THE ACTION OF ELAPIDAE VENOMS ON SMOOTH MUSCLES

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The venoms of Indian Cobra (Naja naja) and the Indian Krait (Bungarus Caeruleus) produce spasm of various smooth muscles of different species of laboratory animals. The spasm is reduced or inhibited in presence of the ganglion blocking agent pentolinium. The action of histamine or acetylcholine on smooth muscles is potentiated after exposure to venom, but that of adrenaline on guinea-pig vas deferens is reduced. The contraction of the nictitating membrane produced by the injection of the Venom in the intact animal, is blocked by previous administration of dibenzyline.

The spasmogenic action of snake venoms on various smooth muscles has been reported by a number of workers (9, 2). The mechanism of such action is however not clear. Histamine has been associated with action of snake venoms for a long time (5, 6, 7). Recent investigations have shown that action of histamine can only account for a minor fraction on such spasmogenic action. The problem has been investigated using a number of smooth muscles from various species. It was also seen that exposure to venom modified the response of smooth muscles to commonly employed drugs. These studies are now being reported.

METHODS

Lyophilised dried snake venoms of two common elapidae species, that is Naja naja (Cobra) and Bungarus Caeruleus (Krait) were obtained from Haffkein's Institute, Bombay. The venoms were reconstituted in distilled water before use.

The rabbit ileum, the guinea-pig ileum, the guinea-pig vas deferens, the rat uterus and the rat colon were used in *in-Vitro* experiments. The bath temperature was 37°C except for rat tissues. The rat colon was perfused at 22°C and the rat uterus at 30°C. The rabbit intestine and the guinea-pig vas deferens were perfused with 'ringer-Locke' solution; the guinea-pig ileum was perfused with tyrode; where as rat tissues were perfused with de Jalon solution. The bath volume was 25 ml.

The contraction of the splenic capsule of the dog, and the nictitating membrane of either the dog, or the cat were recorded in *in-Vivo*, under pentobarbitone anaesthesia (1).

Spot chromatograms on Whatman filter paper No. 1 were developed for histamine by Panly's reagent, for 5-Hydroxytryptamine (5-HT) by Ehrlich's reagent and for adrenaline by potassium ferricyanide.

RESULTS

Studies with Naja naja Venom: The action of graded doses of venom was studied on various smooth muscles. In the initial experiments, each piece of tissue was

exposed to one dose only and then rejected to obviate the influence of one dose on the responses of the same piece to succeeding doses. Repeated doses are given to the same piece in later experiments. The onset, peak action, duration and the pattern of the response were recorded and have been presented in Table - 1. It will be seen that the venom produced contraction of all the smooth muscles studied.

TABLE 1

Pattern of smooth muscles response to cobra venom

S. No.	Tissue	Dose in mgs.	Onset of action	Peak action	Recovery	Pattern of response	Response of repeated doses
1.	Rabbit intestine	0.5	25 sec.	1 min. 50 sec.	Incomplete in 5 min.	Gradual rise in tone and reduction in amplitude	Reduction in amplitude each time
2.	Guinea pig ileum	0.03	10-20 sec.	42 sec to 1 min.	Complete in 2 min.	Sharp rise in tone	Densensitisation
3.	Guinea pig vas deferens	5.0	Immediate	2 mins.	Complete in 10 min.	Marked rise in tone. Rhythmic contractions during relaxation phase	nodesensitisation
4.	Rat colon	0.5	Quick	2 mins	Partial in 5 min	Gradual rise in tone	Desensitisation
5.	Rat uterus	0.5	30 sec.	—	Incomplete recovery	Sharp rise in tone. Irregular rhythmic contractions after washing	Desensitisation
6.	Nictitating membrane (cat)	0.2 mg/kg	15 sec-	—	Complete recovery	Sharp rise in tone whith gradual recovery	—
7.	Spleen volume (dog)	0.1mg/kg.	20-30 sec.	—	Partial recovery	Sustained contraction	—

The response of the nictitating membrane of the cat or the dog to adrenaline or noradrenaline was not altered by exposure to venom. The stimulant action of adrenaline on guinea-pig vas deferens was reduced after envenomation. The venom considerably potentiated the action of histamine or acetylcholine on guinea-pig ileum. The response of smooth muscles to 5-Hydroxytryptamine was not altered after exposure to venom.

The effect of pentolinium tartarate, atropine sulphate, mepyramine maleate and self blocking doses of 5-HT, on the spasmogenic action of the venom is given in Table - 2.

TABLE 2

Action of Naja naja Venom in Relation to Autonomic Blocking Agents

Serial No.	Drug	Concentration gm/ml	Guinea—pig ileum	Rabbit intestines
1.	Pentolinium	10^{-4}	Blocked	Blocked (only slight increase in amplitude-no change in tone)
2.	Atropine	10^{-7}	Not blocked	Not blocked
3.	Mepyramine	10^{-8}	Not blocked	Organ resistant to histamine
4.	5—HT	3×10^{-5}	Not blocked	Blocks 0.25 gm/20 ml bath, does not block 0.5 gm/20 ml bath.

The contraction of the nictitating membrane produced by the venom was blocked by previous administration of dibenzyline to the animal.

Studies on formation of active smooth muscle stimulating substances: The snake venoms are complex substances and contain several types of enzymes. It is possible that some active substance is produced in the plasma as a result of such enzyme action. The venom was incubated with human plasma and the effect of the incubated product was seen on various smooth muscles. Doses of 100 µg of Naja naja venom did not produce any effect on pieces of rabbit ileum. Doses of 0.2 ml of human sera were also inert on the same or similar preparations. When the same doses of the venom and human sera were incubated for 30 minutes, an active substance was produced, which stimulated the rabbit ileum. There was no tachyphylaxis to this active substance. Incubation of human saliva with human serum also produced a similar active substance.

The active substance prepared by incubation of venom with human serum produced contractions of the guinea-pig ileum. The tissue had been previously desensitised by repeated exposures to a similar concentration of the venom, and as such the action could not have been due to the venom content of the mixture. The action of the incubated product was diminished in the presence of atropine, but was unaffected by the presence of pentolinium, mepyramine or self blocking doses of 5-HT

The active substance was produced within 15-30 minutes of commencement of incubation, and did not increase or disappear on prolonged incubation for 2-3 hours. The substance produced was fairly stable and could be preserved in a refrigerator for 7 days without any loss of activity.

Actions of Bungarus Caeruleus Venom : The action of Bungarus Coereleus venom was studied on the rabbit ileum, the splenic capsule of the dog and the nictitating membrane of the cat and found to be similar to that of Naja naja venom.

Chromatographic Studies : Chromatographic studies failed to reveal detectable traces of histamine, 5-HT or adrenaline in the venom.

DISCUSSION

The snake venom produced contraction of all the smooth muscles studied. The stimulation of the smooth muscles could be due to a direct action or could be due to formation or release of some active substance. An active substance actually resulted on incubation of venom with serum. The action of this substance was not identical with that of venom itself, particularly with reference to blocking agents. Histamine has been associated with venom action for a long time, but the importance of histamine appears to be minimal due to the following reasons.

(a) Histamine cannot be detected in the venom in measurable quantity. The venom action is not reduced in presence of mepyramine in a concentration in which the latter blocks the action of histamine.

(b) The smooth muscle of the rat and the rabbit is resistant to action of histamine, but responds to the venom.

Acetylcholine or 5-HT is also not likely to be the mediator, because of the failure of atropine or self-blocking doses of the 5-HT to inhibit the spasmogenic action of the venom.

In the intact animal, the venom is known to release adrenaline (3), and the contraction of the nictitating membrane could have been due to this substance, particularly in view of the fact that dibenzyline blocked the response.

The blocking of the venom action on isolated smooth mucle by pentolinium raises the interesting possibility of ganglionic stimulation. In a different context, we have noted that snake venoms release potassium from the tissues, and it has also been reported that potassium is capable of stimulation of peripheral ganglia (4).

A characteristic action of snake venom is to produce a fall in blood pressure (11) and in recent years a number of substances have been shown to cause stimulation of smooth muscles and a fall in blood pressure (8). Bradykinin is one of such a substance, but it has been reported that Naja naja venom do not produce bradykinin (10). Vogt (13) has identified a lipid soluble acid called SRS-C, which has been shown in perfusates of isolated tissues after injection of Cobra Venom (7). This substance potentiates the response of guinea-pig ileum to acetylcholine and histamine (12). A similar potentiation was seen in experiments reported above. The effect of SRS-C is

slow to develope, but the contraction of guinea-pig ileum on exposure to venom was abrupt, and as such must have been due to some other fraction. It seems likely that multiple factors operate in determining the response of tissues to venom.

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