SHORT COMMUNICATION

SKELETAL NEUROMUSCULAR AND SMOOTH MUSCLE EFFECTS OF WHOLE VENOM OF THE GREEN MAMBA, DENDROASPIS ANGSTICEPS - I

J. WANGAI,* K. THAIRU** AND B.V. TELANG

Division of Pharmacology and Therapeutics (Department of Medicine) and Department of Physiology,** Faculty of Medicine, P.O. Box 30588, Nairobi, Kenya

Summary: Dendroaspis angusticeps venom (75 μg/ml) caused an irreversible blockade of the directly as well as directly evoked contractions of the rat hemidiaphragm and indirectly evoked contractions of the chick biventer cervicis muscle. The venom itself also produced a contraction of the frog rectus abdominis muscle, rat fundal strip, rat uterus and nictitating membrane of the cat; however, it did alter responses of nictitating membrane to preganglionic electrical stimulation and to ganglionic stimulant drugs. The effect was attributed to the presence of acetylcholine-like substance in the venom.

Key words : Dendroaspis angusticeps skeletal muscle smooth muscle nictitating membrane

INTRODUCTION

Envenomation by mambas (genus Dendroaspis) produces flaccid paralysis and high lethality characteristic of elapid snakes. Dendroaspis angusticeps is a poisonous snake found in East Africa. There is very little information in literature about the pharmacological characteristics of the venom of the snake.

The purpose of the paper is to present a preliminary report on some of the actions of the venom on neuromuscular junction, smooth muscle and on ganglionic transmission.

MATERIALS AND METHODS

In vitro experiments:

Skeletal muscle and nerve muscle preparation;
Preparations used were the rectus abdominis of frog, rat phrenic nerve hemidiaphragm (1), and biventer cervicis muscle of 7 day old chicks (2).
Supramaximal single shocks (0.05 and 0.5 msec duration) were used for indirect and direct stimulation of diaphragm respectively. For indirect stimulation of the tendon, supramaximal shocks (0.5 msec) were used in chick experiments.

Smooth muscle preparations:
Female Wistar rats (120-150 g) were injected with stilboesterol (0.1 mg/kg) for 3 consecutive days. The uterus was set up in De Jalon fluid.
Isolated rabbit jejunum was set up in Tyrode solution at 37°C; the rat fundal strips were set up in Krebs solution at 37°C.

In vivo experiments:
Ten cats were chloralose (80/mg) arteries using a Statham pressure transducer. Rectal temperature injections into the caudal efferent nerves were made using saline as described earlier (3). Gras polygraph. Width (0.1 msec)

Drugs used: Dendroaspis angusticeps snake venom was dissolved in saline (Sigma, U.S.A.), sulphate (Sigma, U.S.A.), Laboratories Inc., U.S.A.

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D B V. TELANG

(Department of Medicine)

Morphology, **

88, Nairobi, Kenya

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In vivo experiments:

Ten cats weighing between 3 and 4 kg were anaesthetized with ether followed by

chioralose (80/mg/kg iv). The blood pressure was recorded from one of the femoral

aries using a Statham transducer (P23D). The cats were artificially ventilated using

on an electronic ventilator at a pressure of 15 cm of water/kg and a rate of 20/min. The

rectal temperature was maintained at 36°-37°C throughout the experiments. Intra-arterial

jections into the left superior cervical ganglion were made through a T-shaped cannula

as described earlier (3). Injection volumes did not exceed 0.3 ml. The tone of the nicti-

ating membrane was recorded by a Grass force displacement transducer connected to a

Grass polygraph. The preganglionic nerve was stimulated supramaximally at 10 Hz (pulse

width 0.1 msec) for 10 sec at intervals of 10 min.

Drugs used were: acetylcholine chloride (E. MERCK, Darmstadt), atropine sulphate

Sigma, U.S.A.), histamine diphosphate (Sigma, U.S.A.), 5-hydroxytryptamine creatinine

sulphate (Sigma, U.S.A.), 1, 1-dimethyl-4 phenylpiperazinium iodide (DMPK: K & K Labo-

ratories Inc., U.S.A.), methacholine chloride (Sigma, London). Doses refer to their salts.

Desiccated whole venom of Dendroaspis angusticeps was obtained (Mr. J.N. Leakey,

aringo snake farm, Nakuru, Kenya) and stored at 4°C. Requisite quantity of the venom

was dissolved in the appropriate physiological solution before each experiment.

RESULTS

The venom of D. angusticeps (75 µg/ml) completely blocked the contractile response
to indirect electrical stimulation (n=10) after a latent period of 2–4 min. The time to
100% blockade was 39.0±9.6 min. The effect of direct electrical stimulation was also

totally blocked simultaneously (n=10). No recovery from the block occurred even after

peated washing of the diaphragm for 3 hr.

Addition of neostigmine (1 µg/ml) or choline chloride (400 µg/ml) at the time of

ial blockade did not reverse the neuromuscular block (n=20).

Other concentrations of the venom (25, 50, 125 and 175 µg/ml) produced qualita-
tively similar results although the blocking effect occurred earlier with higher concentration

and vice versa. For instance, when the dose of the venom was increased from 75 µg/ml

to 175 µg/ml the blocking time significantly decreased to 16.4 ± 2.4 min (P<0.01; n=10).

The venom (75 µg/ml) also blocked the effect of indirect electrical stimulation of

the chick biventer cervicis muscle. At this time, the contractile effect of acetylcholine

(50 µg/ml) was also substantially reduced.
Smooth muscles:

The snake venom, 4 μg/ml, elicited contractions of the rabbit jejunum (n=15) which were completely blocked by atropine (10 μg/ml). The contractions of the rat uterus (n=15) were elicited by acetylcholine (2 μg/ml) and a slightly higher dose of the venom (10 μg/ml). These contractions were blocked by atropine (10 μg/ml).

Contractions of the rat fundus (n=15) were elicited by acetylcholine (0.002 μg/ml), 5-HT (0.002 μg/ml), histamine (0.03 μg/ml) and snake venom (0.5 μg/ml). Pretreatment of the tissue with atropine (0.1 μg/ml) abolished the spasmogenic action of acetylcholine and snake venom but had no effect on the contractile responses to 5-HT and histamine.

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The snake venom (300 μg) injected intra-arterially to reach the superior cervical ganglion itself elicited contractions of the nictitating membrane and slightly reduced the blood pressure. The contractile response to ganglion stimulating drugs, DMPP (20 μg), methacholine (20 μg) and potassium chloride (10 mg), however, remained unaltered when they were tested at various intervals following the venom.
DISCUSSION

The venom of *D. angusticeps* blocks the contractile response of the rat hemidiaphragm. This shows a pronounced effect of the venom on the muscle contractility although an effect at the neuromuscular junction cannot be ruled out. The direct action of the venom was further confirmed by the decrease in the contractile response to exogenous acetylcholine in chick muscle experiments.

The irreversible effect of the venom on the muscle may be due to firm binding of the toxins in the venom to the skeletal muscle.

The contractions of the frog rectus abdominis, rabbit jejunum, rat uterus and rat fundus by the snake venom as well as their blockade by specific blocking agents suggests the presence of a substance with cholinergic activity in the venom. This would also explain the contraction of the nictitating membrane seen after close intraarterial injection of the venom. A similar contractile response of the nictitating membrane following *Dendroaspis jamesoni* venom was noted which was ascribed to an acetylcholine-like substance present in the venom (4).

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REFERENCES