Elapid α-toxins have no effect on the cholinergic responses of bivalve myocardia[1]

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Summary. Elapid α-toxins (α-bungarotoxin, α-cobratoxin and crude *Bungarus caeruleus* venom) do not affect the myocardial nicotinic ACh receptors of the following bivalve molluscs: *Mercenaria mercenaria, Chione cancellata, Mya arenaria, Mytilus edulis, Rangia cuneata* and *Crassostrea virginica.*

Acetylcholine (ACh) may either excite or depress hearts of bivalve molluscs; but usually a dual response occurs, and the myocardium is depressed by low and excited by high concentrations[2]. The particular effect, in any species, is attributable to the proportion of 2 isoreceptors in the myocardium, one mediating excitation and the other depression[3]. Both receptors are nicotinic although they are distinguishable pharmacologically from each other and from the 2 main classes of vertebrate nicotinic receptors; i.e., those of the autonomic ganglia and the neuromuscular junction[4].

Since elapid α-toxins are useful in isolating and characterizing vertebrate nicotinic receptors[5], we decided to test their effects on those of molluscs. The bivalves chosen have myocardial ACh responses representative of the class. *Mercenaria mercenaria, Chione cancellata* and *Mya arenaria* hearts are only depressed by ACh, and threshold is low[2,6,7]. *Crassostrea virginica* hearts are also only depressed by ACh, but threshold is high due, in part, to high endogenous acetylcholinesterase (AChE) activity[2,8]. Finally, *Mytilus edulis* and *Rangia cuneata* hearts exhibit the more typical dual response to ACh[2].

Elapid α-toxins bind only to certain nicotinic receptors[9,10]. Moreover, individual α-toxins differ in rates of association and dissociation, in degree of reversibility and in specificity. The differences between long (71–74 amino acids) and short (61–62 amino acids) α-toxins are especially pronounced[11]. Therefore, in addition to 2 long α-toxins – α-bungarotoxin (α-BgT) from *Bungarus multicinctus* venom, and α-cobratoxin (α-CT) from *Naja naja siamensis* venom – short α-toxins from *Bungarus caeruleus* venom (BCV) were selected for experimentation.

Materials and methods. The experiments were carried out in quadruplicate. Each isolated ventricle was suspended in an aerated, temperature-controlled organ bath and attached to a force transducer; mechanical activity was recorded on an inkwriting oscillograph[2]. The hearts were first challenged with a series of ACh concentrations, and a control dose-response relationship established. After a short wash, pure α-toxin or crude venom was added to the bath and left there for 1–16 h, insuring sufficient time for α-toxin-receptor interaction. A second ACh dose-response curve was then determined in the presence of the α-toxin or venom. All doses are expressed as final concentrations in the organ bath. To control for time-dependent changes in myocardial ACh sensitivity, 1 or 2 of the 4 concurrent preparations were treated as described above, except that no α-toxin or venom was added to the bath.

Results and discussion. α-BgT and α-CT, the 2 long α-toxins tested, differ in pharmacology primarily in degree of reversibility at the vertebrate neuromuscular junction[12]. Neither (at 10^{-5} M) had any effect on the mechanical activities or ACh sensitivities of any of the bivalve myocardia tested (table).

The effects of elapid α-toxins on cholinergic sensitivities of representative bivalve myocardia

Species	Agonist[a]	Pretreatment[b] Diastolic arrest	Systolic arrest	Toxin	Posttreatment[c] Diastolic arrest	Systolic arrest
Mercenaria mercenaria	ACh[d]	1×10^{-9} -3×10^{-7}	-	BgT[f]	3×10^{-9} -3×10^{-7}	-
	ACh	3×10^{-9} -1×10^{-8}	-	CT[g]	1×10^{-9} -1×10^{-8}	-
	ACh	3×10^{-9} -1×10^{-7}	-	BCV[h]	3×10^{-4} -5×10^{-4}	-
	4 K[e]	1×10^{-6} -3×10^{-6}	-	BCV	3×10^{-6}	-
Chione cancellata	ACh	5×10^{-10}-7×10^{-10}	-	BgT	5×10^{-10}-7×10^{-10}	-
	ACh	1×10^{-11}-7×10^{-10}	-	CT	9×10^{-11}-3×10^{-10}	-
	ACh	1×10^{-11}-3×10^{-10}	-	BCV	1×10^{-7} -3×10^{-5}	-
Mya arenaria	ACh	1×10^{-9} -1×10^{-7}	-	BgT	1×10^{-9} -1×10^{-7}	-
	ACh	1×10^{-8} -3×10^{-7}	-	CT	1×10^{-8} -3×10^{-7}	-
	ACh	3×10^{-9} -1×10^{-7}	-	BCV	3×10^{-7} -3×10^{-6}	-
Mytilus edulis	ACh	1×10^{-6}	3×10^{-6} -1×10^{-5}	BgT	3×10^{-6}	3×10^{-6}
	ACh	3×10^{-7} -3×10^{-6}	3×10^{-6} -1×10^{-5}	CT	3×10^{-7} -3×10^{-6}	3×10^{-6}-1×10^{-5}
	ACh	1×10^{-7}	3×10^{-7} -1×10^{-5}	BCV	3×10^{-7} -1×10^{-5}	1×10^{-5}-3×10^{-4}
Rangia cuneata	ACh	3×10^{-10}-1×10^{-7}	3×10^{-7} -1×10^{-6}	BgT	3×10^{-10}-1×10^{-7}	1×10^{-6}
	ACh	1×10^{-9} -1×10^{-8}	1×10^{-5}	CT	1×10^{-9} -1×10^{-8}	1×10^{-5}-3×10^{-5}
	ACh	1×10^{-9}	1×10^{-5}	BCV	1×10^{-7} -1×10^{-6}	1×10^{-3}-3×10^{-3}
	4 K	1×10^{-7} -1×10^{-5}	1×10^{-5}	BCV	1×10^{-7} -1×10^{-5}	1×10^{-5}
Crassostrea virginica	4 K	3×10^{-6} -3×10^{-5}	-	BgT	1×10^{-6} -1×10^{-5}	-
	4 K	1×10^{-5} -1×10^{-4}	-	CT	1×10^{-6} -1×10^{-4}	-
	4 K	1×10^{-6} -3×10^{-5}	-	BCV	3×10^{-6} -3×10^{-5}	-
	ACh	3×10^{-4} -5×10^{-4}	-	BCV	3×10^{-4} -4×10^{-3}	-

[a] Cholinergic agonist with which experiments were run. [b] Range of agonist concentrations (final bath concentration expressed in moles) at which systolic or diastolic arrest was observed in a series of hearts. This represents the original sensitivity of the hearts. [c] Range of agonist concentrations (final bath concentration expressed in moles) at which systolic or diastolic arrest was observed in a series of hearts while α-toxin was present in the bath. [d] Acetylcholine chloride. [e] 4-ketoamyltrimethylammonium chloride. [f] α-bungarotoxin, 10^{-5} M final bath concentration. [g] α-cobratoxin, 10^{-5} M final bath concentration. [h] *Bungarus caeruleus* venom, 100 µg/ml final bath concentration.

Doses of BCV less than or equal to 100 µg/ml (approximately 3×10^{-7} M α-toxin) shifted the ACh dose-response curves of *Mercenaria, Chione, Mya, Mytilus* and *Rangia* to the right by 2-5 orders of magnitude; this inhibition was rapidly reversed by washing. The ACh sensitivities of *Crassostrea* hearts, in contrast, were relatively unaffected by BCV. When the experiment was repeated on *Mercenaria, Rangia* and *Crassostrea* hearts with 4-ketoamyltrimethylammonium chloride (4 K; an ACh analog lacking the esteratic linkage hydrolyzed by AChE) as agonist, BCV had no effect on the response sensitivities of any of the 3 (table). Since BCV contains AChE[13], the lowered responsiveness of most of these hearts probably resulted from the presence of the enzyme in the bath rather than from any effect of the α-toxins on the receptors. The high endogenous AChE activity of *Crassostrea* hearts[8] explains both the ineffectiveness of BCV in this species and the greater sensitivity of oyster hearts to 4 K as compared to ACh.

Crude BCV also had an excitatory effect of its own on all of the myocardia tested. Excitation could not have been produced by 5-hydroxytryptamine (5-HT) in the venom since the response was not blocked by methysergide (a potent 5-HT antagonist). And it could not have been due to ACh because of the AChE in the venom[13]. The excitation is not unexpected since, in addition to short α-toxins, the crude venom contains an arsenal of other polypeptides and enzymes[14].

In conclusion, these elapid α-toxins lack any effect on the nicotinic ACh receptors of bivalve mollusc hearts. This is yet another example of the pharmacological heterogeneity of nicotinic receptors in general[9,13,15,16].

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Effect of gum Arabic on aminopyrine demethylation in rats

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Summary. Stimulation of aminopyrine demethylation induced in rats by oral or i.p. administration of phenobarbital was partially inhibited in animals receiving daily treatments of 2×200 mg/kg gum Arabic p.o.

Earlier studies in this laboratory have shown that repeated oral administration of commonly used suspending agents (gum Arabic, gum Tragacanth, methylcellulose and carboxymethylcellulose) affected the function of heart and liver mitochondria and mixed function oxidases in the liver of small laboratory animals[1,2]. For example, 40 and 200 mg/kg gum Arabic given twice daily on 5 days per week to rats caused uncoupling of oxidative phosphorylation of mitochondria isolated from heart and liver, and a progressive inhibition of 2- and 4-biphenylhydroxylase in liver microsomes. In all these studies, ex vivo procedures were used. It was, therefore, interesting to investigate whether comparable biochemical effects of gum Arabic could also be demonstrated in a living animal. For this purpose, the expiratory measurement of maximal aminopyrine demethylation[3] promised to be a suitable approach.

Material and methods. The in vivo demethylation of 4-dimethyl(^{14}C)aminoantipyrine (DAA, The Radiochemical Centre, Amersham, England, sp. act. 15.6 mCi/mmole) was measured by trapping expired $^{14}CO_2$ from an inhalation chamber. The method of Lauterburg and Bircher[3] was used with the following modifications: the air drawn through the inhalation chamber (2 l desiccator) was dried with silica gel and freed from CO_2 with solid KOH. 50 µCi DAA were dissolved in 20 ml 0.9% NaCl. 100 µl of this solution (0.25 µCi) were injected under light ether anesthesia into the subclavial vein of female rats (ZUR SIV-Z strain, initial weight 152-180 g). The expired $^{14}CO_2$ was collected in 8-min fractions for 40 min. The fraction containing the highest radioactivity was taken to calculate the peak demethylation rate. It was expressed as fraction of the total DAA dose expired as CO_2 per min. This measurement was made for each individual rat 1 day before and after treatment, exactly at the same time of day. In the 3rd experiment, an additional measurement was made on the last day of a pretreatment phase. The relative induction of demethylase activity was calculated by dividing the peak rate after treatment by the peak rate before treatment.

1st experiment: 2 groups of 2 rats received 0.2 ml/100 g b. wt of a 10% (w/v) aqueous suspension of gum Arabic (USP, Sigma Chemical Co., St. Louis, Mo.) by gavage at 09.00 h and 17.00 h on 3 consecutive days. A 3rd group received equal volumes of water. Phenobarbital (PB), University Hospital-Pharmacy, Zurich) was dissolved in water and given for the same 3 days at 17.00 h by gavage at a dose of 100 mg/kg to 1 group treated with gum Arabic and to the control group.

2nd experiment: 2 groups of 4 rats received gum Arabic as