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Antibodies to a phospholipase A_2 from *Vipera russelli* selectively neutralize venom neurotoxicity

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SUMMARY

Polyclonal antibodies to a purified neurotoxic phospholipase A_2 (PLA_2), VRV PL-VIII$_a$, from *Vipera russelli* venom were raised in rabbits. Anti-PL-VIII$_a$-Ig (γ-globulin fraction of rabbit antiserum injected with VRV PL-VIII$_a$) selectively neutralized the neurotoxicity of VRV PL-VIII$_a$, VRV PL-V, VRV PL-VI (neurotoxic PLA_2 of *V. russelli* venom) and whole *V. russelli* venom without affecting their PLA_2 activity, which clearly demonstrates that the catalytic site and the neurotoxic site (the site through which the PLA_2 binds to the nervous system) are distinct on a PLA_2 molecule. Anti-PL-VIII$_a$ Ig did not have any effect on the oedema-inducing activity and indirect haemolytic activity of VRV PL-VIII$_a$, VRV PL-V and VRV PL-VI, which are attributed to the PLA_2 activity of the peptide, but inhibited their anti-coagulant potency.

INTRODUCTION

The venom phospholipase A_2 (PLA_2) (EC 3.1.1.4), along with their contribution in digesting the prey, induce pathological symptoms, including neurotoxic, myotoxic, cardiotoxic, haemolytic, anti-coagulant, convulsant and hypotensive effects. The presynaptic neurotoxins are highly potent toxins of snake venom and they are either basic PLA_2 *per se* or contain basic PLA_2 as an indispensable part of their structure (Kini & Iwanaga, 1986a). Attempts are being made to identify the relationship between the pharmacological properties and catalytic activity of PLA_2. Although the dissociation of enzymatic and pharmacological properties has been achieved to a certain extent, there is also considerable evidence in favour of the essential role of PLA_2 activity in toxicity (Kini & Iwanaga, 1986a, b).

Using hydropathy profiles, Kini & Iwanaga (1986a) predicted that the neurotoxic phospholipases have a distinct hydrophobic region around amino acid residues 80–110, which is the neurotoxic potential probably involved in the interaction with the synaptic membrane, which is absent in non-neurotoxic PLA_2.

Recently, a theoretical approach has been used to locate specific pharmacological sites in venom PLA_2 enzymes, and the prediction indicates the presence of specific sites associated with a particular pharmacological activity, and the absence of such sites results in non-exhibition of those pharmacological effects (Kini & Evans, 1989). Their predictions are strengthened by previous results of the present authors. A neurotoxic PLA_2 of *Vipera russelli* venom, upon self-aggregation, loses its lethality and neurotoxicity, whereas there is a remarkable increase in its catalytic activity. Antibodies prepared against the same aggregated PLA_2 nullifies the lethality and neurotoxic symptoms, whereas its catalytic activity remains unaffected. Based on these results, the possible presence of distinct neurotoxic and catalytic sites on a PLA_2 molecule is suggested (Jayanthi, Kasturi & Gowda, 1989; Kasturi & Gowda, 1990).

In the present study a successful attempt is made to demonstrate the presence of a distinct 'neurotoxic potential' involved in the interaction with the nervous system apart from the catalytic site on the venom PLA_2 molecule.

Abbreviations: Ag–Ab; antigen–antibody; i.p., intraperitoneal; ND, not determined; PC, phosphatidyl choline; PLA_2, phospholipase A_2; SDS, sodium dodecyl sulphate; VRV, *Vipera russelli* venom; w/w, weight/weight.

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MATERIALS AND METHODS

Materials

Vipera russelli venom was purchased from Irula Snake Catcher's Industrial Cooperative Society Limited, Madras (Lot no. ISCICS-II). CM-Sephadex-C-25 (4·5 meq/g) and Sephadex-G-50 were from Pharmacia Fine Chemicals, Uppsala, Sweden. Linoleic acid, agarose, Freund's complete and incomplete adjuvants and α-nitroso-β-naphthal were purchased from Sigma Chemical Company, St Louis, MO. All other chemicals and solvents were of analytical grade. The solvents were redistilled before use. Male Swiss Wistar mice, weighing 20–22 g, were used for all pharmacological studies. Female rabbits, weighing $\sim$1·5 g, were used for the preparation of antibodies.

Purification of PLA_2 enzyme VRV Pl-$VIII_a$ from V. russelli *venom*

VRV PL-$VIII_a$ was purified to homogeneity as described elsewhere (Kasturi & Gowda, 1989).

Preparation of VRV PL-$VIII_a$ toxoid and production of anti-VRV Pl-$VIII_a$ antibody

VRV PL-$VIII_a$ toxoid was prepared by formalin treatment, according to the method of Kondo *et al.* (1970). Antibodies were raised in rabbits as described elsewhere (Kasturi & Gowda, 1990)

Preparation of γ-globulin and detection of antibodies

γ-Globulin was prepared from rabbit antiserum injected with VRV PL-$VIII_a$ toxoid, by precipitation with ammonium sulphate as described by Heide & Schwick (1973). The γ-globulin fraction thus obtained is termed anti-PL-$VIII_a$ Ig. The Ouchterlony agar gel double-diffusion technique was used to detect precipitating antibodies, as described by Williams & Chase (1971).

Preparation of synaptosomes from rat brain

The procedure for isolating synaptosomal fraction was based on the method of Cotman *et al.* (1970). Briefly, the cerebral cortices from three to five male rats were used for each experiment. Removal of the cortex was achieved within 40 seconds of decapitation. The cortex was washed thoroughly in ice-cold saline and homogenized in 0·32 M sucrose, pH 7·0 (20% w/v). The homogenate was diluted to 10% w/v in 0·32 M sucrose and centrifuged at 1000 *g* for 5 min. The supernatant was discarded. The precipitate was resuspended in a minimum amount of 0·32 M sucrose and applied to a gradient consisting of 7 ml each of 4%, 6% and 13% w/v Ficoll (Sigma) in 0·32 M sucrose and centrifuged at 64,000 *g* for 45 min. The synaptosomal fraction settled at the 6–13% interface was collected with 4 volumes of 0·32 M sucrose, pH 7·0, and centrifuged at 50,000 *g* for 20 min, which resulted in pellet formation.

Assay of PLA_2 activity using egg phosphatidyl choline (PC) and synaptosomes as substrates

PLA_2 was assayed by estimating the free fatty acid released, as described in earlier papers (Kasturi & Gowda, 1989), using egg PC and synaptosomes as the substrates. PLA_2 activity is expressed in terms of nmoles of fatty acid released per min per mg of protein. In order to study the effect of anti-PL-$VIII_a$ Ig on the PLA_2 activity of VRV PL-$VIII_a$, VRV PL-VI, VRV PL-V and whole *V. russelli* venom, the enzyme samples were incubated with various concentrations of anti-PL-$VIII_a$ Ig (w/w) for different lengths of time at 37° in phosphate-buffered saline (PBS), pH 7·5, and the PLA_2 assay was carried out by using both egg PC and synaptosomes as substrates. The typical reaction mixture (1 ml) contained 400 nmole of egg PC (or synaptosomes, when synaptosomes were used as substrate), in 400 μl of 0·05 M Tris-HCl buffer, pH 7·5, 100 μl of diethyl ether, 40 mM Ca^{2+}, and 50 μg of enzyme sample with different concentrations of anti-PL-$VIII_a$ Ig in 400 μl of PBS, pH 7·5. PLA_2 activity is expressed in terms of amount of fatty acid released in nmoles per min per mg of protein.

Assay of indirect haemolytic activity

A semi-quantitative indirect haemolytic assay was employed to study the effect of anti-PL-$VIII_a$ Ig on the haemolysis induced by PLA_2 enzyme VRV PL-$VIII_a$, VRV PL-V, VRV PL-VI and whole *V. russelli* venom, as described by Viswanath, Kini & Gowda (1987).

Lethal potency

In order to study the effect of anti-PL-$VIII_a$ Ig on the lethal potency of VRV PL-$VIII_a$, VRV PL-V, VRV PL-VI and whole *Vipera russelli* venom, these toxins were preincubated with various concentrations of anti-PL-$VIII_a$ Ig at 37° for 1 hr and injected separately by i.p. route into groups of eight mice at twice their LD_{50} doses. The number of deaths occurring within 24 hr was noted.

Oedema-inducing activity

The effect of anti-PL-$VIII_a$ Ig on the oedema-inducing activity of all the enzymes and whole venom was checked by incubating these proteins with different concentrations of anti-PL-$VIII_a$ Ig for different lengths of time at 37° in PBS (pH 7·5) (Vishwanath *et al.*, 1987). The oedema ratio (the weight of the oedematous leg × 100/wt of the normal leg), at 5 μg concentration of each protein, and minimum oedema dose (amount of protein causing an oedema ratio of 120%) in the presence of different concentrations of anti-PL-$VIII_a$ Ig were calculated.

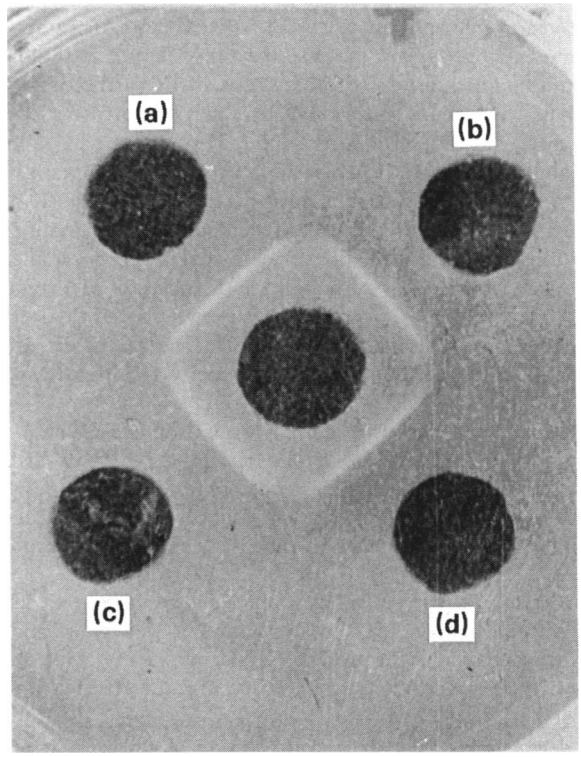

Figure 1. Ouchterlony immunodiffusion of antigens against antibody; immunodiffusion was carried out in 1% Agar gel. The central well contained anti-PL-$VIII_a$ Ig (100 μg). (a) VRV PL-$VIII_a$; (b) VRV PL-V; (c) VRV PL-VI; (d) whole *V. russelli* venom (50 μg each).

Table 1. Effect of anti-PL-VIII$_a$ Ig on the PLA_2 activity of VRV PL-VIII$_a$, VRV PL-V, VRV PL-VI and Crude *V. russelli* venom with egg PC used as substrate

Conc. antibody (anti-PL-VIII$_a$ Ig) added (μg)	Incubation of Ag–Ab: time in hr at 37°	Specific activity*			
		VRV PL-VIII$_a$	VRV PL-V	VRV PL-VI	Crude venom
—	1	111±2	52±5	84±2	56±5
	2	111±3	56±0·5	81±1	57±2
	24	111±33	58±2	84±0·5	5±0·5
50	1	111±2	56±2	86±1	57±2
50	2	106±0·5	55±2	81±3	58±0·5
50	24	111±3	56±0·5	84±1	59±3
100	1	105±0·5	53±0·5	86±0·5	60±3
100	2	113±2	53±3	84±1	60±2
100	24	115±5	58±2	81±2	57±0·5
1000	1	117±7	55±0·5	83±1	57±6
1000	2	110±0·5	54±2	84±0·5	57±0·5
1000	24	107±2	58±0·5	82±1	56±0·5

Values of specific activity are expressed as mean±SD ($n=4$).

*Specific activity is expressed in terms of nmoles of fatty acid liberated/min/mg of protein. 50 μg of enzyme sample were taken per assay.

Table 2. Effect of anti-PL-VIII$_a$ Ig on the indirect haemolytic activity of VRV PL-VIII$_a$, VRV PL-V, VRV PL-VI and whole *V. russelli* venom

Conc. anti-PL-VIII$_a$ Ig added (μg)	Indirect haemolytic activity of antigen* (% haemolysis)			
	VRV PL-VIII$_a$	VRV PL-V	VRV PL-VI	Whole venom
0	18±1	9±2	12±0·5	7±2
25	16±3	10±0·5	11±2	7±0·5
100	17±0·5	9±0·5	12±1	8±1
250	17±2	9±5	12±0·5	7±1
500	18±1	10±2	12±1	7±3

Antigen and anti-PL-VIII$_a$ Ig were incubated together at 37° for 1 hr before the assay.

The complete haemolysis of the erythrocytes by the addition of 10 ml distilled water was taken as 100%. Values are presented as mean±SD of four independent determinations.

*25 μg of antigen were used for all assays.

Coagulation tests

Anti-coagulant activity was routinely determined by prothrombin times, according to the method of Quick (1966). The effect of anti-PL-VIII$_a$ Ig on the anti-coagulant potency of VRV PL-VIII$_a$, VRV PL-V and VRV PL-VI was determined.

RESULTS

When the rabbit antiserum and anti-PL-VIII$_a$ Ig were tested on gel diffusion plates against the antigens VRV PL-VIII$_a$, VRV PL-VI, VRV PL-V and whole *V. russelli* venom, by Ouchterlony immunodiffusion technique, one precipitin line was formed in all the cases (Fig. 1), in contrast to preimmune serum which did not give any precipitin line on agar gel plates with any of these antigens.

Anti-PL-VIII$_a$ Ig could neither inhibit the catalytic activity of VRV PL-VIII$_a$, VRV PL-V, VRV PL-VI and whole *V. russelli* venom, when egg PC was used as a substrate at the concentrations shown in Table 1, nor have any effect on their indirect haemolytic activity at the concentrations indicated in Table 2. When synaptosomes were used as the substrate, anti-PL-VIII$_a$ Ig completely inhibited the PLA_2 activity of all three neurotoxic PLA_2 and of whole *V. russelli* venom when present at an antigen–antibody (Ag–Ab) ratio of 1:5 and 1:10 (w/w) and a preincubation time of 1 hr. However, anti-PL-VIII$_a$ Ig did not have any effect when present at an Ag–Ab ratio of 1:2 (w/w) and a preincubation time of 24 hr (Table 3).

When VRV PL-VIII$_a$ was mixed with anti-PL-VIII$_a$ Ig at the ratio of 1:2 (w/w) and injected to animals at twice the LD_{50} dose (10·6 mg/kg body weight), five out of eight animals died within 5–8 hr of injection. All the animals showed neurotoxic symptoms, such as hindlimb paralysis, respiratory distress, convulsion and lacrimation. Three animals recovered from the neurotoxic effects, as the symptoms vanished slowly 12 hr after injection. When VRV PL-VIII$_a$ and anti-PL-VIII$_a$ Ig were mixed in the ratio of 1:5 and 1:10 (w/w), there was absolutely no mortality and no neurotoxic symptoms observed in any of the animals injected with these Ag–Ab mixtures. When VRV PL-V was mixed with anti-PL-VIII$_a$ Ig in the ratio 1:2 (w/w) and injected at twice the LD_{50} dose (3·6 mg/kg body weight) all the animals exhibited neurotoxic symptoms and died within 3–4 hr of injection; when mixed in the ratio 1:5, six out of eight animals died, but the survival time had increased from 2–3 hr to 4–6 hr. All the animals showed symptoms of neurotoxicity; however, two animals recovered and the neurotoxic symptoms vanished 10 hr after injection. When this antigen and antibody were mixed in the ratio of 1:10, all the animals survived and none of them showed neurotoxic symptoms. VRV PL-VI was mixed with anti-PL-VIII$_a$ Ig in the ratio of 1:2 and 1:5 (w/w) and injected at twice the LD_{50} dose (7 mg/kg body weight). All the animals exhibited neurotoxic symptoms and died within 3 hr of injection. When this Ag–Ab ratio was raised to 1:10, all the

Table 3. Effect of anti-PL-VIII$_a$ Ig on the PLA$_2$ activity of VRV PL-VIII$_a$, VRV PL-V, VRV PL-VI and crude *V. russelli* venom when synaptosome was used as a substrate

Conc. anti-PL-VIII$_a$ Ig added (μg)	Incubation of Ag–Ab: time in hr at 37°	Specific activity			
		VRV PL-VIII$_a$	VRV PL-V	VRV PL-VI	Crude venom
—	1	333	215	499	510
—	24	339	213	489	511
40	1	338	216	491	515
40	24	339	212	498	505
100	1	0	0	0	0
100	24	ND	ND	ND	ND
200	1	0	0	0	0
200	24	ND	ND	ND	ND

20 μg of enzyme sample were taken per assay; specific activity is expressed in terms of nmoles of fatty acid liberated/min/mg of protein.

Values of specific activity are expressed as mean of four independent determinations. The SD in all the cases was less than 1.

Table 4. Effect of anti-PL-VIII$_a$ Ig on the oedema-inducing activity of VRV PL-VIII$_a$, VRV PL-V, VRV PL-VI and whole *V. russelli* venom

Conc. anti-PL-VIII$_a$ Ig added (μg)	Oedema ratio (%) (5 μg)			
	VRV PL-VIII$_a$	VRV PL-V	VRV PL-VI	Whole venom
—	158±4·3	159±3	160±1	166±0·5
10	153±2·8	161±1	151±9	164±2
25	156±9·6	158±0·5	155±3	163±5
50	160±5·7	159±3	156±1	167±0·5

Values of oedema ratio are expressed as mean±SD ($n=6$).

Minimum oedema dose is defined as the amount of protein causing an oedema ratio of 120%. It remained constant in all the cases. Minimum oedema dose of VRV PL-VIII$_a$, 1 μg; VRV PL-V, 0·1 μg; VRV PL-VI, 0·85 μg; whole *V. russelli* venom, 0·2 μg.

Table 5. Effect of anti-PL-VIII$_a$ Ig on anti-coagulant activity of *V. russelli* PLA$_2$ enzymes

	Clotting time (seconds)	
	Without anti-PL-VIII$_a$ Ig	With anti-PL-VIII$_a$ Ig
Control	19±1	19±1
VRV PL-VIII$_a$	55±2	20±2
VRV PL-V	46±2	19±1
VRV PL-VI	41±3	19±1

10 μg of PLA$_2$ enzyme were used for all assays.

The values of clotting time are expressed as mean±SD ($n=6$).

The antigen and antibody were incubated together at 37° for 10 min before the assay.

10 μg of anti-PL-VIII$_a$ Ig were used for all interaction studies.

injected animals survived without any neurotoxic symptoms. When whole *V. russelli* venom was mixed with anti-PL-VIII$_a$ Ig in the ratio of 1:5 (w/w) and injected at twice the LD$_{50}$ dose (8·2 mg/kg body weight), all eight animals died, but the survival time had increased from 3–4 hr to 10–12 hr. All the animals exhibited neurotoxic symptoms after 6 hr. When this Ag–Ab ratio was raised to 1:10 (w/w), although all the animals died 10–12 hr after injection, none of them exhibited symptoms of neurotoxicity.

Anti-PL-VIII$_a$ Ig could not inhibit the oedema-inducing activity of VRV PL-VIII$_a$, VRV PL-V, VRV PL-VI and whole *V. russelli* venom at the concentrations given in Table 4. All three PLA$_2$ (VRV PL-VIII$_a$, VRV PL-V and VRV PL-VI) studied from *V. russelli* venom exhibited anti-coagulant potency by increasing the clotting time of human plasma. The anti-coagulant activity of all the three enzymes were completely inhibited in presence of anti-PL-VIII$_a$ Ig at the concentrations indicated in Table 5.

DISCUSSION

Injection of VRV PL-VIII$_a$ toxoid into rabbits elicited the formation of specific antibodies. Formation of a single precipitin line against VRV PL-VIII$_a$ indicates the presence of more than one antibody to VRV PL-VIII$_a$. The possibility of this being a non-immune precipitate is ruled out because no precipitin lines were ever observed between VRV PL-VIII$_a$ and preimmune serum from the same rabbit. Anti-PL-VIII$_a$ Ig gave a single identical precipitin line against VRV PL-V and VRV PL-VI, which are potent neurotoxins from *V. russelli* venom, suggesting that anti-PL-VIII$_a$ Ig cross-reacts with the other neurotoxins studied from *V. russelli* venom. As the basic PLA$_2$ having neurotoxic effects from *V. russelli* venom are of similar molecular size, they migrate to the same distance on agar gel plates, leading to the formation of a single precipitin line even in case of whole *V. russelli* venom.

Anti-PL-VIII$_a$ Ig could not inhibit the *in vitro* PLA$_2$ activity of VRV PL-VIII$_a$ when egg PC was used as a substrate, suggesting that the region involved in the catalytic activity of VRV PL-VIII$_a$ is poorly immunogenic. It also suggests that the animal which receives PLA$_2$ enzyme as an antigen may have

PLA_2 enzyme with a similar catalytic site in its own body and thus fails to recognize the catalytic site as foreign. Though anti-PL-$VIII_a$ Ig cross-reacted with other neurotoxins studied (VRV PL-V and VRV PL-VI) and whole *V. russelli* venom on agar gel plates, it had no effect on their *in vitro* PLA_2 activity when egg PC was used as the substrate, suggesting that anti-PL-$VIII_a$ Ig cannot recognize the catalytic site of the enzymes. The fact that the anti-PL-$VIII_a$ Ig has no influence on the indirect haemolytic activity of these PLA_2 isoenzymes further confirms that the interaction between the anti-PL-$VIII_a$ Ig and the enzyme does not affect the catalytic site.

Anti-PL-$VIII_a$ Ig offered complete protection against the lethality and symptoms of neurotoxicity of VRV PL-$VIII_a$, VRV PL-V and VRV PL-VI, the potent neurotoxins of *V. russelli* venom and the neurotoxic symptoms of whole venom without affecting the *in vitro* PLA_2 activity when using egg PC as the substrate. This indicates that the anti-PL-$VIII_a$ Ig may be recognizing the molecules as a neurotoxin.

This suggestion is further confirmed by the studies carried out using synaptosomes. Synaptosomes (synaptic bodies) are defined as pinched off nerve endings or synaptic boutons which, after homogenization, reseal to form a discrete particle. Anti-PL-$VIII_a$ Ig inhibited the PLA_2 activity of the neurotoxins and whole *V. russelli* venom when synaptosomes were used as the substrate. This can be explained on the basis of the following fact. In synaptic transmission blockade, there are two functionally separate steps: (i) binding of PLA_2 to the specific synaptic site through a neurotoxic potential, which is unrelated to the enzyme activity; this is a primary prerequisite for neurotoxicity; and (ii) perturbation of the synaptic membrane by PLA_2 action, which is a secondary step (Kini & Evans, 1989). So initially, the neurotoxin will have to bind to a particular site on the synaptosomes, then the PLA_2 activity will bring about the hydrolysis of phospholipids in the synaptosomes. In the absence of anti-PL-$VIII_a$ Ig, the neurotoxins bind to synoptosomes and further facilitate the PLA_2 activity of the toxin. But in the presence of anti-PL-$VIII_a$ Ig, the neurotoxins were incapable of binding to the synaptosomes. The site on the neurotoxin responsible for binding to the nervous system or synaptosome (neurotoxic potential) is being masked in the presence of anti-PL-$VIII_a$ Ig, which in turn has inhibited the neurotoxins from binding to the synaptosomes. Anti-PL-$VIII_a$ Ig is masking this particular site, but the catalytic site is free as the catalytic activity *in vitro* using egg PC as the substrate remained unaltered. So the site on the neurotoxins responsible for binding to the target site of the nervous system or synaptosomes (neurotoxic potential) is independent of the catalytic site. As anti-PL-$VIII_a$ Ig selectively recognizes the neurotoxic potential, this antibody has been termed anti-neurotoxic antibody. Though the importance of PLA_2 activity in neurotoxicity cannot be completely neglected as it plays a secondary role causing the perturbation of the structure of synaptic membrane by hydrolysing the phospholipid, neurotoxic potential, which is distinct from the catalytic site, plays a primary role in the synaptic transmission blockade mechanism.

The fact that the catalytic site and the neurotoxic potential are distinct on a PLA_2 molecule is also strengthened by previous results. (i) The enzyme VRV PL-V, upon aggregating into a pentameric form, loses its lethality and symptoms of neurotoxicity completely, while the catalytic activity is increased by twofold (Jayanthi *et al.*, 1989). (ii) The antibodies prepared against the same aggregated PLA_2 VRV PL-V were capable of inhibiting the lethality and symptoms of neurotoxicity of VRV PL-V, VRV PL-VI and VRV PL-$VIII_a$ without affecting their catalytic activity (Kasturi & Gowda, 1990). Anti-PL-$VIII_a$ Ig, which was unable to inhibit the catalytic activity of the neurotoxic PLA_2 and whole *V. russelli* venom, also could not inhibit its oedema-inducing activity, suggesting that the enzyme activity may be directly responsible for oedema induction. This suggestion is further strengthened by the following observations: (i) Aristolochic acid, an alkaloid [8-methoxy-6-nitrophenanthro (3-4-d)-1,3-dioxole-5-carboxylic acid] from the medicinal plant *Aristolochia radix*, inhibits the catalytic activity and oedema-inducing activity of a PLA_2 TFV PL-X from *Trimeresurus flavoviridis* venom (Vishwanath *et al.*,1987); (ii) inhibition of oedema induction of human synovial fluid PLA_2 in a dose-dependent manner is positively correlated with *in vitro* inhibition of PLA_2 activity. Alkylation of HSF-PLA_2 with p-bromophenacyl bromide concomitantly inhibits both enzyme and oedema-inducing activity (Vishwanath, Fawzy & Franson, 1988). Based on these results, it is suggested that the catalytic activity of PLA_2 is positively correlated with *in vivo* oedema induction.

The fact that anti-PL-$VIII_a$ Ig completely inhibited the anti-coagulant potencies of three neurotoxic PLA_2 isoenzymes without affecting their catalytic activity suggests that PLA_2 activity alone is not responsible for anti-coagulant activity. The present results are in accordance with the results of Kini, Nancy & Evans (1988), as they identified four non-enzymatic polypeptides from *Naja nigricollis crawshawii* venom as having anti-coagulant potency.

Though many attempts are being made to nullify the toxicity of pure toxins or whole venom by using polyclonal antibodies raised against them (Kondo *et al.*, 1979; Rosenfeld & Kelen, 1966; Ownby, Colberg & Odell, 1984; Pakmanee *et al.*, 1987), there are no reports explaining the relationship between the catalytic activity and pharmacological properties making use of these antibodies. However, there are reports on attempts relating PLA_2 activity to the toxicity of the enzymes by chemical modification studies, which suggests that there are two separate but perhaps overlapping regions existing within the molecule and these regions are responsible, respectively, for the pharmacological and catalytic activity of the enzyme (Rosenberg, 1986). The present study, along with previous results (Jayanthi *et al.*, 1989; Kasturi & Gowda, 1990) supports the hypothesis put forward by Kini & Iwanaga (1986a) and Kini and Evans (1989), as it clearly demonstrates the presence of a distinct neurotoxic potential on the neurotoxic PLA_2 apart from the catalytic site.

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