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SENSITIZATION TO POTASSIUM IONS AND DECURARIZATION BY SOME VERATRUM ALKALOIDS

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Bacq (1939a, b) showed that veratrine (5×10^{-7}) increases the response of striped muscle to potassium ions; this property might explain most, if not all, the actions of veratrine, particularly on the muscle fibre.

It is well known that veratrine is not a pure alkaloid, but a mixture of several; a review of the chemistry and pharmacology of different pure alkaloids, isolated from *Veratrum album* and *Schoenocaulon officinale,* has been published by Krayer and Acheson (1946). We wished to know how much these pure alkaloids would increase the response of striated muscle to potassium ions.

Sensitization to potassium ions

All our experiments were made on the same pattern: a muscle (*rectus abdominis*) of our common toad (*Bufo bufo*) or sometimes of the frog (*Rana temporaria*) was immersed in a constant volume of Ringer solution, suitably oxygenated; it was excited by small amounts of KCl (0.2 to 0.5 c.c. 5 per cent (w/v) KCl in a 10 c.c. bath) at first in normal Ringer and afterwards in Ringer containing a veratrum alkaloid. The muscle contraction to K, isotonically recorded, developed quickly, so that the potassium could be washed out thirty seconds to one minute after its introduction into the solution.

All the alkaloids tested increased the sensitivity of the muscle to K ions, but to different degrees; their action was easily reversible by washing with normal Ringer (Figs. 1 and 2).

We first determined the lowest concentrations of each alkaloid, which increased the muscle reaction to K. Our results are given in Table I and compared with the toxicities of the pure alkaloids, measured as LD50 by Krayer and Acheson (1946).

It will be seen that a certain relation exists between the toxicities and the sensitizing concentrations, but this relation is not striking enough to allow definite conclusions. For instance, the lowest sensitizing concentrations of cevadine and jervine are about the same, although the latter substance is ten times less toxic than the former. It is, indeed, illogical to compare lethal doses with threshold sensitizing concentrations. When using higher concentrations, we could observe

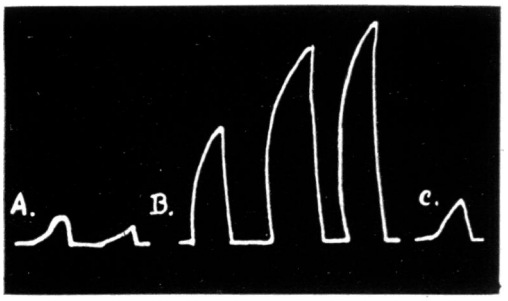

FIG. 1.

FIG. 1.—Frog's rectus in a 10 c.c. bath. Contractions provoked by 0.2 c.c. 5% KCl. A, in pure Ringer; B, in 1×10^{-6} cevadine after 12, 35, and 60 min.; C, 30 min. after return to pure Ringer.

FIG. 2.—Same as in Fig. 1. Contractions provoked by 0.2 c.c. 5% KCl. A, in pure Ringer; B, in 1×10^{-6} veratridine after 10 and 20 min.

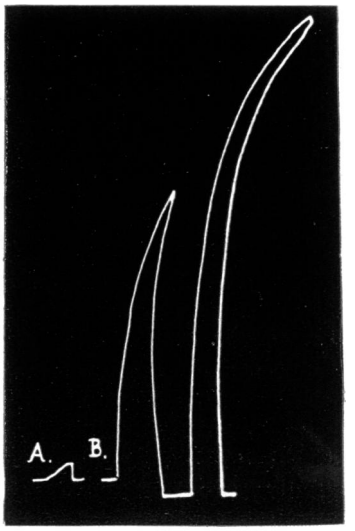

FIG. 2.

a closer relation between the LD50 and the sensitizing power to K. Unfortunately, we had at our disposal only a small quantity of each drug and were not able to make enough experiments with all of the alkaloids mentioned in Table I.

If ten times the lowest sensitizing concentrations of jervine and cevadine are used, jervine, though acting twice as long, is now half as active as cevine (Table II, A and B).

In the same way cevadine, at a low concentration, close to the sensitizing threshold, potentiates more strongly than germine at a concentration which is double the lowest active one, and more strongly than cevine at five times its threshold concentration (Table II, C, D, and E).

Substances, such as germine and cevine, which have a rather high threshold, show only a slight augmentation in sensitizing power, when their concentration is increased, whereas the other alkaloids, which are active in much lower concentrations, show a marked increase in sensitizing power when their concentration is increased (Tables I and II).

TABLE I

Alkaloid	LD50 mg./kg. mouse, i.v.	Lowest sensitizing concentration
Protoveratrine	0.048	2×10^{-7} to 5×10^{-7}
Veratridine	0.42	1×10^{-7}
Cevadine	1	5×10^{-7} to 1×10^{-6}
Jervine	9.3	1×10^{-6} to 2×10^{-6}
Rubijervine	70	1×10^{-5}
Cevine	87	2×10^{-5}
Germine	139	5×10^{-5}

TABLE II

Alkaloid and concentration used	Time of action in minutes	Increase in the contraction provoked by 5% KCl
A. Jervine 2.10^{-5}	10	× 10
B. Cevadine 1.10^{-5}	5	× 20
C. Germine 1.10^{-4}	15	× 2
D. Cevadine 1.10^{-6}	10	× 5
E. Cevine 1.10^{-4}	13	× 3

Time is also a matter of importance. At its lowest active concentration, cevadine (1×10^{-6}) still induces increased response to potassium after 45 minutes of action ; jervine, however (2×10^{-6}), shows a maximum effect after 20 minutes and cevine (2×10^{-5}) after 10 minutes.

In order to have a full picture of the sensitization to K, not only must the lowest sensitizing concentration be considered, but also the action of higher concentrations and the time factor.

Decurarization

When applied to a curarized frog's sartorius, veratrine provokes intense repetitive impulses from both end-plates and muscle fibres in response to a single nerve impulse (Coppée, 1943). Pure veratrum alkaloids have the same action. Our technique is the following: An isolated *sartorius* muscle of a frog (*Rana temporaria*) with its motor nerve is set up, and the muscular action potentials recorded by means of two electrodes, one of which is placed on a chosen spot of the muscle and the second on the upper ligated end. The action potentials are observed by means of the cathode ray oscillograph built and described by Coppée (1943), and eventually recorded. The muscle is indirectly stimulated by condenser discharges.

Our first experiments had exhausted the samples of several alkaloids so that we were only able to test veratridine, cevadine, and cevine. These three drugs have a remarkable decurarizing action on the frog's sartorius, curarized with "Merck" curare, and, like veratrine, they produce an asynchronous repetitive response (Fig. 3). We observed the same phenomena as those carefully described with veratrine by Coppée (1943).

Fifteen minutes after the addition of a veratrum alkaloid (5×10^{-5} cevine ; 1×10^{-5} cevadine ; 4×10^{-6} veratridine), the curarized muscle develops a stronger and more prolonged contraction than before curarization. When the muscle is in this state and is stimulated at a rather high frequency (one stimulus every one or two seconds), fatigue

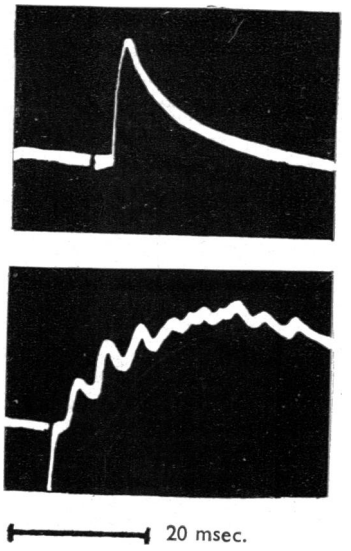

FIG. 3. — Frog's sartorius after complete curarization. Indirect stimulation. 1, End-plate potential. 2, The same potentials fifteen minutes after 1×10^{-5} cevadine.

rapidly occurs: first, mechanical response and muscle fibre potentials disappear; afterwards, the few steps of the staircase-like repetitive end-plate potential (see Fig. 3) may also disappear one by one. This phenomenon was particularly well-marked with veratridine: the repetitive response induced by a single nerve stimulus lasted over ten seconds, the tension developed being double the normal one; none of the later stimuli were able to induce repetitive response of the end-plates.

Summary

1. Pure veratrum alkaloids (protoveratrine, veratridine, cevadine, jervine, rubijervine, cevine, and germine) sensitize isolated amphibian striped muscle to potassium ions; this action parallels their general toxicity.

2. Cevine, cevadine, and veratridine are decurarizing substances.

We are indebted to Professor Krayer for specimens of the pure veratrum alkaloids. We also wish to thank Professor Coppée for his interest in the decurarization experiments.

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