EFFECT OF THE BLACK SNAKE TOXIN ON THE GASTROCNEMIUS-SCIATIC PREPARATION

BY A. H. MOHAMED AND O. ZAKI

Physiology Department, Faculty of Medicine, Abbassia, Cairo

(Received 3 June 1957)

When the toxin of the black snake (*Walterinnesia aegyptea*) was injected subcutaneously into albino rats, one of the common symptoms which appeared was the paralysis which takes place in the limb near the site of injection and extends to other limbs just before death.

Such observations led us to examine the changes produced in a stimulated gastrocnemius-sciatic preparation of *Bufo* to show the effect of the toxin and the site of its action.

No work had been done on the action of the black snake toxin on the nerve-muscle preparation. Yet much work has been published about such data for other poisonous snakes.

Lauder, Brunton & Fayrer (1874) were the first to demonstrate a curare-like action of cobra venom in dogs.

Epstein (1930) observed complete loss of excitability of voluntary muscles brought about by cape cobra venom and Kellaway & Holden (1932) demonstrated the direct action of venom on muscles.

Sarker (1951) has reported the action of Indian cobra venom both on the muscle and neuromuscular junction, the paralysis of the latter setting in much earlier and with smaller concentrations of the venom.

The aim of this work was to study the following points:

1. The effect of the toxin of the black snake on the contraction height of gastrocnemius muscle when stimulated directly and/or indirectly.
2. The site of action of the toxin.
3. The mechanism of action of the toxin.

METHODS

The toxin was prepared according to methods previously described (Mohamed & Zaki, 1956).

The gastrocnemius-sciatic preparation of the toad *Bufo* was employed.

A muscle trough was divided into two chambers by interposing a paraffin wall across the trough having a small groove for the transit of the nerve across the wall. The trough was so constructed that the bathing solution could be changed readily. Special care was necessary to avoid even small injuries to muscle fibres during dissection. Frog Ringer's fluid was used. The solution was freshly prepared for each day's experiment from stock solutions. Curare was used throughout in
Black snake toxin effect on gastrocnemius-sciatic preparation

concentration of $1 \times 10^{-4}$. With this concentration complete neuromuscular block was obtained and so direct stimulation could be applied to a curarized muscle.

The sciatic nerve was stimulated through submersible electrodes by supramaximal rectangular pulses of 300 $\mu$ sec. duration delivered at a rate of six per minute from an electronic square wave stimulator. The frequency was such as to keep the muscle contracting with no fatigue and so a constant contraction height was obtained. The duration used was the shortest effective duration so as to avoid repetitive excitation in nerve and summation in muscle.

A spring-loaded lever was used to record the contraction on a slowly revolving drum. The preparation was always stimulated for 1$\frac{1}{2}$-1$\frac{1}{2}$ hr. before starting an experiment to allow stretching of the muscle to become complete, and for the preparation to settle down to a steady level of contraction.

In control experiments the gastrocnemius continued to contract for at least 3-4 hr. without appreciable decline.

RESULTS

A. Site of action of the toxin

(1) Effect of toxin on the nerve

The nerve of the gastrocnemius-sciatic preparation was immersed in toxin solution and the muscle in Ringer's solution. The soaking of the nerve in $1/5000$ toxin produced no change in the contraction of muscle over the next 200 min. This shows that the toxin has no effect on the excitability of the nerve.

(2) Effect of toxin on the muscle

The gastrocnemius muscle was immersed in the toxin and the nerve in Ringer's solution.

Using three different concentrations of toxin $1/5000$, $1/10,000$ and $1/20,000$ the time required to cause paralysis of the muscle by the solutions, as determined by the response both to direct and indirect stimulation was noted.

With the lower concentrations $1/10,000$, $1/20,000$ of toxin the latency was increased in duration and the rate of paralysis was slowed. With the very low concentration of $1/10,000$ no change was detected in the preparation run for about 2 hr.

Results of the effects of three concentrations are shown in Table 1.

It will be noticed from the table that at the time when the stimulation of the nerve fibres fails to evoke any contraction the muscle still shows some contraction on direct stimulation. This means that although the toxin has slight depressant action on the contractility of the muscle, yet its main action is not direct on the muscle but is on the neuromuscular junction.

(3) Post-tetanic potentiation

Brown & v. Euler (1938) showed that in normal and partially curarized muscle a short tetanus of the motor nerve increased the tension developed in the succeeding single twitches.
Table 1. Action of the black snake toxin on the toad gastrocnemius-sciatic preparation
(The toxin is in the muscle chamber.)

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Concentration of toxin</th>
<th>Time required for no contraction of muscle when stimulated (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Indirectly</td>
</tr>
<tr>
<td>1</td>
<td>1/5,000</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>1/10,000</td>
<td>65</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>65</td>
</tr>
<tr>
<td>1</td>
<td>1/20,000</td>
<td>105</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>95</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>210</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>195</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>180</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Post-tetanic potentiation in gastrocnemius-sciatic preparation. Indirect stimulation six per min. At points $T$, $T_{15}$, $T_{30}$, $T_{45}$ and $T_{55}$ the rate of stimulation was increased to 50/cyc./sec. for 10 sec.

After the addition of toxin to the muscle bath (1/5000 concn.), the post-tetanic potentiation was tested at different intervals during the progress of paralysis (Fig. 1). At ($T$), before the addition of toxin, the post-tetanic potentiation was in the ratio 5:4. At ($T_{15}$), 15 min. after addition of the toxin, the post-tetanic potentiation was in the ratio 3:2. At ($T_{30}$), 30 min. after the addition of toxin, the potentiation was in the ratio 4:1. At ($T_{45}$), 55 min. after the toxin, the potentiation was in the ratio 9:1.

The degree of potentiation increases as the paralysis progresses.

As Brown and v. Euler (1938) showed that the post-tetanic potentiation was essentially a muscular phenomenon and was present on direct stimulation of the
Black snake toxin effect on gastrocnemius-sciatic preparation

Fully curarized or denervated muscle, it is not surprising that it should persist in the case of poisoning by black snake toxin, and this fact offers further evidence of the lack of strong direct action on the muscle, and that the main effect of the toxin is on the neuromuscular junction.

B. Mechanism of the neuromuscular block produced by black snake toxin

The neuromuscular block may be either a result of

(a) reduction in the sensitivity of the end-plate to the depolarizing action of acetylcholine, i.e. curare-like action; or

(b) change in the amount of the acetylcholine liberated at the nerve ending.

These possibilities were examined by means of the following tests:

(1) Genesis of tetanus.
(2) Effect of potassium chloride.
(3) Effect of prostigmine.
(4) Effect of acetylcholine.

As 1/50,000 concentration of toxin can be relied upon to produce paralysis regularly in a convenient time, this dose has been used for all experiments.

Fig. 2. Gastrocnemius-sciatic preparation. Indirect stimulation at 50 cyc./sec. for 30 sec. at $T_{10}$, $T_{10}$ and $T_{44}$.

METHODS AND RESULTS

(1) Genesis of tetanus

When the sciatic nerve is stimulated at 50 cyc./sec. there is a rapid build-up of muscle tension in the first 5–10 sec. and the tetanus is then well maintained.

After partial paralysis with the toxin the response to tetanus remains perfectly normal in shape, but with declining amplitude as long as poisoning is prolonged. Figs. 2A–C show 30 sec. tetanus at different stages of poisoning. In a typical
experiment with a muscle in which curare had reduced the sciatic tension by 40%, stimulation of the sciatic nerve at 50 cyc./sec. for 30 sec. resulted in an initial strong contraction followed by a rapid fall in the tension. Thus no tetanus was produced.

(2) Effect of potassium chloride

It is a well-known fact that KCl has an anticurare action (Wilson & Wright, 1936). When added to the muscle-bath while the muscle is partially curarized it causes immediate recovery. The action is a maintained one. In the present experiments KCl added to the bath in concentrations of 2–15 mg./ml. produced in normal and poisoned preparations very rapid depression. This shows that while KCl improves the condition of a muscle acted upon by curare, yet it depresses the condition of a muscle acted upon by the black snake toxin.

![Graph showing the effect of potassium chloride on muscle tension](image)

Fig. 3. Lack of beneficial effect of prostigmine upon the paralysis caused by black snake toxin.

Prostigmine 0.01 μg./ml. added 25 min. after toxin. 0.1 μg./ml. added 30 min. after toxin. 0.3 μg./ml. added 35 min. after toxin. 0.5 μg./ml. added 40 min. after toxin.

![Graph showing the effect of acetylcholine on muscle contraction](image)

Fig. 4. Effect of acetylcholine 10 μg./ml. added to muscle chamber 25, 40 and 55 min. after addition of 1:5000 toxin solution.

(3) Effect of prostigmine

The blocking action of curare at the end-plate region can be overcome by administering the anticholinesterase prostigmine. If prostigmine is added to a curarized muscle after the contraction height has diminished, gradual recovery
**Black snake toxin effect on gastrocnemius-sciatic preparation**

... takes place and the normal contraction height is retained. In a typical experiment (Fig. 3), after 25 min. paralysis, the effect of successive doses of 0.01, 0.1 and 0.3 mg. prostigmine is shown. The low dosage produced no effect and the contraction height remained constant in the following twitches up to 5 min. Larger amounts of prostigmine did not hinder the progress of the paralysis and there was gradual fall in the contraction height.

(4) **Effect of acetylcholine**

The neuromuscular block produced by curare results from its action on the motor end-plates which become insensitive to the acetylcholine released from the motor nerve endings (Dale, Feldberg & Vogt, 1936). During the neuromuscular block caused by black snake toxin, however, the motor end-plates remain sensitive to acetylcholine (10 μg.) as shown by the Fig. 4.

**DISCUSSION**

Since the gastrocnemius muscle responds when stimulated through the nerve when this is treated with the black snake toxin and the contractility remains nearly the same, it may be concluded that the toxin has no paralysing action on the nerve. When the solutions are interchanged, i.e. when the muscle is dipped into the toxin, it gradually goes into paralysis. The marked difference in time required for no contraction with direct stimulation as compared with stimulation through the nerve suggests that the black snake toxin acts mainly on the neuromuscular junction. That the muscle still shows the phenomenon of post-tetanic potentiation after being immersed in the toxin is further evidence for the neuromuscular effect of the toxin (Brown & v. Euler, 1938).

The mechanism by which the toxin produces block is entirely different from the mechanism by which curare does so. The normal type of tetanic response and the lack of response to anti-curare drugs suggest that the preparation partially poisoned with toxin contains some motor units which are susceptible to stimulation.

The end-plate is normally sensitive to acetylcholine, but the output of acetylcholine from the nerve endings is greatly diminished due to the presence of the toxin. It may be that the power to synthesize acetylcholine by the nerve endings would gradually decline, until the amount of acetylcholine fell below the threshold needed to activate the end-plate. A diminished acetylcholine output due to increased cholinesterase activity, and hence greater breakdown can be excluded, since prostigmine, a cholinesterase inhibitor, does not improve the condition of the muscle.

The release of acetylcholine may be decreased perhaps by a permeability change in the nerve endings. This is now under examination in our laboratory by a wide study of the effect of increase and decrease of the different cations in Ringer's solution. It may be that the toxin hinders the entry of Na ions into the nerve terminal and so prevents the equivalent efflux of acetylcholine ions from the interior of the nerve endings (Fatt & Katz, 1952).
SUMMARY


2. The rate of paralysis is slowed by lowering the concentration of toxin.

3. The paralysis produced by the black snake toxin is due to neuromuscular block. Conduction in the nerve is unaffected and the muscle is only slightly affected.

4. The neuromuscular block produced by the toxin differs from that of curare in that tetanus is well maintained and that paralysis is unaffected by prostigmine or potassium.

5. The neuromuscular block produced by the toxin differs from that of curare in that the motor end-plates remain sensitive to acetylcholine.

REFERENCES


