SOME PHARMACOLOGICAL STUDIES WITH SCORPION (PANDINUS EXITIALIS) VENOM

M. ISMAIL*, O. H. OSMAN, K. A. GUMAA and M. A. KARRAR

Departments of Pharmacology and Biochemistry, University of Khartoum, Khartoum, Sudan

(Accepted for publication 6 July 1973)

Abstract—Venom from the scorpion Pandinus exitialis was fractionated on Cellogel membranes into at least 14 bands in acetate buffer pH 4.2, and into 16 bands, 4 of which exhibited cathodic mobility, in barbitone buffer, pH 8.6. The venom was fractionated into 6 fractions using Biogel P 100. The LD50 of the venom following i.p. injection into mice was estimated to be 40 mg/kg. The cardiovascular effects of the venom appeared to be mediated through stimulation of the autonomic nervous system with a predominance of sympathetic stimulation, and release of tissue catecholamines. On the rat uterus the venom produced a powerful contraction which was greatly attenuated by methysergide and completely blocked by meclofenamic acid indicating that the contraction is mediated partly by the serotonin content of the venom and partly through the release of kinins, prostaglandins and/or slow reacting substance.

INTRODUCTION

Pandinus exitialis (Poc) is a large black scorpion about 14 cm long which inhabits the western part of the Sudan. It usually burrows in the cool muddy areas and emerges during the rainy seasons. It is also reported to be present in Arabia (BÜCHERL, 1971). Although it is dreaded by the local inhabitants, reports concerning the Pandinus species have described its venom as being mild (BÜCHERL, 1971). In view of the scanty information available in the literature, it was thought of interest to investigate some biochemical and pharmacological properties of P. exitialis venom.

MATERIALS AND METHODS

Venom, obtained from mature P. exitialis by electrical stimulation of the telson, was suspended in water, centrifuged and the supernatant freeze-dried. The dried venom was kept in a desiccator over CaCl2 at room temperature, and when required, was reconstituted by the addition of distilled water.

Electrophoretic separation of the venom was performed in a horizontal Shandon tank using either acetate buffer (0.1 ionic strength, pH 4.2) or barbitone buffer (0.05 ionic strength, pH 8.6) and Cellogel strips (Chemetron-Milano). Twenty-five μl of crude venom solution (10 mg/ml) were applied along a 2 cm line marked on the strip. Electrophoresis was conducted at room temperature (22-25°C) for 75 min applying 20 V/cm. At the end of the run the strips were stained with Ponceau S (0.2 per cent in 3 per cent trichloroacetic acid).

Twenty mg of the venom were dissolved in 5 ml distilled water and the solution was dialysed in cellophane tubes (2.5 cm dia) against 150 ml water. Dialysis was continued for

*Visiting lecturer, Department of Pharmacology, Faculty of Pharmacy, University of Alexandria, Egypt.

TOXICON 1974 Vol. 12
48 hr at 4°C with 4 changes of water. The dialysate was pooled and concentrated in a rotary film evaporator under reduced pressure to a final volume of 3 ml.

Two-dimensional chromatography of the dialysate was performed on Whatman No. 1 paper using the solvent system butan-1-ol-acetic acid–water (12:3:5, v/v) for the first direction and phenol–water (4:1, w/v) for the second direction. At the end of the run, the chromatogram was dried and stained with ninhydrin.

Ten g Biogel P 100 (Calbiochem, Los Angeles, California, U.S.A.) were allowed to swell in distilled water at room temperature for 5 days, placed into a glass column giving bed dimensions of 2.4 × 30 cm and equilibrated with 200 ml 1 mM phosphate buffer (pH 7.5). Twenty mg of the venom were dissolved in 3 ml buffer and after centrifugation and removal of mucus were applied to the column. Elution was continued with phosphate buffer and 5 ml fractions were collected at a flow rate of 15 ml/hr. Protein was estimated spectrophotometrically at 280 nm.

The LD₅₀ of the crude venom was determined by the method of KÄRBER (1931) using 60 albino mice (20–30 g body weight). The toxicity of the protein fractions separated by gel filtration of the crude venom was determined in mice, using 2 animals for each fraction. Generally 100 μg of protein were injected i.p. with the mice being observed for 24 hr and post mortem examinations being made.

The venom was tested on the following preparations: the isolated rabbit heart (Langendorff’s preparation), the cat blood pressure (BURN, 1952), the isolated phrenic nerve–hemidiaphragm of the rat (BÜLBRING, 1946), the tibialis anterior muscle of the cat (BROWN, 1938), the isolated rabbit’s intestine and guinea-pig ileum (MAGNUS, 1904), the isolated rat uterus (DE JALON et al., 1945) and the spontaneously contracting rat uterus in Ringer–Locke solution (TOTTILL, 1965).

In some experiments, reserpine was administered to cats, and in other experiments, hearts were isolated from reserpine-treated rabbits (ISMAIL et al., 1972).

The following drugs were used: acetylcholine chloride (Roche), atropine sulphate (B.D.H.), hexamethonium chloride (May & Baker), 5-hydroxytryptamine creatinine sulphate (B.D.H.), isoprenaline sulphate (Burroughs Wellcome), meclofenamic acid (2-6-dichloro-m-tolyl anthranilic acid, Parke Davis), methysergide (Deseril, Sandoz), nicotine tartrate (B.D.H.), propranolol (Inderal, I.C.I.), reserpine (Serpasil, Ciba) and tolazoline (Priscol, Ciba).

RESULTS

Electrophoresis of the crude venom at pH 4.2 (Fig. 1) revealed 14 fractions which migrated towards the cathode, while a dark band remained at the line of application. On the other hand, electrophoresis at pH 8.6 revealed 16 fractions, 11 of which migrated towards the anode, one remained stationary at the line of application and 4 moved towards the cathode (Fig. 2).

Two-dimensional chromatography of a sample of the dialysate revealed 11 ninhydrin stainable spots and two u.v. fluorescent spots. Eight of the ninhydrin stainable spots were identified as Asp, Ser, Gly, Ala, Lys, Norval, Leuc and Phe. The remaining three ninhydrin stainable spots and the two u.v. fluorescent spots were not identified.

Using Biogel P 100, it was possible to fractionate the venom into 6 fractions (Fig. 3). The LD₅₀ of the crude venom injected intraperitonally into mice was 40 mg/kg. The mice showed irritation, restlessness, excitation, fighting behaviour, difficulty in respiration, convulsions and death 1–24 hr after injection. Autopsy revealed no signs of bleeding. The
Pandinus exitialis venom

Fig. 1. Electrophoretic separation of scorpion (P. exitialis) venom on cellogel using acetate buffer pH 4.2.
B, Buthus minax venom; P, P. exitialis venom; L, Leiurus quinquestriatus venom; Or, origin.

Fig. 2. Electrophoretic separation of scorpion (P. exitialis) venom on cellogel using barbitone buffer pH 8.6.
B, Buthus minax venom; P, P. exitialis venom; L, Leiurus quinquestriatus venom; Or, origin.
dialysed venom, on the other hand, in doses up to 100 mg/kg was not lethal to the mice. Similarly, neither the dialysate nor any of the fractions separated on the Biogel column were lethal when injected into mice.

Pharmacological investigations

The effects reported below are typical responses, each obtained in at least three experiments.

The isolated rabbit heart

The typical effects of the venom are shown in Fig. 4, and consisted of a marked positive inotropic effect which started immediately after injection of the venom and lasted for more than 30 min. The heart rate, on the other hand, was only slightly increased. This cardiac stimulant effect was not preceded by bradycardia and was produced by doses ranging between 200 and 600 µg. Perfusion of the heart with propranolol at a rate of 0.5–1.0 µg/min for 30 min before injection of the venom greatly attenuated the cardiac stimulant effect of the venom. On hearts of reserpine-treated rabbits, the venom caused initial negative inotropic and chronotropic effects followed by an increase in both force and rate of contraction. The initial cardiac depressant effect of the venom was partially blocked by atropine. Treatment with cyproheptadine greatly attenuated the subsequent cardiac stimulation.

Cat blood pressure and respiration

*P. exitialis* venom (100–300 µg/kg) produced a hypertensive response which started 10–30 sec after injection, reached a peak within 1–2 min and was over within 5–20 min. After repeated injection of the same dose of the venom, signs of tachyphylaxis occurred.
FIG. 4. EFFECT OF SCORPION (P. exitialis) VENOM ON THE ISOLATED RABBIT'S HEART. At the solid circles P. exitialis venom (300 µg). At Prop, propranolol was infused at a rate of 0.5 µg/min for 30 min. Ip, isoprenaline (3 µg). At DS, the drum was stopped and where the individual contractions are clearly seen the drum speed was increased to 225 mm/min for 5 sec.
FIG. 5. EFFECT OF SCORPION (P. exitialis) VENOM ON THE RAT UTERUS.
Uterus in natural oestrus. At the solid circles P. exitialis venom (2 × 10^-8 g/ml) was added
W, wash. At, atropine sulphate (10^-4 g/ml) added 10 min before venom. Met, methysergide
(2 × 10^-4 g/ml) added 10 min before venom. Mecl, meclofenamic acid (2 × 10^-4 g/ml) added
10 min before venom. Contractions magnified 4 times under a constant load of 2 g using an
auxotonic lever. Cm = centimeters in original tracings.

TOXICON 1974 Vol. 12
The hypertensive effect of the venom was completely blocked by treatment with tolazoline and was nearly absent in reserpinized cats.

The venom produced an increase in depth of respiration, however, after repeated injection of the venom or injection of large doses (more than 300 μg/kg), there was observed a 50 per cent or greater decrease in both rate and depth of respiration.

*Neuromuscular junctions*

On the rat phrenic nerve-hemidiaphragm preparations, the venom (40–80 μg/ml) produced a gradual block of twitch activity in only one out of the six preparations; no effect being observed in the other preparations. On the other hand, on the cat tibialis anterior muscle preparation, the venom in a dose of 1 mg injected close arterially produced first and increase in twitch height that was followed by a gradual decrease until complete block of twitch activity occurred.

In young chicks, the intravenous injection of the venom (1–2 mg/kg) rapidly produced a flaccid paralysis which was followed 1 min later by spastic paralysis; however, the chicks recovered within 5–10 min.

*Rat uterus*

The crude venom produced an increase in both frequency and tone of contraction of the spontaneously contracting rat uterus. This action was produced by concentrations as low as $2 \times 10^{-8}$ g/ml. The action of the venom started 5–20 sec after addition; and following washing, the spontaneous activity of the uterus was much reduced. After repeated additions of the venom, the spontaneous activity was abolished (Fig. 5). The response of the uterus to the venom remained constant, however, with no signs of tachyphylaxis. This stimulant activity was not blocked by a dose of atropine sulphate ($10^{-6}$ g/ml) sufficient to block the action of acetylcholine without influencing the intrinsic rhythm of the uterus. Treatment with methysergide (1 to $4 \times 10^{-6}$ g/ml), 10 min before adding the venom greatly reduced the uterine stimulant action of the venom. Meclofenamic acid ($2 \times 10^{-6}$ g/ml) added 10 min before the venom completely blocked the stimulant action of the venom (Fig. 5).

The dialysed venom was much less active on the spontaneously contracting rat uterus than the crude venom. Doses as high as $10^{-6}$ g/ml were needed before any appreciable stimulation occurred. The dialysate from 2.5 mg crude venom stimulated the rat uterus, though to a lesser extent than that obtained with 50 μg doses of crude venom.

All six peak fractions obtained by Biogel fractionation of the crude venom stimulated the spontaneously contracting rat uterus; however, peaks II and V were the most potent. In case of peak V there was a delay of about 4 min before any stimulation occurred. Again, the spontaneous activity of the uterus was completely abolished after washing out the fractions.

Addition of the venom ($1.6 \times 10^{-6}$ g/ml) to the rat uterus (in de Jalon solution) produced a powerful contraction which was not blocked by atropine ($10^{-6}$ g/ml) but completely blocked by methysergide ($10^{-6}$ g/ml).

*Rabbit intestine and guinea pig ileum*

On the isolated rabbit duodenum the venom produced an initial relaxation which was followed by contraction. The relaxant effect, though resistant to block by either tolazoline ($10^{-6}$ g/ml) or propranolol ($10^{-6}$ g/ml), was blocked by a mixture of both drugs. The subsequent contraction was blocked by nicotine ($10^{-6}$ g/ml).

On the guinea-pig ileum the venom produced a contraction that was completely blocked by nicotine ($10^{-6}$ g/ml) and atropine ($10^{-6}$ g/ml).
DISCUSSION

Few reports have been found in the literature describing the toxicity of the venom from the *Pandinus* species. This might be due to the general belief among workers that this species is only mildly toxic. However, due to the large amount of venom ejected per scorpion and the fact that *P. exitialis* is dreaded by the local inhabitants, it was thought worthwhile to investigate some of the biochemical, toxicological and pharmacological properties of its venom.

*P. exitialis* venom possesses pharmacological properties similar to those of the venoms from the more commonly occurring scorpions, *Leiurus quinquestriatus* (H & E) and *Buthus minax* (L. Koch) (ISMAIL et al., 1972, 1973). However, *P. exitialis* venom has an LD₅₀ about 40 times higher than that of *L. quinquestriatus* (ZLOTKIN et al., 1971) and 10 times higher than that of *B. minax* (EL-ASMAR et al., 1973). The amount of dry venom obtained by electrical stimulation from *P. exitialis* is about 2 mg per scorpion, which is approximately 10 times more than that obtained from *B. minax* and 5 times that from *L. quinquestriatus*. The larger amount of venom ejected from *P. exitialis* might compensate in part for the low toxicity of the venom.

The cardiovascular effects of *P. exitialis* venom appear to be mediated through stimulation of both parts of the autonomic nervous system with predominance of sympathetic stimulation and release of tissue catecholamines, similar to those of the venoms from *L. quinquestriatus* and *B. minax* (ISMAIL et al., 1972, 1973). However, some qualitative and quantitative differences exist between the three venoms. Thus while *P. exitialis* venom resulted in a positive inotropic effect with slight increase in rate, *L. quinquestriatus* venom increased both force and rate while *B. minax* venom increased the force with no change in the heart rate (ISMAIL et al., 1972, 1973). In addition, the negative inotropic effect caused by *P. exitialis* venom in the reserpinized hearts was only partly blocked by atropine, while that of *L. quinquestriatus* and *B. minax* venoms are completely blocked by atropine. Quantitatively, *P. exitialis* venom was 10–20 times less potent on the cardiovascular system than either *L. quinquestriatus* or *B. minax* venom.

On the rabbit intestine, *P. exitialis* venom caused an initial relaxation that was followed by contraction. The relaxation was blocked by a mixture of tolazoline and propranolol, the α- and β-adrenergic receptors blocking agents, indicating that it is mediated through adrenergic receptors stimulation. This is in contrast to the effects of both *L. quinquestriatus* and *B. minax* venom which only cause stimulation (MOHAMMED, 1950; ISMAIL et al., 1973). The stimulant effect of *P. exitialis* venom was blocked by nicotine, indicating that it may be mediated through ganglionic stimulation.

Venom from *P. exitialis* produced a powerful contraction of the rat uterus, an action similar to that of *L. quinquestriatus* venom (OSMAN et al., 1972). This action appears to be produced partly by the serotonin content of the venom as evident from the blocking effect of methysergide, a serotonin antagonist, and partly through the release of kinins, prosta-glandins and/or slow-reacting substance as evident from the blocking effect of meclofenamic acid. Meclofenamic acid is reported to antagonise kinins and slow-reacting substance (SRS-A) in the guinea pig lung (COLLIER and JAMES, 1967) and to antagonise prosta-glandins (SORRENTINO et al., 1972). When *P. exitialis* venom was washed out, there remained an inhibition of the spontaneous activity of the uterus. This is in contrast to the action of *L. quinquestriatus* venom (OSMAN et al., 1972). It appears probable that *P. exitialis* venom contained factor(s) that produce both stimulation and inhibition of the spontaneous activity.
of the rat uterus. Washing probably removes the stimulant factor(s), revealing the action of the depressant factor(s) whose action is not readily reversible.

Electrophoretic separation of *P. exitialis* venom on cellogel at pH 4.2 revealed 14 bands which migrated towards the cathode. This indicates that they have isoelectric points above pH 4.2. Under similar conditions, the venom from *L. quinquestriatus* revealed 17 bands and that of *B. minax* 15 bands. It is interesting to note that the bands corresponding to the lethal fractions present in the venoms of *L. quinquestriatus* and *B. minax* (El-Asmar et al., 1972, 1973) are lacking from the venom of *P. exitialis*. Fractionation at pH 8.6 showed that *P. exitialis* venom was separated into 16 bands, 11 of which migrated towards the anode while 4 bands moved towards the cathode, indicating their strongly basic nature. Under similar conditions *L. quinquestriatus* venom revealed 18 bands, 4 of which exhibited cathodic mobility, and that of *B. minax* showed 15 bands, 4 of which exhibited cathodic mobility. Until purified toxins are obtained from *P. exitialis* venom as has been accomplished with the other scorpion venoms (El-Asmar et al., 1972; Zlotkin et al., 1972), there is little point in further testing bands which may still not represent purified venom components.

Earlier workers were unable to fractionate venoms from *L. quinquestriatus* and *B. minax* at pH 8.6, using paper or cellulose acetate electrophoresis (Adam and Weiss, 1959a, b; Nitzan and Shulov, 1966; El-Asmar et al., 1972). Zlotkin et al. (1972), however, have reported good separation of *L. quinquestriatus* venom at pH 8.6 using starch gel zone electrophoresis. The good separation reported here for both *L. quinquestriatus* and *B. minax* venoms at pH 8.6 might be due to the superior resolving properties of Cellogel membranes.

REFERENCES


