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specifically cleave synaptic proteins required for neurotransmitter release. VAMP/synaptobrevin is the specific target of TeNT and BoNT/B, D, F and G, SNAP25 is cleaved by BoNT/A and E, and syntaxin is attacked by BoNT/C. Besides this proteolytic activity, it has been proposed that TeNT might affect neurotransmission via a stimulation of neuronal transglutaminases (TGases) and subsequent cross-linking of the synapsin-I, a synaptic vesicle membrane protein. In order to investigate a possible involvement of intraneuronal TGases in the inhibitory action of TeNT, we examined the action of TGases inhibitors on TeNT-induced blockade of neurotransmission. In *Aplysia*, preincubation of cholinergic neurones with various TGases inhibitors such as monodansylcadaverine was shown to induce a reproducible delay (45–100 min) in the induction of blockade of acetylcholine release by TeNT. Moreover, a similar effect was observed when presynaptic neurones were preinjected with specific antibodies directed against TGases. Taken together, these observations strongly suggest that activation of neuronal TGases is involved in the blocking action of TeNT. In addition, the presence of a delay before TeNT acts indicates that TGase/TeNT interaction occurs before the proteolytic attack of VAMP/synaptobrevin.

Effects of apamin on learning and memory in rats. O. Deschaux[1], J. C. Bizot[1] and M. Goyffon[2] ([1]Laboratoire de Pharmacologie du Comportement, Centre d'Etudes du Bouchet-ETCA-91710 Vert le Petit, France; [2]LERAI, Muséum national d'Histoire naturelle, 57 rue Cuvier 75005 Paris, France).

Apamin, an active principle of the *Apis mellifera* honey-bee venom, is a centrally acting toxin that selectively blocks the small conductance Ca^{2+}-activated K^+-channel (SK). This channel is involved in the after-hyperpolarization that follows action potentials in many excitable cells. Some results show circumstantial evidence that apamin can improve learning and memory processes in rodents. In order to better delineate the implication of apamin sensitive K^+ channel in memory processes, we investigated the effects of apamin injections on learning, as assessed in various tasks.

Apamin did not modified acquisition of one-trial passive avoidance task. In Morris water maze, apamin did not modify spatial learning. In an other hand, Apamin at the doses of 0.2 and 0.4 mg/kg has improved learning in an object recognition task. Futhermore, apamin (0.4 mg/kg) improved extinction of a previously learned operant task in Skinner box.

These results could indicate that inhibition of apamin sensitive K^+ channels improves learning of non aversive tasks—such as object recognition or extinction—but has no effects in stressful experimental situations—such as passive avoidance or Morris water maze.

C-terminal amidation of apamin is important for biological activity as revealed by recombinant technology and chemical synthesis. C. Devaux[1], M. Knibiehler[2], M.-L. Defendini[1], K. Mabrouk[1], H. Rochat[1], J. Van Rietschoten[1], D. Baty[2] and C. Granier[1] ([1]Laboratoire d'Ingénierie des Protéines, CNRS URA 1455, Faculté de Médecine-Nord, Bd P. Dramard, 13916 Marseille Cedex 20, France; [2]Laboratoire d'Ingénierie et Dynamique des Systèmes Membranaires du CNRS, CBBM, 31 chemin Joseph Aiguier, 13402 Marseille Cedex 20, France).

Apamin, a bee venom neurotoxin, specifically blocks a class of Ca^{2+}-activated K^+ channels of small conductance. It is a small polypeptide of 18 amino acids reticulated by two disulfide bridges and its C-terminal amino acid is amidated. Solid-phase synthesis has contributed to structure–activity and structure–antigenicity relationships studies. We used apamin as a toxin model in the secretion system based on a colinogenic plasmid which encode a lysis protein. This system could be of great value for production of soluble fusion protein in *E. coli* culture medium. A gene fusion was constructed encoding a hybrid molecule containing the first 171 amino acids of colicin A, an additional methionine residue and the apamin sequence. Biochemical and immunological characterization of the recombinant protein suggested correct folding. However, in biological tests it expressed less activity than natural toxin. Further studies using a non amidated chemical analog show that the amidation of the C-terminal residue is more important for activity than the nature or presence of this residue.

Effects of paradoxin, a ß-neurotoxin from Australian Inland Taipan snake (Oxyuranus microlepidotus) *venom: studies in rodent central nervous system*. F. Dorandeu, I. Pernot-Marino, G. Brochier, E. Delorme and G. Lallement (Centre de Recherches du Service de Santé des Armées, Unité de Neurotoxicologie, 24 Av. des Maquis du Grésivaudan, BP87, F-38702 La Tronche Cedex, France).

Paradoxin (PDX), purified from the Australian Elapid snake *Oxyuranus microlepidotus* venom is a little-studied toxin that belongs to the ß-neurotoxin family, a group of potent snake neurotoxins having PLA_2 catalytic properties. PDX was first described 15 years ago as a taipoxin-like toxin (taipoxin is a three chain ß-neurotoxin isolated from the Taipan snake, *O. s. scutellatus*). Since 1979, very few studies have been done on PDX and that led us to investigate its neurotoxic effects after central administration, with a particular focus on its potential convulsive activity as some ß-neurotoxins have been observed to induce convulsions, and sometimes seizures.

After intracerebroventricular (icv, lateral ventricle) injection of different doses through a stereotaxically implanted cannula, a four-step neurotoxic syndrome developed in rats. In most of the rats treated we did not notice seizures (ECoG recording) nor convulsions (visual observation). The mean lethal dose by icv route was inferred to be *c.* 1.2 μg/kg for these animals. A very similar kind of neurotoxic effect was observed in mice, stunned with ether and then injected directly through the skin near or in their third ventricle. LD_{50} for mice was calculated to be 1.17 ± 0.08 μg/kg ($n = 60$), that is very close to that reported after iv injection (2 μg/kg). Gross histological examination (H and E staining) of the brain of the rats intoxicated by PDX did not show major target areas. Biochemical brain damage assessment is currently under investigation. *In vitro* experiments using rat hippocampal and striatal mini-slices were then carried out to determine the major neurochemical disturbances induced by PDX. In a preliminary superfusion experiment, PDX (100–200 nM) showed a slight tendency to increase ACh and Glu efflux from hippocampal mini-slices preincubated with [^{3}H]choline and [^{14}C]Glu. This effect did not seem to be related to a major leakage and is presently under investigation using a chemiluminescent technique. On the other hand PDX significantly increased the K^+-evoked efflux of [^{3}H]DA from rat striatal mini-slices after 6 or 16 min of contact with the toxin during the superfusion. Testing the hypothesis that PDX could impair the uptake of the same neurotransmitters, we found that PDX actually did so, in an apparently calcium-independent way. Comparison with taipoxin is presented. Using a colorimetric assay PDX proved to be a weak enzyme as it is reported for taipoxin. In conclusion, PDX shares some of its pharmacological effects with other ß-neurotoxins but the *in vitro* results are not sufficient to explain the complex neurotoxic syndrome observed that might involve several types of neurotransmission.

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Biogenic amines (noradrenaline, dopamine and tryptophan) in stonefish venom. Identification of noradrenaline in Synanceia verrucosa. P. Garnier[1], J.-M. Grosclaude[2], V. Gervat[3], F. Goudey-Perrière [1], P. Gayral [1], C. Jacquot[2] and C. Perriere[1] ([1]Laboratoire de Biologie et Contrôle des Organismes Parasites, (Equipe Toxines et Insectes, [2] Laboratoire de Pharmacodynamie et Pharmacologie, [1,2]Faculté de Pharmacie, Université Paris-Sud, 92296 Châtenay-Malabry Cedex, [3] Laboratoire de Chimie Structurale Organique et Biologique, EP 103, Université Pierre et Marie Curie, 4 place Jussieu, 75252 Paris Cedex 05, France).

Stonefish venom exerts a positive inotropic response on the frog heart as does adrenaline, and this effect is inhibited by propranolol, a ß receptor antagonist (Sauviat *et al.*, 1995). This suggests the presence of an adrenergic substance in the venom. Biogenic amines were detected in *Synanceia verrucosa* and *Synanceia horrida* venoms, by HPLC associated with electrochemical detection. Venom extracted from anaethetized fish was immediately used. After proteic precipitation by perchloric acid, the supernatant was injected on Kromasil C18 column (phosphate buffer; mobile phase: methanol 7.5%, pH 3.76). Three peaks were observed with the retention time of noradrenaline, dopamine and tryptophan used as reference substances. Their concentration expressed in μg/mg proteins are indicated in table

Biogenic amines	*Synanceia verrucosa*	*Synanceia horrida*
Noradrenaline	8	1.2
Dopamine	0.03	2.37
Tryptophan	0.16	0.54

The most abundant compound, noradrenaline, was identified in *S. verrucosa* venom by gas chromatography/mass spectrometry. After proteic precipitation, the extract was derivated to increase the compound volatility. The spectra of standard noradrenaline and venom were compared. They have same MH^+ ion with identical fragmentation. That allows us to assert noradrenaline in *Synanceia verucosa* venom.

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Sauviat *et al.* (1995), *Toxicon*, **33**, 1207–1213

Verrucotoxin and isoforms, lethal and hemolytic proteins from stonefish (Synanceia verrucosa) *venom*. P. Garnier, F. Petek, F. Goudey-Perrière and C. Perrière (Laboratoire de Biologie et Contrôle des Organismes Parasites (Equipe Toxines et Insectes), Faculté de Pharmacie, Université de Paris-Sud, 92296 Châtenay-Malabry Cedex, France).

From biological activities of the stonefish venom, three (letal activity for mice, hypotension in rats, hemolytic activity for rabbit erythrocytes) may be attributed to the verrucotoxin (VTx). VTx was purified by three steps chromatography (Garnier *et al.*, 1995). In some attempts of purification (preparative electrophoresis, CM-Sepharose column, etc.), we detect the activity in other fractions. The separation obtained by a Resource 15S column (Pharmacia, FPLC system) with NaCl gradient showed three peaks with hemolytic activity. (i) The