cell degranulation induced by two phospholipase A_2 homologues: Dissociation between enzymatic and biological activities

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Abstract

Bothropstoxin-I and bothropstoxin-II are phospholipase A_2 homologues isolated from *Bothrops jararacussu* snake venom. The former is devoid of phospholipase A_2 activity whereas the latter has very low enzymatic activity. In this study, we have investigated the in vivo (rat paw and skin oedema) and in vitro (mast cell degranulation) inflammatory effects caused by bothropstoxin-I and bothropstoxin-II. Bothropstoxin-I (25–100 μg/paw) and bothropstoxin-II (12.5–50 μg/paw) caused dose-dependent rat paw oedema. The intradermal injection of bothropstoxin-I (0.125–5 μg/site) and bothropstoxin-II (0.125–5 μg/site) into rat skin also resulted in dose-dependent oedema formation. These oedematogenic activities were largely reduced in animals pretreated with the histamine/5-hydroxytryptamine (5-HT) receptor antagonist cyproheptadine (2 mg/kg, i.p. 0.5 h before). Similarly, *p*-bromophenacyl bromide, a compound known to inhibit phospholipase A_2 activity, significantly inhibited rat paw and skin oedema induced by both phospholipase A_2 homologues. The polyanion heparin (5 IU/site) significantly reduced the rat skin oedema induced by either bothropstoxin-I or bothropstoxin-II as well as the paw oedema (50 IU/site) induced by the former. When assayed in the rat peritoneal mast cells in vitro, both bothropstoxin-I (10 and 100 μg/ml) and bothropstoxin-II (3 and 10 μg/ml) significantly caused [^{14}C]5-HT release. The [^{14}C]5-HT release caused by these phospholipase A_2 homologues were reduced by *p*-bromophenacyl bromide and heparin (50 IU/ml). Our results indicate that oedema formation induced by bothropstoxin-I and bothropstoxin-II is mostly dependent on in vivo mast cell degranulation. Since heparin greatly reduced the oedematogenic activity of these phospholipase A_2 homologues, it is likely that the cationic charge of these substances plays a major role in the mast cell activation. Our results also indicate that *p*-bromophenacyl bromide may not be a suitable pharmacological tool to investigate the correlation between enzymatic activity and the inflammatory effects of phospholipases A_2. © 1998 Elsevier Science B.V.

Keywords: Bothropstoxin; Phospholipase A_2; Inflammation; Mast cell degranulation; Snake venom

1. Introduction

Phospholipase A_2 activity is significantly enhanced during inflammation and seems to play an important role in the generation of inflammatory events in several diseases. For instance, circulating phospholipase A_2 enzyme activity is raised in conditions such as experimental endotoxaemia (Vadas and Hay, 1983), experimental subchronic inflammation (Moreno, 1993), clinical sepsis (Vadas, 1984), rheumatoid arthritis (Pruzanski et al., 1988) and pancreatitis (Schroder et al., 1980). The ability of phospholipase A_2 to induce inflammation is thought to parallel the protein's enzymatic activity. Thus, phospholipase A_2 with high enzymatic activity (*Naja naja* venom phospholipase A_2) causes potent inflammatory effects whereas those with low enzymatic activity, such as pancreatic phospholipase A_2, have little or no inflammatory effects (Cirino et al., 1989; Moreno et al., 1992; Lloret and Moreno, 1993). However, this is still controversial. Early observations suggested that phospholipases A_2 contain separate sites for the catalytic and pharmacological activities (Rosenberg et al., 1983).

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Later, Kini and Evans (1989) described a hypothetical model to explain the mechanisms by which venom phospholipases A_2 induce different pharmacological effects. According to these authors, phospholipases A_2 bind to a particular tissue (or cell) which could be a protein (or glycoprotein) on the surface of the target cell. The targeting of phospholipases A_2 would also be determined by a specific complementary region of the phospholipase A_2 molecule, the pharmacological site, which displays high affinity towards the target molecule.

Bothropstoxin-I and bothropstoxin-II are phospholipase A_2 homologues with myotoxic activity isolated from *Bothrops jararacussu* venom (Homsi-Brandeburgo et al., 1988; Cintra et al., 1993). Although both bothropstoxin-I (MW 13 720) and bothropstoxin-II (MW 13 975) resemble phospholipase A_2 in their amino acid composition and N-terminal region sequences, the former is devoid of enzymatic activity in egg yolk lecithin whereas the latter has only low phospholipase activity in this substrate (Homsi-Brandeburgo et al., 1988). Despite the lack of phospholipase A_2 activity, bothropstoxin-I has conserved His-48 and Asp-99 residues, both of which are involved in the proposed catalytic mechanism of phospholipase A_2 activity (Van den Berg et al., 1988).

In this study, we have investigated the oedematogenic activity of both bothropstoxin-I and bothropstoxin-II in the rat paw and dorsal skin. This activity was compared to that of phospholipase A_2 from *Naja naja* venom (known to contain high enzymatic activity; Waite, 1987) and with mammalian pancreatic phospholipase A_2 (known to contain low enzymatic activity; Waite, 1987). Rat mast cell degranulation in vitro, as assessed by the release of [^{14}C]5-hydroxytryptamine, was also studied.

2. Materials and methods

2.1. Measurement of rat paw oedema

Male Wistar rats (120–150 g) were used. Hind paw oedema was induced by a single subplantar injection (0.1 ml) of the desired phospholipase A_2 (dissolved in 0.9% w/v sterile saline). Paw volume was measured immediately before the injection of the inflammatory agents and at selected times thereafter using a hydroplethysmometer (model 7150, Ugo Basile, Italy). The results are expressed as the increase in paw volume (ml) calculated by subtracting the basal volume. In some cases, the area under the time-course curve (AUC) was calculated using the trapezoidal rule and the results expressed as the % change in the total oedema volume by comparison with the control animals.

2.2. Measurement of rat skin oedema

Local oedema formation was measured in male Wistar rats (120–150 g) as the accumulation of i.v. injected ^{125}I-human serum albumin into skin sites, as described previously (Brain and Williams, 1985). The animals were anaesthetised with sodium pentobarbitone (Sagatal, 30–40 mg/kg; i.p.). ^{125}I-Human serum albumin (10 μCi/kg) and Evans blue dye (1.5 ml/kg, of a 2.5% w/v solution) were injected via the tail vein. The test agents were made up in Tyrode bicarbonate solution and injected in volumes of 100 μl into the shaved dorsal skin according to a balanced site pattern with two replicates per agent.

15 min after the injection of ^{125}I-albumin and Evans blue dye, a 5 ml cardiac blood sample was taken into heparin and the animal killed with a barbiturate overdose. The dorsal skin was removed and the injection sites punched out (12 mm diameter) and their radioactivity determined in a β counter. The oedema formation at each site was expressed as plasma volume (μl), calculated from the counts in 1 ml of plasma.

2.3. Mast cell isolation and incubation

Mast cells were obtained from 4–6 male Wistar rats (200–300 g) by injecting 10 ml of Krebs–Ringer phosphate solution (KRP; pH 7.3) into the peritoneal cavity. The abdomen was then carefully massaged and the fluid withdrawn and placed in polypropylene tubes prior to centrifugation at 300 g for 5 min at room temperature. The cell suspension was washed twice in KRP and subsequently incubated with 40 nCi of [^{14}C]5-hydroxytryptamine (5-HT)/ml at 37°C for 60 min. The cells were then washed three times and resuspended in 6.5 ml of KRP (Coleman et al., 1981). Aliquots of the peritoneal cell suspension (0.5 ml) were warmed at 37°C for 20 min prior to stimulation (final volume of 1.0 ml). After 20 min at 37°C, the cells were centrifuged (300 g, 10 min) and the supernatant removed for the determination of released [^{14}C]5-HT. Krebs–Ringer solution (1.0 ml) was added to the cell pellet to release residual [^{14}C]5-HT. The [^{14}C]5-HT concentrations were determined by measuring the luminescence in a β counter. [^{14}C]5-HT release was expressed as a percentage of the total cellular content of the amine. All values were corrected for the spontaneous [^{14}C]5-HT release which occurred in the absence of stimulus.

2.4. Modification of the protein with p-bromophenacyl bromide

Native bothropstoxin-I and/or bothropstoxin-II (900 μl) were dissolved in 0.2 M Tris–HCl pH 8.0, containing 1.4 mM EDTA and mixed with 100 μl of *p*-bromophenacyl bromide to give a final inhibition concentration of 0.08 mg/ml. The mixture was incubated for 20 h at 37°C. The non-reacted inhibitor was removed by ultrafiltration using an Amicon model 8 MC apparatus and a filter with a cut off of 3 kDa. The ultrafiltration process resulted in a protein loss of approximately 32%.

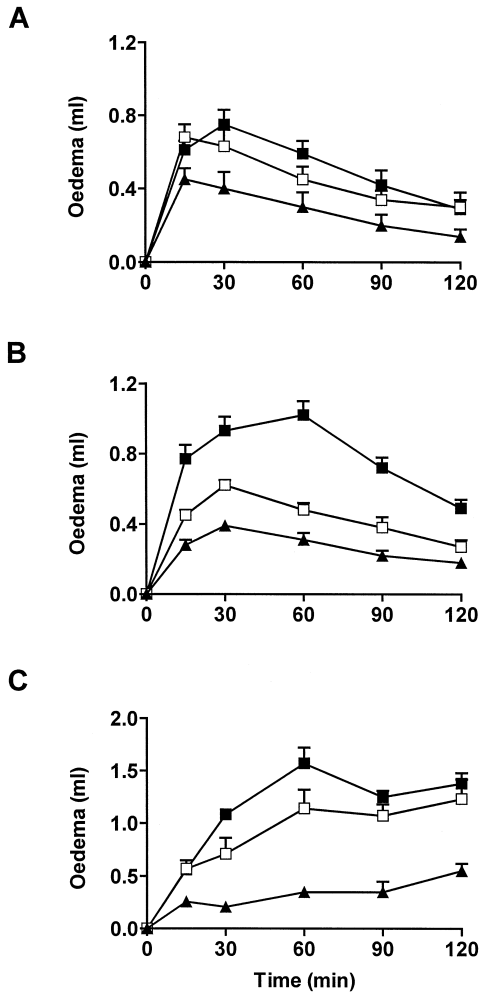

Fig. 1. Dose-dependent rat paw oedema induced by bothropstoxin-I (panel A: ▲ 25; □ 50 and ■ 100 μg/paw), bothropstoxin-II (panel B; ▲ 12.5; □ 25 and ■ 50 μg/paw) and *Naja naja* phospholipase A_2 (panel C; ▲ 1; □ 5 and ■ 10 μg/paw). The oedema is expressed as the increase in volume (ml) of the injected paw compared to its basal volume. Each point represents the mean of 5–15 rats; S.E.M. is shown by the vertical bars.

2.5. Reagents

Bothropstoxin-I and bothropstoxin-II were isolated and purified as previously described (Homsi-Brandeburgo et al., 1988; Cintra et al., 1993). Phospholipase A_2 from *Naja naja* venom and bovine pancreas, *p*-bromophenacyl bromide, EDTA, compound 48/80 and cyproheptadine were obtained from Sigma Chemical Co. (St. Louis). ^{125}I-Human serum albumin (50 μCi/ml, 20 mg albumin/ml) and [^{14}C]5-HT were obtained from Amersham International (UK). Evans blue dye was obtained from Merck (Darmstadt, Germany). Pentobarbitone sodium (Sagatal) was obtained from May and Baker (Dagenham, Essex, UK). Heparin was obtained from Roche (Rio de Janeiro, Brazil). All the salts were obtained from Merck (Darmstadt, Germany).

2.6. Statistical analysis

Results are expressed as mean ± S.E.M. 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.

3. Results

3.1. Rat paw oedema

The subplantar injection of either bothropstoxin-I (25–100 μg/paw, $n = 5$; Fig. 1A) or bothropstoxin-II (12.5–50 μg/paw, $n = 5$; Fig. 1B) induced dose-dependent rat paw oedema. The oedema induced by both phospholipase A_2 homologues (50 μg/paw) was rapid in onset (0.7 ± 0.07 ml and 0.8 ± 0.08 ml within 15 min for bothropstoxin-I and bothropstoxin-II, respectively) and of short duration (0.4 ± 0.04 and 0.5 ± 0.05 ml after 120 min, respectively). Phospholipase A_2 from *Naja naja* venom (1–10 μg/paw, $n = 15$) also induced dose-dependent and sustained rat paw oedema (Fig. 1C). The area under the time-course curve (AUC; ml · min) clearly showed the following order of potency: *Naja naja* phospholipase A_2 > bothropstoxin-II > bothropstoxin-I (Table 1). In contrast to the above phospholipase A_2, bovine pancreatic phospholipase A_2 did not induce significant oedema in doses up to 100 μg/paw (0.1 ± 0.03 and 0.04 ± 0.02 ml after 15 and 120 min, respectively, $n = 5$).

Previous treatment of the animals with the histamine/5-HT receptor antagonist cyproheptadine (2 mg/kg, i.p., 30 min before; $n = 5$) virtually abolished the oedema formation induced by either bothropstoxin-I or bothropstoxin-II

Table 1
Rat paw oedema induced by *Naja naja* phospholipase A_2, bothropstoxin-I and bothropstoxin-II. The area under the time-course curve (AUC; ml · min) was calculated using the trapezoidal rule. Each point represents the mean ± S.E.M. of 5–15 rats

Phospholipase A_2	Dose (μg/paw)	AUC (ml · min)
Naja naja	1.0	1.6 ± 0.2
	5.0	4.8 ± 0.7
	10.0	6.0 ± 0.2
Bothropstoxin-I	25	1.5 ± 0.3
	50	2.4 ± 0.3
	100	2.8 ± 0.3
Bothropstoxin-II	12.5	1.4 ± 0.1
	25	2.3 ± 0.2
	50	4.1 ± 0.3

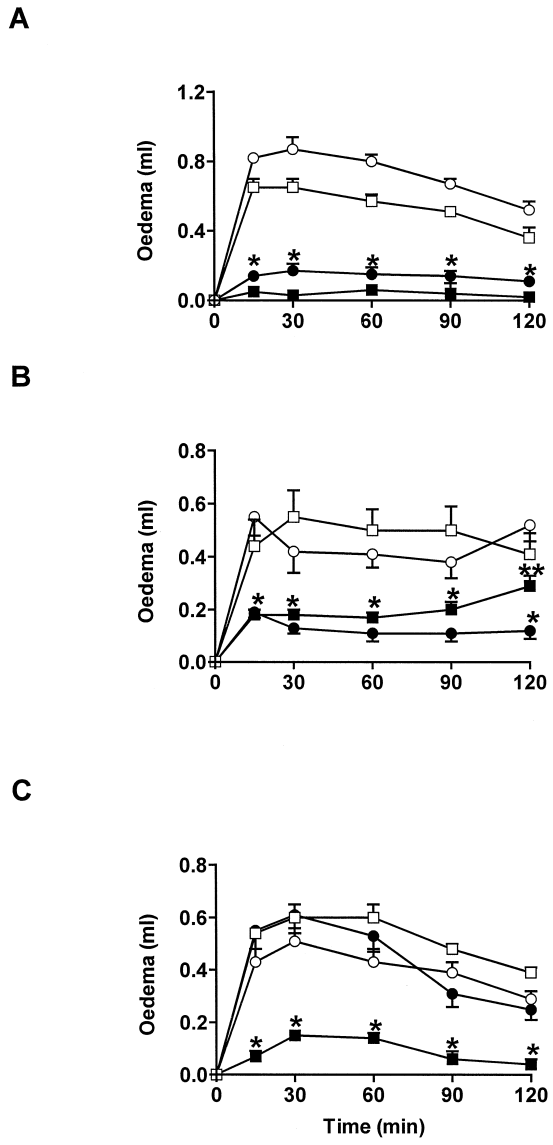

Fig. 2. Effect of cyproheptadine (2 mg/kg, i.p., 30 min before; panel A), *p*-bromophenacyl bromide (panel B) and heparin (50 IU/paw; panel C) on the rat paw oedema induced by bothropstoxin-I (100 μg/paw; ●) and bothropstoxin-II (50 μg/paw; ■). The effect of bothropstoxin-I and bothropstoxin-II in control animals is represented by the symbols ○ and □, respectively. *p*-Bromophenacyl bromide was employed as described in Section 2. The oedema is expressed as the increase in volume (ml) of the injected paw compared to its basal volume. Each point represents the mean of 5–10 rats; S.E.M is shown by the vertical bars. $^{*}P < 0.01$, $^{**}P < 0.05$ compared to their respective control values.

(Fig. 2A). Cyproheptadine (same dose) also markedly reduced the paw oedema induced by both the *Naja naja* phospholipase A_2 (1.2 ± 0.2 and 0.07 ± 0.04 ml · min, AUC for control and treated animals, respectively, $n = 5$) and the mast cell degranulating compound 48/80 (30 min: 0.9 ± 0.05 and 0.2 ± 0.1 ml, for control and treated animals, respectively, $n = 5$).

Fig. 2B shows that previous incubation of bothropstoxin-I and bothropstoxin-II with *p*-bromophenacyl bromide (see Section 2) markedly inhibited the rat paw oedema induced by these phospholipase A_2 homologues. This inhibition was specific for these proteins since the paw oedema induced by compound 48/80 (3 μg/paw; 2.2 ± 0.1 ml · min, $n = 5$) was not significantly affected when this agent was prepared in the presence of *p*-bromophenacyl bromide (2.3 ± 0.3 ml · min, $n = 5$).

Heparin (50 IU/paw) significantly reduced the paw oedema induced by bothropstoxin-I (100 μg/paw, $n = 10$; Fig. 2C) but it had no significant effect on the bothropstoxin-II-(50 μg/paw)-induced oedema ($n = 10$; Fig. 2C).

3.2. Rat skin oedema

The intradermal injection of bothropstoxin-I (0.125–5 μg/site, $n = 8$), bothropstoxin-II (0.125–5 μg/site, $n = 8$) or *Naja naja* phospholipase A_2 (0.125–5 μg/site, $n = 8$) caused dose-dependent oedema formation in the rat skin, as assessed 15 min after the injection (Fig. 3).

Previous treatment of the animals with cyproheptadine (2 mg/kg, i.p., 30 min before) markedly reduced the oedema formation induced by either bothropstoxin-I (5 μg/site; $n = 4$) or bothropstoxin-II (5 μg/site, $n = 4$; Fig. 4A). At this dose, cyproheptadine reduced the compound 48/80 (1 μg/site)-induced rat skin oedema by 90% ($n = 10$).

p-Bromophenacyl bromide significantly inhibited the skin oedema induced by bothropstoxin-I (5 μg/site, $n = 4$) and bothropstoxin-II (5 μg/site, $n = 4$; Fig. 4B) without affecting that caused by compound 48/80 (0.5 μg/site; 97 ± 3.5 and 90 ± 13 μl, plasma extravasation for 48/80 prepared in saline and in a solution of *p*-bromophenacyl bromide, respectively, $n = 10$).

Heparin (5 IU/site) significantly reduced the skin oedema induced by both bothropstoxin-I (5 μg/site, $n = 7$) and bothropstoxin-II (5 μg/site, $n = 7$; Fig. 4C).

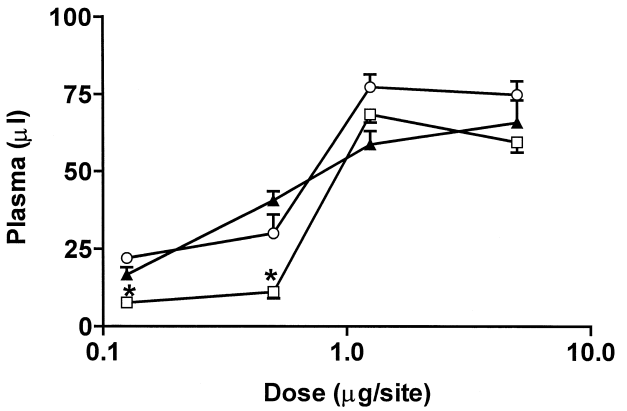

Fig. 3. Dose-dependent rat skin oedema induced by bothropstoxin-I (□), bothropstoxin-II (○) and *Naja naja* venom phospholipase A_2 (▲). The oedema was measured 15 min after the intradermal injection of the above described agents and is expressed as μl of plasma protein extravasation. Each point represents the mean of 8 rats; S.E.M. is shown by the vertical bars. $^{*}P < 0.05$ compared to bothropstoxin-II or *Naja naja* phospholipase A_2.

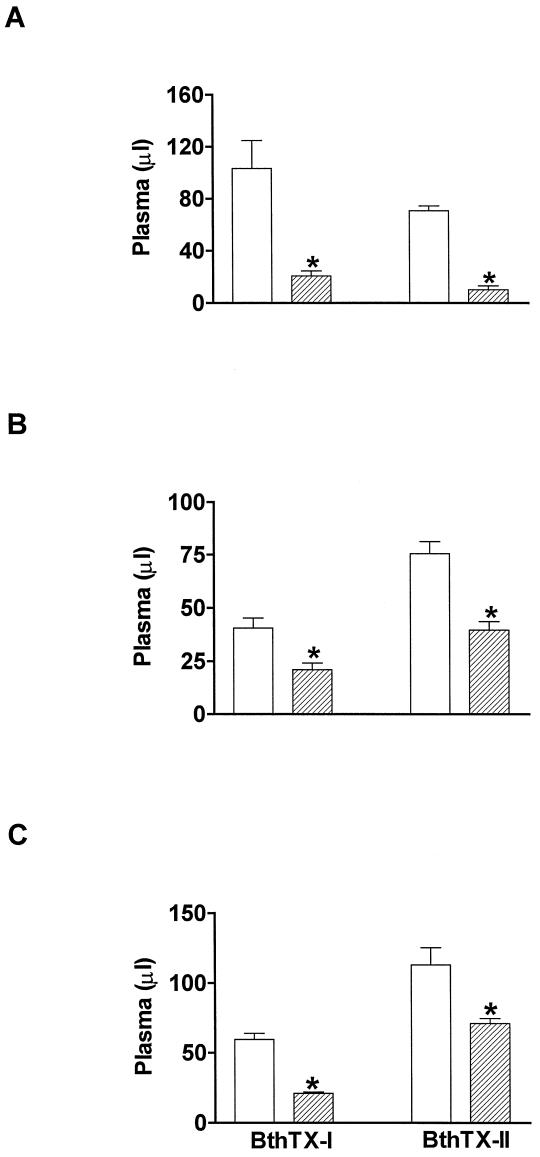

Fig. 4. Inhibition by cyproheptadine (2 mg/kg, i.p., 30 min before; panel A), *p*-bromophenacyl bromide (panel B) and heparin (5 IU/site; panel C) of the rat skin oedema induced by either bothropstoxin-I (5 μg/site; striped bars) or bothropstoxin-II (5 μg/site; striped bars). The effect of bothropstoxin-I and bothropstoxin-II in control animals is represented the open bars. Each point represents the mean of 4–7 rats. S.E.M. is shown by the vertical bars. $^{*}P < 0.01$ compared to their respective control values.

3.3. Mast cell degranulation in vitro

Bothropstoxin-I (10 and 100 μg/ml, $n = 3$), bothropstoxin-II (3 and 10 μg/ml, $n = 5$), *Naja naja* phospholipase A_2 (3 μg/ml) and compound 48/80 (10–30 μg/ml, $n = 3$) induced a significant release of [^{14}C]5-HT from rat peritoneal mast cells (Table 2).

p-Bromophenacyl bromide significantly inhibited the [^{14}C]5-HT release induced by both bothropstoxin-I (100 μg/ml; 50 ± 6 and 31 ± 2%, release for control and *p*-bromophenacyl bromide-treated bothropstoxin-I, respectively, $n = 5$) and bothropstoxin-II (100 μg/ml; 44 ± 7 and 3 ± 1%, release for control and *p*-bromophenacyl bromide-treated bothropstoxin-II, respectively, $n = 5$; $P < 0.01$).

Heparin (50 IU/ml) significantly inhibited the [^{14}C]5-HT release induced by both bothropstoxin-I (100 μg/ml; 37 ± 9 and 0.7 ± 0.6% release for control and heparin-treated mast cells, respectively, $n = 3$; $P < 0.05$) or bothropstoxin-II (100 μg/ml; 51 ± 8 and 17 ± 9%, release for control and heparin-treated mast cells, respectively, $n = 4$; $P < 0.05$).

Table 2
[^{14}C]5-HT release from rat peritoneal mast cells induced by compound 48/80 (3–30 μg/ml), *Naja naja* phospholipase A_2 (3 μg/ml), bothropstoxin-I (10 and 100 μg/ml) and bothropstoxin-II (3 and 10 μg/ml). Results represent the mean ± S.E.M. of 3–5 experiments

Substance	Dose (μg/ml)	[^{14}C]5-HT (%)
48/80	3	31 ± 1
	10	56 ± 2
	30	63 ± 3
N. naja phospholipase A_2	3	62 ± 2
Bothropstoxin-I	10	33 ± 3
	100	67 ± 4
Bothropstoxin-II	3	32 ± 6
	10	56 ± 4

4. Discussion

Our results demonstrate that the phospholipase A_2 homologues bothropstoxin-I and bothropstoxin-II increased microvascular permeability in both the rat paw and skin in vivo leading to local oedema formation. The finding that the histamine/5-HT antagonist cyproheptadine abolished the bothropstoxin-I and bothropstoxin-II-induced oedema and that these substances also caused the release of [^{14}C]5-HT from rat peritoneal mast cells in vitro indicate that the oedema formation is largely dependent on in vivo mast cell activation, as previously observed for other snake phospholipases A_2 (Cirino et al., 1989; Wang and Teng, 1990; Moreno et al., 1992). Since bothropstoxin-I has no phospholipase activity whereas bothropstoxin-11 presents residual phospholipase activity (Homsi-Brandeburgo et al., 1988; Cintra et al., 1993), our results suggest that the hydrolysis of phospholipids by phospholipase A_2 is not essential for oedema formation. This concept is further supported by the findings of Rodrigues-Simioni et al. (1995) who demonstrated that although bothropstoxin-I has low level of phospholipase activity in primary cultures of human and rat skeletal muscle, the contracture and blockade of the mouse isolated diaphragm induced by this toxin is unrelated to this activity. Indeed, mechanisms either dependent or independent of their catalytic activity

was proposed by Kini and Evans (1989) to explain the actions of phospholipases A_2.

Mast cells are located close to blood vessels, nerves, epithelia and smooth muscle. Interestingly, they express and release type II phospholipase A_2, which is thought to modulate mast cell degranulation (Murakami et al., 1993). *p*-Bromophenacyl bromide inhibits phospholipases A_2 enzymatic activity by alkylating the histidine-48 residue located in the active site (Dennis, 1987). This inhibitor is widely employed to evaluate the involvement of the catalytic activity of different phospholipases A_2 in biological phenomena (Yang, 1994). *p*-Bromophenacyl bromide markedly inhibited both the oedema formation in vivo and the mast cell degranulation in vitro induced by bothropstoxin-I (and bothropstoxin-II) even though these phospholipase A_2 homologues have no (or present residual) enzymatic activity. This suggests that besides alkylating the histidine residue (and hence inhibiting enzymatic activity), *p*-bromophenacyl bromide may have additional effects on the phospholipases A_2 structure which may result in conformational changes in the molecule. Actually, it is known that the ability of phospholipase A_2 inhibitors to suppress histamine release is independent of either cyclo-oxygenase and lipoxygenase products (Cirino et al., 1989), further supporting the concept of the dissociation between catalytic and degranulating activity. Alternatively, histidine-48 may also be important for the pharmacological domain activity of phospholipase A_2, independent of the catalytic site (Diaz et al., 1993). Furthermore, *p*-bromophenacyl bromide is not considered an specific phospholipase A_2 inhibitor since it may interact irreversibly with many other enzymes and can also irreversibly bind thiol groups (see Blackwell and Flower, 1983). Thus, caution should be exercised when using *p*-bromophenacyl bromide to ascertain the involvement of enzymatic activity in the biological responses to phospholipase A_2.

Compounds rich in cationic charges such as polyarginine and polylysine activate rat mast cells in vitro (Padawer, 1970; Ennis et al., 1980; Coleman et al., 1981) and in vivo (Needham et al., 1988; Antunes et al., 1990, Santana et al., 1993) and this effect is largely reduced by polyanionic molecules including heparin and its analogues. Since plasma membranes from different cell types (including mast cells) contain fixed anionic sites constituted mainly by sulphated glycosaminoglycans (Lane and Lindahl, 1989; Schwartz, 1994), it is thought that mast cell activation by these polycations takes place by electrostatic interactions with these cell surface anionic sites. The finding that heparin inhibited mast cell activation by bothropstoxin-I and bothropstoxin-II both in vivo (except the bothropstoxin-II-induced paw oedema) and in vitro suggests that the cationic charge content of these molecules is the main factor responsible for their pharmacological properties. Similar observations have been observed with other snake phospholipases A_2 (Wang and Teng, 1990; Murakami et al., 1993). Heparin most likely competes with the anionic sites on the mast cell surfaces for binding to the cationic charges of these phospholipases A_2. A recent study showed that human nonpancreatic secretory phospholipase A_2 interacts with proteoglycans via their glycosaminoglycan moiety (Sartipy et al., 1996). Furthermore, the eosinophil-derived major cationic protein, an endogenous cationic protein, is thought to cause inflammation through this type of electrostatic interaction (Barker et al., 1991; Fredens et al., 1991).

Interestingly, *Naja naja* phospholipase A_2 was more potent than the bothropstoxins in its ability to cause rat paw oedema and skin oedema, and bothropstoxin-II was more potent than bothropstoxin-I despite the high degree of homology and similar cationic content of the latter two proteins (Gutierrez and Lomonte, 1995). This discrepancy probably indicates that in addition to electrostatic forces, other factors such as the helical conformation of the molecules may be of importance. Hydrophobic bonding and chain stiffness also seem to modulate interactions mediated by cationic substances (Ichimura and Zama, 1977; Ichimura et al., 1978).

The above results indicate that anionic substances such as heparin inhibit oedema formation. Thus, anionic substances devoid of anticoagulant activity such as heparan and other proteoglycans may be useful for the treatment of inflammatory diseases (Antunes et al., 1990)

References

Antunes, E., Mariano, M., Cirino, G., Levi, S., De Nucci, G., 1990. Pharmacological characterisation of polycation-induced rat hind-paw oedema. Br. J. Pharmacol. 101, 986–990.

Barker, R.L., Gundel, R.H., Gleich, G.J., Checkel, J.L., Loegering, D.A., Pease, L.R., Hamann, K.J., 1991. Acid polyamino acids inhibit human eosinophil granule major basic protein toxicity. J. Clin. Invest. 88, 798–805.

Blackwell, G.J., Flower, R.J., 1983. Inhibition of phospholipase. Br. Med. Bull. 39, 260–264.

Brain, S.D., Williams, T.J., 1985. Inflammatory oedema induced by synergism between calcitonin gene-related peptide (CGRP) and mediators of increased vascular permeability. Br. J. Pharmacol. 86, 855–860.

Cintra, A.C.O., Marangoni, S., Oliveira, B., Giglio, J.R., 1993. Bothropstoxin-I: Amino acid sequence and function. J. Protein Chem. 12, 57–64.

Cirino, G., Peers, S.H., Wallace, J.L., Flower, R.J., 1989. A study of phospholipase A_2-induced oedema in rat paw. Eur. J. Pharmacol. 166, 505–510.

Coleman, J.W., Holgate, S.T., Church, M.K., Godfrey, R.C., 1981. Immunoglobulin E decapeptide-induced 5-hydroxytryptamine release from rat peritoneal mast cells. Biochem. J. 198, 615–619.

Dennis, E.A., 1987. Regulation of eicosanoid production: Role of phospholipase and inhibitors. BioTechnology 5, 1294–1300.

Diaz, C., Gutierrez, J.M., Lomonte, B., Nunez, J., 1993. *p*-Bromophenacyl bromide modification of *Bothrops asper* myotoxin II, a lysine-49 phospholipase A_2, affects its pharmacological activities. Toxicon 31, 1202–1206.

Ennis, M., Pearce, F.L., Weston, P.M., 1980. Some studies on the release of histamine from mast cells stimulated with polylysine. Br. J. Pharmacol. 70, 329–334.

Fredens, K., Dahl, R., Venge, P., 1991. In vitro studies of the interaction between heparin and eosinophil cationic protein. Allergy 46, 27–29.

Gutierrez, J.M., Lomonte, B., 1995. Phospholipase A_2 myotoxins from *Bothrops* snake venoms. Toxicon 33, 1405–1424.

Homsi-Brandeburgo, M.l., Queiroz, L.S., Santo-Neto, H., Rodrigues-Simioni, L., Giglio, J.R., 1988. Fractionation of *Bothrops jararacussu* snake venom: Partial chemical characterization and biological activity of bothropstoxin. Toxicon 26, 615–627.

Ichimura, S., Zama, M., 1977. The interactions of 8-anilinonaphtalene-sulfonate with polylysine and polyarginine. Biopolymers 16, 1449–1464.

Ichimura, S., Mita, K., Zama, M., 1978. Conformation of poly (L-arginine). I. Effects of anions. Biopolymers 17, 2769–2782.

Kini, R.M., Evans, H.J., 1989. A model to explain the pharmacological effects of snake venom phospholipase A_2. Toxicon 27, 613–635.

Lane, D.A., Lindahl, U., 1989. Heparin: Chemical and Biological Properties, Clinical Application, Edward Arnold, London.

Lloret, S., Moreno, J.J., 1993. Oedema formation and degranulation of mast cells by phospholipase A_2 purified from porcine pancreas and snake venoms. Toxicon 31, 949–956.

Moreno, J.J., 1993. Time-course of phospholipase A_2, eicosanoid release and cellular accumulation in rat immunological air pouch inflammation. Int. J. Immunopharmacol. 15, 597–603.

Moreno, J.J., Ferrer, X., Ortega, E., Carganico, G., 1992. Phospholipase A_2-induced oedema in rat skin and histamine release in rat mast cells. Evidence for involvement of Iysophospholipids in the mechanism of action. Agents Actions 36, 258–263.

Murakami, M., Hara, N., Kudo, I., Inoue, K., 1993. Triggering of degranulation in mast cells by exogenous type 11 phospholipase A_2. J. Immunol. 151, 5675–5684.

Needham, L., Hellewell, P.G., Williams, T.J., Gordon, J.L., 1988. Endothelial functional responses and increased vascular permeability induced by polycations. Lab. Invest. 59, 538–548.

Padawer, J., 1970. The reaction of rat mast cells to polylysine. J. Cell Biol. 47, 352–372.

Pruzanski, W., Keystone, E.C., Sternby, B., Bombardier, C., Snow, K.M., Vadas, P., 1988. Serum phospholipase A_2 correlates with disease activity in rheumatoid arthritis. J. Rheumat. 15, 1351–1356.

Rodrigues-Simioni, L., Prado-Franceschi, J.A.C., Cintra, A.C., Giglio, J.R., Jiang, M.S., Fletcher, J.E., 1995. No role for enzymatic activity or dantrolene-sensitive Ca^{2+} stores in the muscular effects of Bothropstoxin, a Lys-^{49} phospholipase A_2 myotoxin. Toxicon 33, 1479–1489.

Rosenberg, P., Condrea, E., Rapuano, B.E., Soons, K.R., Yang, C.C., 1983. Dissociation of pharmacological and enzymatic activities of snake venom phospholipase A_2 by modification of carboxylate groups. Biochem. Pharmacol. 32, 3525–3530.

Santana, A., Hyslop, S., Antunes, E., Mariano, M., Bakhle, Y.S., De Nucci, G., 1993. Inflammatory responses induced by poly-L-arginine in rats lungs in vivo. Agents Actions 39, 104–110.

Sartipy, P., Johansen, B., Camejo, G., Rosengren, B., Bondjers, G., Hurt-Camejo, E., 1996. Binding of human phospholipase A_2 type II to proteoglycans: Differential effect of glycosaminoglycans on enzyme activity. J. Biol. Chem. 271, 26307–26314.

Schroder, T., Kivilakso, E., Kinnunen, P.K.J., Lempinen, M., 1980. Serum phospholipase A_2 in human acute pancreatitis. Scand. J. Gastroenterol. 15, 633–636.

Schwartz, L.B., 1994. Mast cells: Function and contents. Curr. Opin. Immunol. 6, 91–97.

Vadas, P., 1984. Elevated plasma phospholipase A_2 levels correlation with the hemodynamic and pulmonary changes in Gram-negative septic shock. J. Lab. Clin. Med. 104, 873–877.

Vadas, P., Hay, J.B., 1983. Involvement of circulating phospholipase A_2 in the pathogenesis of the hemodynamic changes in endotoxin shock. Can. J. Physiol. Pharmacol. 61, 561–567.

Van den Berg, C.J., Slotboom, A.J., Verheij, H.M., De Haas, G.H., 1988. The role of Asp-49 and other conserved amino acids in phospholipases A_2 and their importance for enzymatic activity. J. Cell. Biochem. 39, 379–390.

Waite, M., 1987. The phospholipases. In: Hanahan, D.J. (Ed.), Handbook of Lipid Research, vol. 5. Plenum Press, New York, p. 155.

Wang, J.P., Teng, C.M., 1990. Comparison of the enzymatic and edema-producing activities of two venom phospholipase A_2 enzymes. Eur. J. Pharmacol. 190, 347–354.

Yang, C.C., 1994. Structure–function relationship of phospholipase A_2 from snake venoms. J. Toxicol. 13, 125–177.