BRAZILIAN SCORPION VENOM (TITYUS SERRULATUS),
AN UNUSUAL SYMPATHETIC POSTGANGLIONIC STIMULANT

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Accepted for publication July 27, 1968

ABSTRACT

CORRADO, A. P., A. ANTONIO AND C. R. DINIZ: Brazilian scorpion venom (Tityus serrulatus), an unusual sympathetic postganglionic stimulant. J. Pharmacol. Exp. Ther. 164: 253-258, 1968. An extract of Brazilian scorpion venom was assayed upon the isolated heart of the guinea pig. The typical effect of the venom, in final concentrations of the order of $10^{-4}$ g/ml, is a short-lasting bradycardia followed by a conspicuous increase in the force and the frequency of cardiac contractions. The venom also produces a marked rise in the phosphorylase activity of the perfused heart. The bradycardia is blocked by atropine ($10^{-5}$ g/ml) and potentiated by neostigmine ($10^{-6}$ g/ml). The cardiac stimulation is abolished by the beta sympatholytic agent propranolol ($10^{-5}$ g/ml) and by the adrenergic blocking agent bretylium ($10^{-5}$ g/ml); the cardiac stimulation is also absent in the hearts of reserpine-treated guinea pigs. Hexamethonium ($10^{-4}$ g/ml) does not affect either the bradycardia or the cardiac stimulation elicited by the venom in doses which abolish the effects of nicotine. It is concluded that 1) both effects of the venom are indirect and due to a local release of acetylcholine and norepinephrine, and 2) the site of action of the venom is the postganglionic nerve terminals.

The exact mechanism of the pronounced arterial hypertension which follows the i.v. administration of scorpion venom has not yet been identified. Most of the reports dealing with the subject refer to a sympathetic stimulating action which, according to the authors, could be 1) peripheral—sympathetic nerve endings (Mohammed, 1942); 2) central—spinal cord and sympathetic preganglionic fiber (del Pozo, 1956); 3) central and peripheral—central nervous system, ganglia and sympathetic nerve endings (Ramos and Corrado, 1954; Freire-Maia and Ferreira, 1961).

However, no attempt has been made to see whether such a sympathetic action is a direct one and caused by some component of the venom extract or an indirect one mediated through the release or preservation of endogenous catecholamines.

In contrast to the aforementioned sympathomimetic action, it has been demonstrated that the venom produces a contraction of the isolated guinea-pig ileum through the release of acetylcholine (Diniz and Valeri, 1959); such a release could explain, at least in part, the slowing of the heart rate, which is simultaneous with the hypertensive effect of the venom (Ramos and Corrado, 1954).

Since the venom seems to affect the two major components of the autonomic nervous system, we have decided to use in our experiments the isolated guinea-pig heart, which is a very simple preparation and yet shows opposite responses to adrenergic or cholinergic agents.

MATERIAL AND METHODS. Langendorff's preparation. The guinea pigs were sacrificed with a blow on the head and exsanguinated by cutting the vessels of the neck; the thorax was opened, the heart was dissected from its connections and the perfusion with a modified Ringer-Locke solution ($9.0$ g of NaCl, $0.42$ g of KCl, $0.25$ g of CaCl$_2$, $0.15$ g of NaHCO$_3$, $0.01$ g of NaH$_2$PO$_4$ and $1.0$ g of glucose per liter) was started immediately. The perfusion fluid was kept at $37^\circ$C, and a pressure of $50$ mm Hg was maintained under constant oxygenation.

The heartbeats were registered by means of a
spring-loaded isotonic lever on a smoked drum. The injections were given through a thin polyethylene tube at an approximate distance of 0.5 cm from the coronary openings, and the volume injected never exceeded 0.2 ml. In many cases we studied the influence of a drug continuously infused at a constant rate of 0.1 ml/mm through an independent opening of the perfusion apparatus by means of a Phipps and Bird infusion pump.

**Determination of phosphorylase.** The hearts were perfused for 10 to 15 min prior to the injection of the drugs or saline to permit a stable baseline for heart contractions. All drugs were quickly injected in a volume of 0.2 ml. As soon as the maximal inotropic effect was obtained (10-60 sec), the aorta was cut and the heart was dropped into a Dry Ice-ethanol slush; the frozen heart was rapidly blotted with a filter paper, weighed and subsequently ground in a mortar (kept in an ice bath) containing 4 ml of a 0.02 M NaF-0.001 M disodium ethylenediamine tetracacetate solution. After complete homogenization, more NaF-ethylenediamine tetracacetate solution was added until a concentration of 33.3 mg of tissue/ml was reached. After a 15-min period of centrifugation (4000 r.p.m. at 5°C), the supernatant was analyzed for phosphorylase a and total phosphorylase activity by the method of Cori and Illingworth (1956) as modified by Haugaard et al. (1961). The final concentration in the reaction mixture was 3.3 mg of tissue/ml.

The guinea pigs treated with reserpine received a total of 2 mg/kg equally divided in two doses given i.p. 48 and 24 hr before the experiments.

The drugs used were: reserpine (Serpasil, Ciba Pharmaceutical Products, Inc.), acetylsalicylic acid, neostigmine sulfate, l-epinephrine hydrochloride, nicotine, propranolol hydrochloride, bretylium tosylate, hexamethonium chloride, tyramine hydrochloride, hemicholinium bromide, atropine sulfate and crude dried scorpion venom obtained by electrical stimulation of the telson and drying over calcium chloride in vacuum (Bücherl, 1955). All dosages are expressed as the salt except for the nicotine and venom.

**RESULTS.** The typical effect of the venom on the isolated heart is a short-lasting bradycardia followed by a conspicuous increase in the heart rate and force of contraction; these effects are seen with total doses of 10 to 20 µg, which correspond to a final concentration of the orcler of 2 × 10⁻⁶ to 4 × 10⁻⁶ g/ml; sometimes a higher dose of venom (20-40 µg) is necessary to produce the bradycardic effect (figs. 1 and 2). When the doses are further increased (40-80 µg), the bradycardia becomes more pronounced and interferes with the positive inotropic effect; as shown in table 1, only the duration of the positive inotropic effect is increased with doses above 40 µg of the venom.

The positive inotropic effect of the venom is very resistant to tachyphylaxis (fig. 2), completely blocked when the heart is infused during 30 min with 0.5 µg/min of propranolol (fig. 3) or during 40 to 60 min with 100 µg/min of bretylium (fig. 4); the positive inotropic effect is absent in the hearts of reserpine-treated guinea pigs (fig. 5). When any of the mentioned procedures to block the positive inotrope...
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20 20 20 20 20 20 20

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Fm. 2. Isotonic contraction of the isolated heart of the normal guinea pig. The numbers represent the total doses in micrograms. The cardiac stimulation produced by 20 μg of the Brazilian scorpion venom extract (VE) is highly reproducible. When the dose is doubled, the bradycardic effect is disclosed.

TABLE 1
The effect of scorpion venom on the isotonic force of contraction of the perfused guinea-pig heart

<table>
<thead>
<tr>
<th>Doses (μg)</th>
<th>N(1)</th>
<th>POSITIVE INOTROPIC EFFECT</th>
<th>PRESENCE OF BRADYCARDIA(2)</th>
<th>MEAN ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MAXIMAL EFFECT</td>
<td>TOTAL DURATION</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(%) OF CONTROL</td>
<td>(MINUTES)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>89.2 ± 9.2</td>
<td>6.0 ± 0.9</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>185.0 ± 17.9</td>
<td>12.2 ± 1.4</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>282.0 ± 37.5</td>
<td>16.4 ± 1.9</td>
<td>- or *</td>
</tr>
<tr>
<td>40</td>
<td>5</td>
<td>121.6 ± 19.5</td>
<td>34.0 ± 3.1</td>
<td>++ or +++</td>
</tr>
<tr>
<td>80</td>
<td>5</td>
<td>108.0 ± 8.5</td>
<td>46.8 ± 5.8</td>
<td>+++</td>
</tr>
</tbody>
</table>

(1) = Number of experiments

(2) = The signal (−) denotes absence of bradycardia. The signal (+) denotes the intensity of the bradycardia.

Doses of the troponic effect are employed, the bradycardia is unmasked and lasts longer (figs. 3 and 5); as in the nontreated hearts, the bradycardia in the reserpine-, propranolol- or bretylium-treated hearts is only seen with doses above 20 μg of the venom.

Figure 6 shows that the positive inotropic effect of tyramine is not blocked by doses of bretylium which abolish the effects of venom on nicotine.

The venom produces also a marked rise in the phosphorylase α activity of the perfused heart; the results in table 2 are reported as percentage phosphorylase α of total phosphorylase.

Nicotine (NIC) and venom (VE) was completely blocked, and their bradycardic effects were disclosed. The effect of 0.5 μg of acetylcholine (AC) is also shown. The kymograph was stopped as in figure 1.
FIG. 4. Isotonic contractions of the isolated heart of the normal guinea pig. The numbers represent total doses in micrograms. The infusion of hexamethonium (HEXA), started at the arrow, blocks both effects of nicotine (NIC) but not the cardiac stimulation by the venom (VE) or epinephrine (EP). If bretylium (BRE) is also infused, the cardiac stimulation by the venom is blocked. Observe the bradycardic effect of 40 μg of venom even in the presence of both bretylium and hexamethonium. The kymograph was stopped as in figure 1.

FIG. 5. Isotonic contractions of the isolated heart of the reserpine-treated guinea pig. The numbers represent total doses in micrograms. The tracing shows that bradycardia is the only effect seen with both nicotine (NIC) and venom extract (VE). The lower tracing was obtained during an infusion of hexamethonium (HEXA); only the effect of nicotine was blocked. The cardiac stimulation by epinephrine (EP) is still present. Between A and B, a 3-min interval was observed. The kymograph was stopped as in figure 1.

Hexamethonium, infused at the rate of 50 μg/min, did not affect the cardiac actions of the venom in doses which abolished the effects of nicotine (figs. 4 and 5).

Discussion. When assayed on the isolated heart of the guinea pig, the aqueous extract of scorpion venom produces a very marked cardiac stimulation and a rise in phosphorylase α activity; these effects are quite similar to those evoked by epinephrine (Hess and Haugaard, 1958).

The cardiac stimulation is completely blocked by the beta sympatholytic agent propranolol in a dose smaller than that required for local
anesthesia, according to Morales Aguilera and Vaughan Williams (1966), and is not seen in hearts of guinea pigs previously treated with reserpine. These results are in favor of an indirect action of the venom, probably through the release of tissue catecholamines. It has also been observed that the venom potentiates the hypertensive response of the dog to injected catecholamines and to carotid occlusion, indicating its participation in the mechanism of inactivation of catecholamines (R. Neto, A. P. Corrado and A. Antonio, unpublished results).

The site of the indirect action of the venom is, however, different from those of other compounds which act on the heart releasing catecholamines; in fact, we found that hexamethonium blocks the cardiac effects of nicotine without affecting the responses to the venom and to tyramine. The effects of these agents are also different because 1) the cardiac stimulation by nicotine and venom is stronger and more resistant to tachyphylaxis than that of tyramine and 2) the cardiac stimulation by nicotine and venom is usually preceded by bradycardia.

The bradycardia which usually precedes the cardiac stimulating effect of the venom is cholinergic in nature, since it is blocked by atropine and potentiated by neostigmine. The bradycardic effect of the venom is better seen when the cardiac stimulation is blocked by the use of propranolol or bretylium or in the hearts of reserpine-treated guinea pigs. Evidence that the cholinergic effects of the venom are also indirect has been reported by Torres and Diniz (1964) working with the isolated guinea-pig ileum. Moreover, the venom is not an inhibitor of cholinesterase activity (Diniz and Gonçalves, 1956). Our results show that the site of the cholinergic effect of the venom is unlike that of nicotine; it is not blocked by hexamethonium in doses which abolish a similar effect of nicotine. The results with hexamethonium are in agreement with the re-

**TABLE 2**

The effect of epinephrine and scorpion venom on the phosphorylase activity of the perfused guinea-pig heart

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>N</th>
<th>PER CENT PHOSPHORYLASE &quot;O&quot; Mean ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL (saline)</td>
<td>8</td>
<td>34.2 ± 2.9</td>
</tr>
<tr>
<td>EPINEPHRINE (0.1)</td>
<td>8</td>
<td>64.4 ± 4.1</td>
</tr>
<tr>
<td>SCORPION VENOM (25.0)</td>
<td>8</td>
<td>70.1 ± 5.9</td>
</tr>
</tbody>
</table>

N = Number of experiments.
ports that the ganglion-blocking agents do not affect either the bradycardic effect of the venom in the intact dog (Ramos and Corrado, 1954) or the contraction of the guinea-pig ileum (Diniz and Valeri, 1959).

When we consider all these facts, they indicate the nerve endings of both sympathetic and parasympathetic systems as the possible sites of action of the venom. One more point in favor of the necessary integrity of the sympathetic nerve ending in the release of catecholamines by the venom is the absence of such release from isolated granules of the adrenal medulla incubated in the presence of venom (Celeste-Henriques et al., 1967).

Preliminary experiments with a highly purified toxic component isolated from the venom (Gomes and Diniz, 1966) have disclosed both pharmacologic responses (i.e., bradycardia followed by cardiac stimulation), which suggests that they are due to the same component of the venom extract (Corrado et al., 1966).

Finally, we should mention that when assayed on the isolated heart the venom is a more potent releaser of catecholamines than is tyramine, and that its mechanism of action may represent a new tool for a better understanding of the events at the sympathetic nerve terminal. The effect of the venom resembles that of sympathetic nerve ending stimulation, and probably it releases norepinephrine by activating the physiologic release in the same way as has been proposed for guanethidine (Costa and Brodie, 1964).

REFERENCES


