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Effect of a sea snake (*Enhydrina schistosa*) venom on the ganglionic nicotinic actions of acetylcholine

PENG N. YEOH AND M. J. A. WALKER*

Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

The effects of venom from a common sea snake, *Enhydrina schistosa*, on the actions of acetylcholine in the superior cervical ganglion, the adrenal medulla and in the atropinized cat were investigated. The crude venom reduced nictitating membrane responses to preganglionic nerve stimulation and intra-arterial injection of acetylcholine, without lowering the responses to postganglionic nerve stimulation or intra-arterial adrenaline. In the eviscerated cat, the venom also antagonized rises in heart rate and blood pressure induced by injections into the coeliac artery of acetylcholine but not those of histamine. Pressor effects due to splanchnic nerve stimulation and carotid artery occlusion were blocked. In the atropinized cat, the venom depressed heart rate and pressor responses to intravenous acetylcholine, but not the responses to adrenaline. It was concluded that the venom antagonized the actions of acetylcholine at autonomic ganglia and the adrenal medulla.

The dried, crude venom of a sea snake, *Enhydrina schistosa* (E.S.), was reported to block nictitating membrane contractions induced by acetylcholine injected into the superior cervical ganglion of the cat. Similar responses initiated by stimulation of the preganglionic nerve were not blocked (Chan & Chang, 1971). Those authors postulated that preganglionic nerve stimulation initiated postganglionic events on a different basis from injected acetylcholine.

We have attempted to verify this by examining the effects of several doses of the venom using the same preparation. In addition, the influences of the venom on the nicotinic actions of acetylcholine on the suprarenal gland and in the atropinized cat were studied.

METHODS

Cats of either sex (1·9 to 4·0 kg) anaesthetized with a chloralose (80 mg kg^{-1})-pentobarbitone sodium (10 mg kg^{-1}) mixture, administered intraperitoneally, were artificially respired (Palmer respiration pump, 80 strokes per min, delivering 60 ml of air per stroke).

The drugs used were: (−)-adrenaline bitartrate, acetylcholine chloride, atropine sulphate, histamine phosphate and a single batch of crude venom of *Enhydrina schistosa* (Snake and Venom Research Institute, General Hospital, Penang, Malaysia) dried over P_2O_5 at 30°. The doses were expressed as amounts of the salts. All drugs were dissolved in saline and solutions were freshly prepared.

Each dose level of the venom was studied on 5 or more cats, unless otherwise

* Present address: Department of Pharmacology, Faculty of Medicine, University of British Columbia, Vancouver 8, Canada.

stated. Only cats that demonstrated at least two sets of reproducible control responses (i.e. less than 10% variation) were treated with the venom.

Cat superior cervical ganglion—nictitating membrane preparation

The procedure as described by Chan & Chang (1971) was generally followed with slight modifications. In a few cats contractions of both nictitating membranes were recorded. For nerve stimulation, rectangular pulses (0·2 ms at 40 Hz) were applied for 10 s. Voltages, which varied from 0·5 to 5·0 V for preganglionic nerve stimulation and 3·0 to 5·0 V for post-ganglionic nerve stimulation were used to produce submaximal, reproducible contractions of the nictitating membrane. These were recorded via a pressure transducer on a Grass polygraph.

Drugs were injected through the lingual artery according to Trendelenburg (1959), except the venom, which was administered intravenously.

At least 2 sets of reproducible control responses induced by acetylcholine (2 or 5 μg kg^{-1}), adrenaline (2 μg kg^{-1}) and by stimulation of the preganglionic and postganglionic nerves were recorded before injection of venom. At varying times after venom injection responses due to these stimuli were again recorded. Several doses of venom (200 μg per cat and 0·1 to 5·0 mg kg^{-1}) were studied.

Eviscerated cat—suprarenal gland preparation

The anaesthetized cat was artificially respired and the viscera removed and weighed (8–9% body weight) after ligation of the major blood vessels in the region. These were then severed, except the left coeliac artery, which was isolated and cannulated for drug administration. Cannulae were also inserted in the right femoral vein for drug injection and in the left carotid artery for blood pressure recording via a Statham pressure transducer. The heart rate was monitored by a tachograph (preamplifier model 7P4A; driver amplifier model 7DAC). Both blood pressure and heart rate were recorded on a Grass polygraph.

The left splanchnic nerve plexus was also isolated and stimulated with bipolar platinum electrodes, using rectangular pulses (0·2 ms at 40 Hz) for 10 s with voltages from 3 to 10 V. Again only submaximal responses were elicited.

Drugs were administered intravenously except acetylcholine and histamine, which were injected through the coeliac artery. The dose administered was based on the weight of the eviscerated cat.

Carotid blood pressure and heart rate were recorded following occlusion (30 s) of the right carotid artery, injection of acetylcholine (2 or 5 μg kg^{-1}, i.a.) and histamine (2 μg kg^{-1}, i.a.). The venom (0·5–10 mg kg^{-1}, i.v.) was then administered at varying intervals after which cardiovascular responses to carotid artery occlusion, acetylcholine and histamine were investigated.

The atropinized cat

The cat was prepared for artificial respiration, injection through the femoral vein and for blood pressure and heart rate recordings as previously stated. Atropine (2 mg kg^{-1}, i.v.) was administered and 30 min later, responses to 0·2 or 0·5 mg kg^{-1} acetylcholine and 2 μg kg^{-1} adrenaline were obtained, the venom (0·5–1·0 mg kg^{-1}) was then given, followed by acetylcholine and adrenaline at varying intervals.

Statistics

In each cat the test observations at the time of maximum venom action were

expressed as percentages of their respective control values obtained before venom administration. The data from all animals in the study were pooled. Means and standard errors of the means (s.e.) were calculated. The results at the time of maximum venom action were compared to control results using the Student's *t*-test for paired data.

RESULTS

Action of the venom on transmission in the superior cervical ganglion

Cats receiving single doses of venom (200 μg) demonstrated no significant difference in their nictitating membrane responses after venom. In 5 venom-treated cats, the mean and s.e. after preganglionic nerve stimulation were 96 $\pm$ 4% of control. The corresponding acetylcholine response was 91 $\pm$ 9% of its control value.

The venom administered at 1·0 mg kg^{-1} (i.v.) and above significantly reduced nictitating membrane contraction initiated by intra-arterial acetylcholine (Fig. 1).

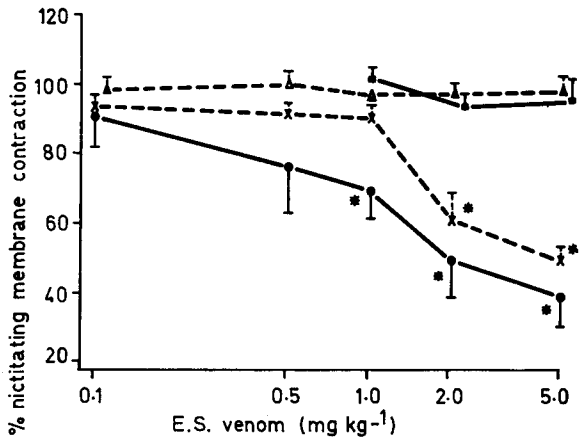

FIG. 1. Effect of venom on nictitating membrane contraction. In this and subsequent figures the response at the time of maximum venom action is expressed as a percentage of the control. ●——●, i.a., acetylcholine (2 or 5 μg kg^{-1}); x - - x, preganglionic nerve stimulation; ▲- ▲, postganglionic nerve stimulation; ■——■, i.a., adrenaline (2 μg kg^{-1}). Each point is the mean of observations from 5–11 cats. Vertical lines indicate $\pm$s.e.; *, significantly different from control using the Student's *t* test for paired data ($P \leqslant 0{\cdot}05$).

Following 1·0, 2·0 and 5·0 mg kg^{-1} venom, the response was reduced to 70, 49 and 40% of the control value, respectively. Nictitating membrane contractions induced by stimulation of the preganglionic nerve were significantly reduced by higher doses of venom. After 2·0 and 5·0 mg kg^{-1} venom, the responses fell to 62 and 51% of their respective control values. However, responses due to adrenaline (i.a.) and stimulation of the postganglionic nerve remained essentially unchanged in the presence of venom (Fig. 1).

The effect of the venom on nictitating membrane contractions induced by acetylcholine or nerve stimulation was seen at 10 min after injection. This is consistent with the observation by Chan & Chang (1971). Its maximum blocking action developed between 15 and 20 min and lasted for 35 to 60 min. Under the same recording conditions, the heights of nictitating membrane contraction elicited in these cats not treated with venom were: preganglionic nerve stimulation, 20 to 54 mm; postganglionic nerve stimulation, 9 to 42 mm; acetylcholine, 7 to 20 mm; adrenaline, 12 to 24 mm.

Action of the venom on the suprarenal gland

Ten min after administration of venom (5 mg kg^{-1}), the cardiovascular response to acetylcholine was depressed and remained so at a constant level for more than 90 min. Similar results were obtained with the other doses of venom tested (Fig. 2).

The pressor effect due to electrical stimulation of the splanchnic plexus was significantly reduced in the presence of 0·5 to 10·0 mg kg^{-1} venom (Fig. 3). After treatment with 0·5 mg kg^{-1} venom, the mean blood pressure rise was 80% of the control.

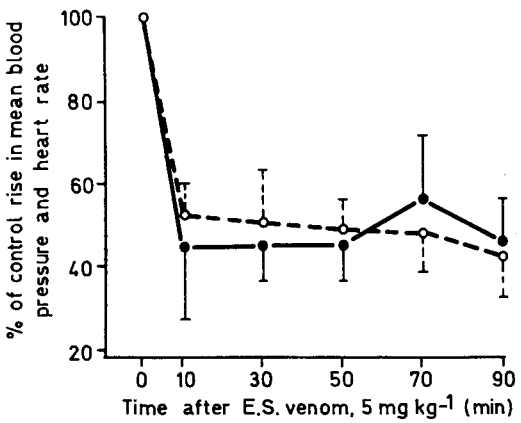

FIG. 2. Onset and duration of venom (5 mg kg^{-1}) action on cardiovascular response induced by acetylcholine (2 or 5 μg kg^{-1}) in the eviscerated cat. ●——●, blood pressure; ○ - - ○, heart rate. Each point is the mean of observations from 5 cats. Vertical lines indicate ±s.e. All values were significantly different from the control at time ○, using the Student's *t*-test for paired data ($P \leqslant 0{\cdot}05$).

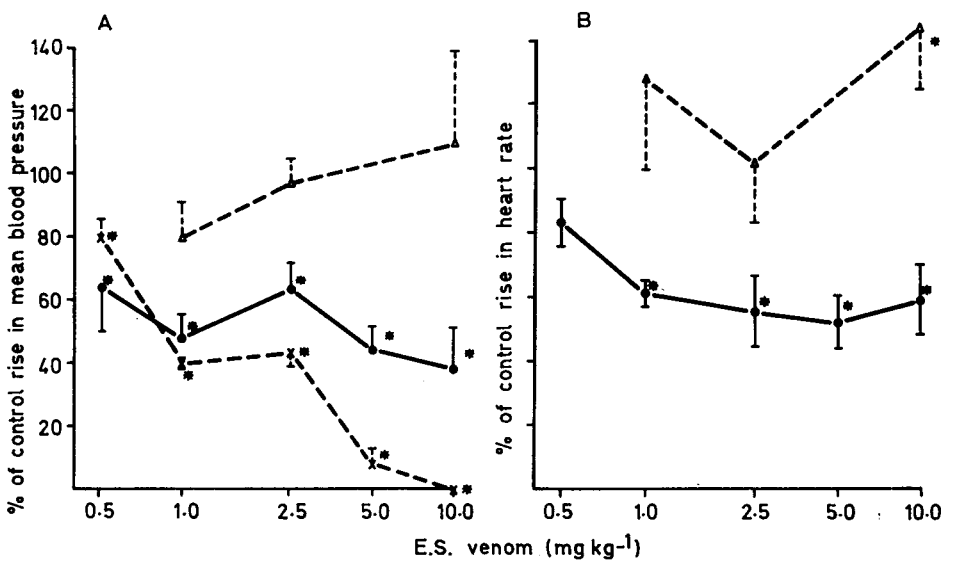

FIG. 3. Effect of venom on (A) the pressor response and (B) the rise in heart rate due to stimulation of the suprarenal gland in the eviscerated cat. x - - x, splanchnic nerve stimulation; ●——●, i.a., acetylcholine (2 or 5 μg kg^{-1}); ▲ - - ▲, i.a., histamine (2 μg kg^{-1}). Each point is the mean of observations from 5–11 cats. Vertical lines indicate ±s.e.; *, significantly different from control, using Student's *t*-test for paired data ($P \leqslant 0{\cdot}05$).

The pressor response was further depressed by higher doses of the venom; so that 10·0 mg kg^{-1} of the venom abolished the response completely. It also reduced the pressor response due to acetylcholine injected into the coeliac artery. Doses of the venom from 0·5 to 10·0 mg kg^{-1} lowered the effect to mean values from 63 to 38% of controls. With these doses, the suprarenal response to histamine was not significantly different from its control values, although the response tended to rise.

Tachycardia initiated by acetylcholine injected through the coeliac artery was also reduced by the venom (Fig. 4A). Doses from 1·0 to 10·0 mg kg^{-1} significantly decreased the acetylcholine response by 40 to 48%, although apparently not in a dose-related manner. In the same study, histamine-induced tachycardia was not reduced by the venom, on the contrary, after 10·0 mg kg^{-1} venom, it rose. The change in heart rate following stimulation of the splanchnic nerve plexus was too small to be recorded.

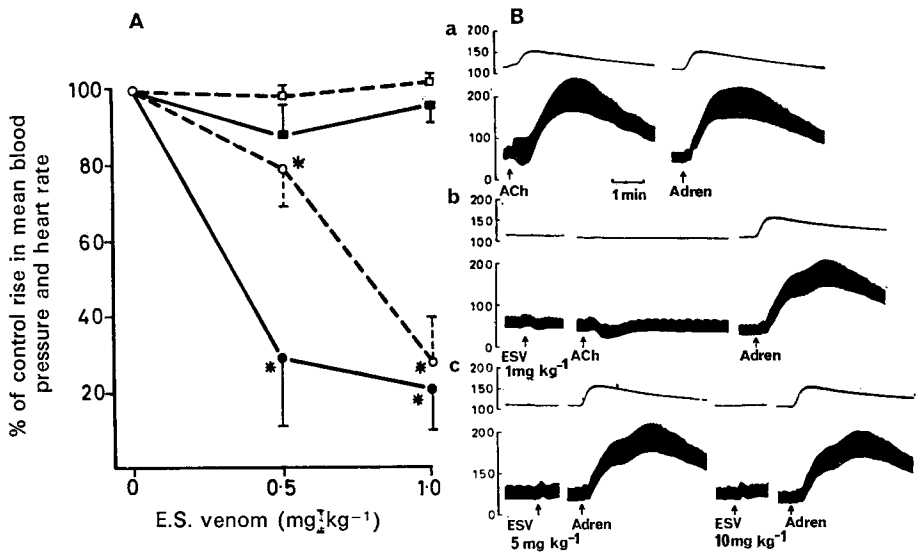

FIG. 4. Effect of venom on cardiovascular responses due to acetylcholine and adrenaline in the atropinized cat.

A. ●——●, increase in mean blood pressure after acetylcholine (0·5 mg kg^{-1}); ○ - - ○ increase in heart rate after acetylcholine; ■——■, increase in mean blood pressure after adrenaline (2 μg kg^{-1}); □ - - □, increase in heart rate after adrenaline. Each point is the mean of observations from 5–7 cats. Vertical lines indicate ± s.e.; *, significantly different from control, using Student's *t*-test ($P \leqslant 0{\cdot}05$).

B. Panel a—control responses; b—acetylcholine, 15 min and adrenaline, 20 min after venom (1 mg kg^{-1}); c—adrenaline, 15 min after higher doses (5 and 10 mg kg^{-1}) of venom.

Occlusion of the right carotid artery produced a reflex rise in the mean blood pressure of 10 to 25 mm Hg. This was not significantly altered by 0·5 mg kg^{-1} venom (111 ± 37%, n = 3). However, 1·0 mg kg^{-1} significantly ($P \leqslant 0{\cdot}05$) reduced the pressor response to 45 ± 5% of the control value (n = 3).

Enhydrina schistosa venom *per se* did not alter the heart rate or mean blood pressure significantly.

Effect of the venom on nicotinic actions of acetylcholine in the atropinized cat

In cats previously treated with 2 mg kg^{-1} atropine, both the mean blood pressure

and the heart rate were increased after acetylcholine (0·5 mg kg^{-1}). Following the injection of the venom, these cardiovascular responses were reduced significantly (Fig. 4B). The acetylcholine-induced pressor responses were reduced to 28 and 21% of control values after 0·5 and 1·0 mg kg^{-1} venom, respectively. The corresponding tachycardia was 79 and 27% of control values. In these animals, cardiovascular effects induced by adrenaline remained essentially unaltered after venom. Higher doses of the venom (5 and 10 mg kg^{-1}) also failed to reduce the action of adrenaline.

DISCUSSION

The venom appears to be mainly protein in nature (Tu & Toom, 1971; Karlsson, Eaker & others, 1972) containing up to 16 neurotoxins which account for 45% of its dry weight. Its ability to block the neuromuscular junction is well recognized (Carey & Wright, 1961; Chan & Geh, 1967).

The results confirm the observations of Chan & Chang (1971) that the venom blocked acetylcholine action in the superior cervical ganglion. However, in the present investigation, although 1 mg kg^{-1} significantly antagonized the acetylcholine-induced response without decreasing the response to preganglionic nerve stimulation, higher doses (2 mg kg^{-1} and above) did lower the response to nerve stimulation. The degree of antagonism produced by the same dose of venom was greater for responses induced by acetylcholine than for responses due to nerve stimulation. However, it is difficult to quantitate the relative sensitivities of these two types of responses to venom block because their initial controls were submaximal responses, unequal in magnitude. Ideally, maximal responses or responses of equal magnitude should be employed, but nerve injury and desensitization tend to occur with maximal stimulations over long periods.

In comparison with the earlier study, higher doses of the venom were required to elicit a blocking effect. Chan & Chang (1971) saw antagonism of the acetylcholine action with 200 μg (i.a.) of the venom. Using cats of the same weight range, we found no significant change in acetylcholine response with a 200 μg dose given intravenously. This could be a reflection of the difference in the route of venom administration. It is also possible that some variation in potency exists between different batches of the venom in its ganglion blocking action.

Since the venom did not interfere with nictitating membrane responses to postganglionic nerve stimulation or direct effector excitation with adrenaline, its action appears to be at the ganglion. The presence of cholinergic sites in the superior cervical ganglion is well established (Eccles & Libet, 1961; Libet, 1970). Since the venom antagonized acetylcholine-induced contractions of the nictitating membrane, it must act on cholinergic sites in the ganglion.

We have also demonstrated that the crude venom blocked the ganglionic action potential recorded from the isolated, desheathed cat superior cervical ganglion (Yeoh & Walker, 1971). These findings confirm the ability of venom to block ganglionic transmission.

Further evidence is provided from the eviscerated cat. Stimulation of the splanchnic nerve plexus releases catecholamines from the adrenal medulla, causing both the blood pressure and the heart rate to rise (Bülbring & Burn, 1949; Butterworth & Mann, 1957; Douglas, Kanno & Sampson, 1967). We find the venom to antagonize the pressor effect induced by both stimulation of the splanchnic plexus and by acetylcholine (the transmitter at these nerve endings), administered through the coeliac

artery. Acetylcholine-induced tachycardia was also depressed. This action of venom on the adrenal medulla seems specific for acetylcholine, as it did not lower histamine-induced pressor effect or tachycardia. Changes in the cardiovascular system subsequent to histamine result from the release of adrenaline by the adrenal medulla (Staszewska-Barczak & Vane, 1965), probably by stimulation of chromaffin cells (Douglas & others, 1967). Since this response remained the same or even elevated, the venom does not appear to interfere with the release or cardiovascular effect of adrenaline. It probably acts by antagonism at cholinergic sites in the adrenal medulla.

In several cats treated with venom, the cardiovascular response to histamine rose beyond control values. This could be a real effect of the venom or could reflect an increase in sensitivity of the adrenal medulla after repeated doses of histamine (Staszewska-Barczak & Vane, 1965).

The venom also suppressed the pressor effect of carotid artery occlusion. Moreover, in cats whose muscarinic sites were first blocked by atropine (Innes & Nickerson, 1970), the venom reduced the nicotinic effects of acetylcholine, i.e. it depressed tachycardia and rise in blood pressure due to high doses of acetylcholine. This cannot be attributed to a direct antagonism of catecholamine action, since the venom did not alter cardiovascular responses to intravenous adrenaline in the same preparation. These findings provide additional support for blocking action of the venom at nicotinic sites in autonomic ganglia.

The venom blocked acetylcholine action at autonomic ganglia at doses much higher than those required to block nicotinic sites in the skeletal muscle. In the cat tibialis anterior muscle, 10 to 200 μg (i.a.) was sufficient to block acetylcholine-induced muscle contractions (Chan & Geh, 1967). This is consistent with the hypothesis of Khromov-Borisov & Michelson (1966) that nicotinic sites in muscles from more advanced animals on the evolutionary scale are more easily blocked by nicotinic blocking agents than such sites in the muscles of more primitive animals (e.g. leech). They regarded ganglionic sites to be more like primitive skeletal muscle sites.

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