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SHORT COMMUNICATIONS
A NON-PROTEINACEOUS TOXIN FROM THE VENOMOUS SPINES OF THE LIONFISH *PTEROIS VOLITANS* (LINNAEUS)

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M. S. R. NAIR, P. CHEUNG, I. LEONG and G. D. RUGGIERI. A non-proteinaceous toxin from the venomous spines of the lionfish *Pterois volitans* (Linnaeus). Toxicon **23**, 525 – 527, 1985. — The venomous spines of *P. volitans* contain a non-proteinaceous ichthyotoxin of low molecular weight. This toxin could be isolated only from spines excised from the living fish; the toxin is apparently destroyed following death, as the extracts of the spines of the dead fish were non-toxic.

IN THE COURSE of our investigation of marine phyla for potential therapeutic agents we isolated several biologically active compounds (NAIR *et al.*, 1982, 1984; GOLDBERG *et al.*, 1982). Here we report the isolation of a low molecular weight ichthyotoxin from the poisonous spines of the lionfish, *Pterois volitans* (Linnaeus).

P. volitans is found in the Red Sea, Indian Ocean and in waters around Japan, China, the Philippines and Australia. Hidden among their feather-like fins are 18 slender pointed spines, 13 dorsal, three anal and two pelvic, which are used to deliver the venom. The pectoral fins contain no spines. The sting of the fish causes very painful, swollen wounds, which can result later in gangrene, delirium, convulsions and cardiac failure. RUSSELL (1965, 1967) reported that the venoms of the scorpion fish *P. volitans* and the stonefishes *Synanceja guttata* and *S. horrida* are similar to those of weeverfish venoms and to one another, and all were proteinaceous. AUSTIN *et al.* (1965), using ultracentrifugal techniques, studied the venoms of several *Synanceja* species and reported them to be proteins having a molecular weight around 150,000.

We became interested in the poison-delivering spines of *P. volitans* when the extracts of the spines of a rather large fish (*c.* 25 cm in length) removed within minutes after its death were found in our assay (*vide infra*) to be not toxic to killifish (*Fundulus heteroclitus*) at 20 mg dry weight dosage. Therefore, we collected more *P. volitans* from the Pacific Ocean off the coast of the Philippines, which were kept in our exhibit aquaria along with about 20 other species of tropical fish. Four young fish, *c.* 13 cm in length were anesthetized using a 0.1% aqueous solution of tricaine (3-aminobenzoic acid ethyl ester methanesulfonate, MS – 222; Sandoz) and their venomous spines were excised at half-length (*c.* 5 cm) using forceps and scissors. The excised portions of the spines were quickly put into 200 ml *n*-butanol and were immediately homogenized in a blender and filtered.

The fish were returned to the holding tank after having been dipped in a tetracycline bath. The spines grew back to their original length in about six months. The *n*-butanol extract was dried *in vacuo* to obtain a residue (*c.* 50 mg), which was dissolved in 5 ml of ethanol (95%). To test for ichthyotoxic activity (NAIR *et al.*, 1982), three killifish each (*c.* 4.5 cm in length) were placed in two liter beakers containing 200 ml of seawater pre-aerated for 10 min. The ethanol solution of the spine extract (0.5 ml, i.e. 5 mg dry weight) was added to the test beaker and 0.5 ml of ethanol was added to the control beaker. The test fish started gasping almost immediately, darkened in color, swam erratically and then became paralyzed and completely inert and stayed at the bottom of the beaker in 16 min. At this stage the test solution was drained off and the fish were washed with charcoal-filtered seawater and were put in a flowing seawater tank. All three fish revived in 2 min and started swimming agilely. The control fish showed no effect, even after 2 hr.

The remainder of the extract (4.5 ml) was dried under a nitrogen stream and was distributed, on a 50 tube (15 ml) countercurrent chromatographic (CCC) unit, between chloroform and water. Tubes 20–26 gave positive ichthyotoxic activity when 5 ml portions of both the layers were combined, the solvents removed under vacuum, the residue dissolved in 0.5 ml ethanol and tested as before, though the fish reacted much more slowly than was the case with the original extract. The contents of the tubes which showed activity were combined and dried to obtain the toxin, *c.* 20 μg. It showed u.v. absorption at λ_{max}^{MeOH} 287 nm and a molecular weight of 327 in D/CI–MS (desorption chemical ionization mass spectrometry Ribermag R – 10 mass spectrometer). The total ion current (TIC) curve showed that the compound was essentially pure. The toxin (4 μg) was more active than the original extract in our assay, as the onset of paralysis in the fishes occurred much sooner.

Later, when their spines had fully grown back, all four fish were sacrificed and the spines were extracted with *n*-butanol and the solvent was removed to obtain a residue (200 mg). This extract (20 mg dry weight) dissolved in ethanol was inactive in our killifish assay. The extract was separated by chromatography* on 50 times (w/w) silica gel (SG 60, 230–400 mesh; E. Merck) in a 50 cm × 3.5 cm column using chloroform and chloroform – methanol (1:1) as eluants. The only compounds isolated in pure form were cholesterol, cholesteryl palmitate and a third inactive compound not yet identified. Neither the crude extract nor any of the fractions showed a u.v. maximum at 287 nm nor a peak at M/Z 328 (M+H) in D/CI–MS, the diagnostic features of the toxin. These methods, especially the mass spectra, being very sensitive, this indicated the absence of the toxin in the dead fish.

The *P. volitans* used in the studies were kept in an aquarium with about 20 other species of fish and none of them showed any deleterious effect. Therefore, it is very unlikely that an ichthyocrinotoxin is secreted by the epidermis of the spines. A connection between the distal end of the venom glands and epidermis was described as a permanent feature in *P. lunulata* by TANGE (1953), but it was not noticed in *Pterois* by PAWLOWSKY (1911, 1914) nor by HALSTEAD *et al.* (1955) in *P. volitans*. CAMERON and ENDEAN (1972) report that it exists in *P. volitans*, even though they do not claim that it is a general feature. Discharge of the venom occurs only on physical violation of the anatomic integrity of the fish. As the spines are the path through which envenomation takes place, it is logical to assume that their excision, even under anesthesia, should result in the discharge of a certain amount of venom into the spines. Thus, though it is entirely possible that this toxin is

*At the time of this experiment a CCC unit was not available to us.

present only in the spines, the likelihood that it may be a component of the venom cannot be ignored.

The procedure we developed, namely excision of the spines from living fish under anesthesia, appears to be an excellent method to study their contents, since the spines grow back in several months and thus provide a reliable, steady and reproducible source. We were not able to show that the regenerated spines actually produced the toxin, because the fish were sacrificed in the second experiment, to ascertain that the toxin can be obtained only from living fish. However, as no visible dissimilarity was observed between the original and regenerated spines, we believe that it is very likely that they will produce the toxin as well. We are collecting more specimens of *P. volitans* and using this procedure we hope to obtain the toxin in sufficient amounts to elucidate its structure, since if this procedure is workable it will obviate the necessity of using a large number of fish to isolate the compound present only in minute quantities. We are also exploring the usefulness of this method in the study of other acanthotoxic fishes.

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REFERENCES

AUSTIN, L., GILLIS, R. G. and YOUATT, G. (1965) Stonefish venom: some biological observations.. *Aust. J. exp. Biol. med. Sci.* **43**, 79.

CAMERON, A. M. and ENDEAN, R. (1972) The venom glands of teleost fishes. *Toxicon* **10**, 301.

GOLDBERG, A. S., WASYLYK, J., RENNA, S., REISSMAN, H. and NAIR, M. S. R. (1982) Isolation and structural elucidation of an ichthyocrinotoxin from the trunkfish (*Lactophrys triqueter*, Linnaeus). *Toxicon* **20**, 1069.

HALSTEAD, B. W., CHITWOOD, M. J. and MODGLIN, F. R. (1955) The anatomy of the venom apparatus of the zebrafish *Pterois volitans* (Linnaeus). *Anat. Rec.* **122**, 317.

NAIR, M. S. R., GOLDBERG, A. S., DAYAL, V, K., ARKEL, Y. S., OBIEDZINSKI, G. and VAN ALLEN, M. A. (1984) Isolation of two platelet aggregation inhibitors from the extract of sea urchin gonads. 25th Annual Meeting of the American Society of Pharmacognosy, Abstract 75.

NAIR, M. S. R., LEONG, I. and NAYAR, M. S. B. (1982) Ichthyotoxins from the oyster toadfish, *Opsanus tau* (Linnaeus). *Toxicon* **20**, 933.

PAWLOWSKY, E. N. (1911) Ein Beitrag zur Kenntniss des Baues der Giftdrüsen einiger Scorpaeniden. *Zool. Jb.* **31**, 529.

PAWLOWSKY, E. N. (1914) Über den Bau der Giftdrüsen bei *Plotosus* und anderen Fischen. *Zool. Jb.* **38**, 427.

RUSSELL, F. E. (1965) Marine toxins and venomous and poisonous animals. *Adv. mar. Biol.* **3**, 255.

RUSSELL, F. E. (1967) Comparative pharmacology of some animal toxins. *Fedn Proc. Fedn Am. Socs exp. Biol.* **26**, 1206.

TANGE, Y. (1953) Beitrag zur Kenntniss der Morphologie des Giftapparates bei den Japanischen Fischen – II. Uber den Giftapparat bei *Pterois lunulata* Temminck et Schlegel. *Yokohama med. Bull.* **4**, 178.

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POSTSYNAPTIC BLOCKING OF GLUTAMATERGIC AND CHOLINERGIC SYNAPSES AS A COMMON PROPERTY OF ARANEIDAE SPIDER VENOMS

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P. B. USMANOV, D. KALIKULOV, N. G. SHADYEVA, A. B. NENILIN and B. A. TASHMUKHAMEDOV. Postsynaptic blocking of glutamatergic and cholinergic synapses as a common property of Araneidae spider venoms. *Toxicon* 23, 528–531, 1985. — Venom effects of eight Araneidae spider species were studied using locust and frog neuromuscular junctions. The spider venoms irreversibly blocked miniature excitatory postsynaptic potentials and excitatory postsynaptic potentials of locust neuromuscular junction. The frog miniature end-plate potentials and end-plate potentials were also blocked, but they recovered upon washing of the preparation with physiological solution.

THE VENOMS of several *Latrodectus* spider species belonging to the Theridiidae family have profound physiological effects on vertebrate and invertebrate neuromuscular junctions. At frog and lobster neuromuscular junctions these venoms cause a massive increase in the frequency of miniature postsynaptic potentials, followed by depletion of synaptic vesicles and neuromuscular block (LONGENECKER *et al.*, 1970; FRITZ *et al.*, 1980). Recently we found that the venom of the spider *Argiope lobata* belonging to the Araneidae family acts on a postsynaptic element and blocks neuromuscular transmission in vertebrate and insects, reducing the sensitivity to the transmitter (USMANOV *et al.*, 1983). In the present paper we investigate the effects of eight Araneidae spider venoms on frog and locust neuromuscular junctions.

Spiders of the species *Argiope lobata, Agalenatea redii, Mangora acalypha, Neoscona adianta, Neoscona cruciferoides, Nuctenea folium* and *Zygiella caspica* were collected in Central Asia and those of *Araneus diadematus* in the Caucasus. Spiders were kept in the laboratory at room temperature on a mealworm diet. *Agriope lobata* spiders could be obtained in large numbers. Their venom glands were removed and homogenized in distilled water. After centrifugation at 10,000 *g* for 30 min the resulting supernatant was dried for use later. This whole extract contained 45% protein or 76 μg protein per venom gland, as determined by the Lowry method (LOWRY *et al.*, 1951). Two to ten venom glands of other spider species were removed and homogenized in 0.5 ml of the corresponding physiological solution just before the experiments. The protein contents ranged from 15 μg to 60 μg per venom gland. Depending on the venom potency, 0.01 – 0.1 ml of this homogenate was added to the 3 ml bathing solution. For every venom from 5 to 20 experiments were performed, depending on the particular venom availability. The *Argiope lobata* venom was fractionated by gel filtration on a Sephadex G-75 column