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Influence of veratrine on the muscle contracting action of potassium

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Abstract

1. Veratrine greatly sensitizes the action of potassium ions on frog's rectus muscle. Quinine inhibits the sensitizing action of veratrine. 2. Perfusate of the preparation from the stimulated veratrinized hind limbs evokes a contraction of the veratrinized test muscle. 3. The other purfusates of the preparations perfused with Ringer's solution or with veratrine-Ringer's solution do not affect the test muscle. But a similar contraction is observable if potassium chloride of a certain strength, is added to the perfusates. 4. It can be shown that the chemical agent causing the muscular contraction is potassium ions, 5. The above experimental results may be wholly explained by the assumption that veratrine increases the permeability of muscle cell membrane to potassium ions.

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Influence of veratrine on the muscle contracting action of potassium

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Introduction.

In my previous communication, I reported that potassium ion inhibited the contracting effect of veratrine on muscle tissue. It was discovered by *Ringer* (1884-85) for heart muscle and by *Buchanan* (1899) for skeletal muscle that potassium ions neutralise the contraction due to veratrine. Later *Bottazzi* (1901), *Lamm* (1911), and *V. Frey* (1912) performed experiments in this connection and confirmed the above.

As against this, *Bacq* (1939) has recently published a number of reports, in which he states that veratrine sensitizes the muscular contraction evoked by the application of potassium chloride to frog's muscle. He has shown by ingenious experimental methods that there is a humoral transmission of muscular contraction in the presence of veratrine.

Interested by these apparently contradictory actions, I performed the present investigation.

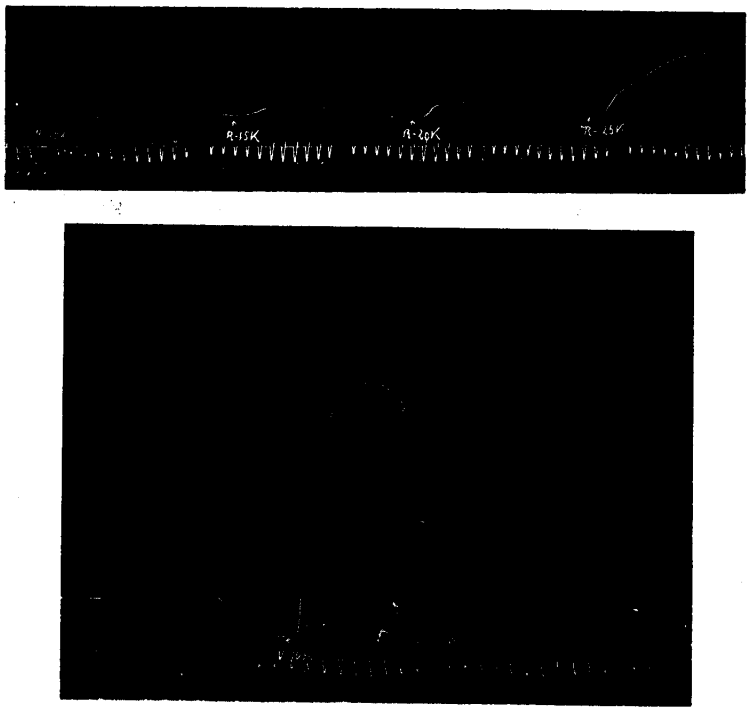
1. Effect of veratrine on muscular contraction provoked by potassium salt.

Frog's rectus abdominis muscle was used, the isolated muscle with a light recording lever, attached being immersed in *Ringer*'s solution. After a lapse of about 30 minutes, the muscle was submitted to the action of KCl-*Ringer*'s solution of strengths 5×N, 10×N, 15×N, 20×N (composition of *Ringer*; NaCl 0.6 g, KCl 0.01 g, $CaCl_2$ 0.02 g, H_2O 100 cc). The contractions were recorded on a rotating smoked drum.

The muscle was then poisoned with veratrine (usually $1:10^5$ or $1:2\times10^5$) for 5 minutes, and immersed in the above described KCl-*Ringer*'s solution.

Comparing these two series of contractions before and after the veratrinization, a marked difference in contraction is noticeable, i. e. indicating that veratrine sensitized highly the muscular contracture evoked by the action of potassium salt (see Fig. 1). This sensitizing action of veratrine appeared until the solution had been diluted to $1:5\times10^6$.

Fig. 1.



Time signal: 5 secs.

Upper figures: potassium contractures of normal rectus abdominis muscle.
Lower figures: those of veratrinized rectus abdominis muscle.

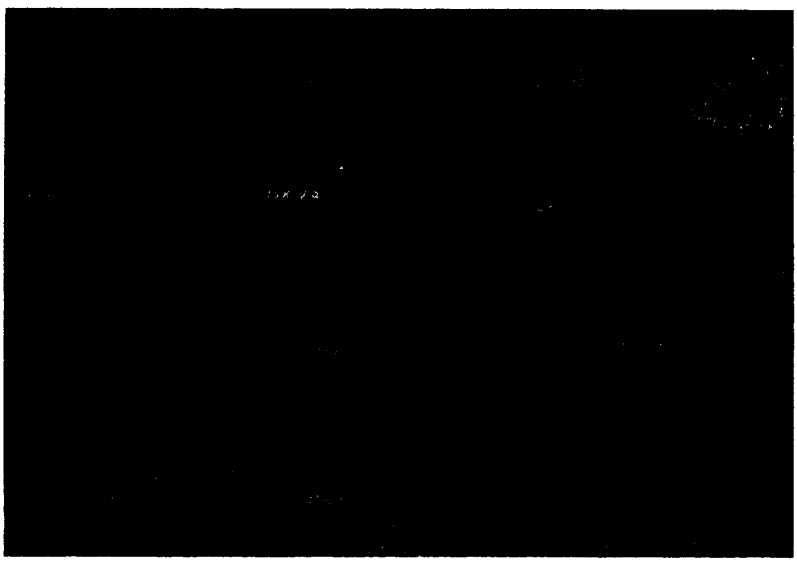
2. Influence of quinine on the potassium contracture of veratrinized muscle.

Quinine is generally accepted as a protoplasmic toxin, at first, stimulating, but inhibiting rapidly the functional activity of the cells. This holds also for muscular activity. In my previous report, I confirmed that veratrine contracture was totally neutralised by quinine

of a certain concentration. In this paper, the question to be decided is whether quinine inhibits the sensitizing action which veratrine has on potassium contracture.

Experimental procedure and materials were the same as before. The muscle was at first submitted to the action of potassium chloride in solutions of varying concentration, then immersed in veratrine-*Ringer* ($1:2\times10^5$) and again returned to the original potassium solution. It was then immersed in quinine-veratrine-*Ringer* (quinine, $1:10^4$; veratrine, from $1:10^5$ to $1:2\times10^5$) for 10 minutes, and put back into the original remained potassium solution.

Fig. 2.



Upper figures: potassium contractures of veratrinized rectus abdominis muscle under quinine treatment.
Middle figures: those of veratrinized rectus muscle.
Lower figures: those of normal rectus muscle.

It can be seen clearly from a comparison of the muscle contractions in these solutions (Fig. 2), that quinine greatly inhibited the sensitizing action of veratrine on potassium contracture.

3. Liberation of potassium ions by veratrinized muscle contraction.

Szent-Györgyi et al (1939) confirmed that perfusates collected from a frog previously veratrinized and electrically stimulated, caused irregular contractions in a second frog, perfused with the liquid

collected from the first. They insisted that this would constitute experimental proof of the humoral transmission of muscular contraction. The chemical agent in this case is not acetylcholine, because

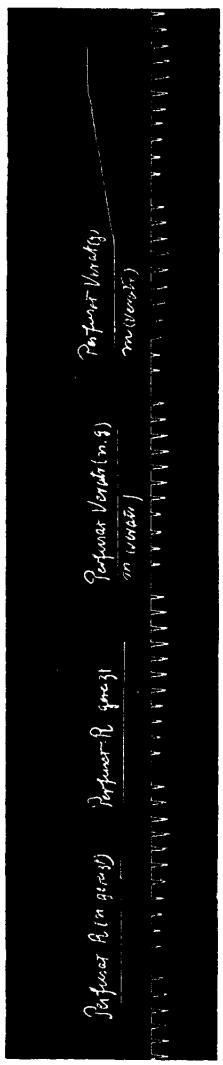


Fig. 3. A.

N.B.: Only perfusate from stimulated veratrinized preparation is effective.

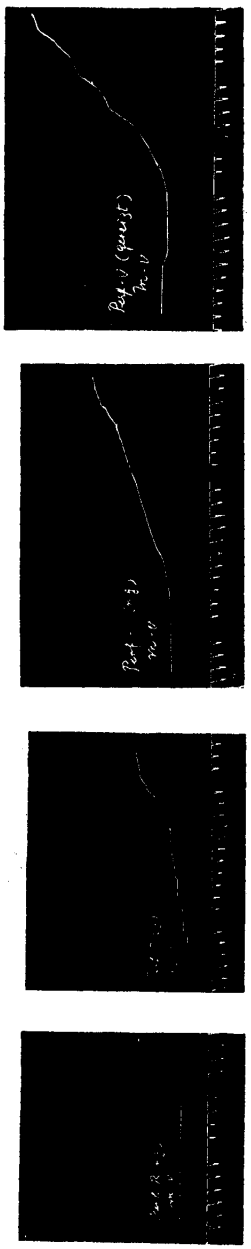


Fig. 3. B.

1. *Ringer*-perfusate from non-stimulated animal.
2. *Ringer*-perfusate from stimulated animal.
3. Veratrine-*Ringer*-perfusate from non-stimulated animal.
4. Veratrine-*Ringer*-perfusate from stimulated animal.

N.B.: Perfusate from the non-stimulated normal preparation always ineffective.
Perfusate from the stimulated normal preparation and perfusate from the non-stimulated veratrinized preparation usually ineffective, but sometimes effective.
Perfusate from the stimulated veratrinized preparation always effective, and the contracture most prominent of all.

(1) veratrine has no anticholinesterase action (*Bacq* and *Brown*), and (2) the transmission is still demonstrable in the presence of curare (*Bacq*). It is quite probable that potassium ions are liberated in

larger amounts from the veratrinized muscle through its irregular contractions. In order to elucidate the above assumption, the following experiments were undertaken.

The hind limbs of Rana nigromaculata were perfused with normal *Ringer*'s solution or alternatively with veratrine $1:2\times10^5$ in *Ringer* (*Trendelenburg*'s method), the perfusate being collected from the abdominal vein. Direct electrical stimulations were applied to the hind limbs 60 times per minute. These perfusates were tested on the frog's rectus muscle preparation, which was treated as before with veratrine of $1:2\times10^5$ concentration.

Results (see Fig. 3):

Perfusates from non-stimulated normal and veratrinized frogs are ineffective. Perfusates from stimulated normal frogs and perfusates from non-stimulated veratrinized frogs are usually ineffective, but sometimes cause a slight contracture of the test muscle. Perfusates from stimulated veratrinized frogs always evoke a marked contraction of the rectus muscle preparation. The same contracture is observable when a potassium chloride of a definite strength is added to the perfusates from non-stimulated preparations as to the perfusates from a stimulated normal frog.

Discussion.

According to the opinion of *Szent-Györgyi*, veratrine stabilizes the so-called "substance de contraction" produced in stimulated muscle just as eserine does for the acetylcholine. This substance of contraction is believed to pass through the membrane of the muscle cell and to appear in the perfusate. It was determined by *Bacq* that the active agent is not acetylcholine, because, it is easily destroyed by cholinesterase (*Marnay* and *Nachmansohn*). Moreover, veratrine has no anticholinesterase action (*Bacq* and *Brown*), and the transmission is still demonstrable in the presence of curare (*Bacq*). I suppose that potassium ion is liberated from the muscle through electrical stimulation which causes the contraction of the rectus muscle preparation. This fact is proved experimentally, for the perfusate from the stimulated veratrinized preparation was the only one to cause the contracture of the rectus muscle, the other perfusates from normal and veratrinized frogs evoked similar contractions only by the addition of potassium chloride to the perfusates. How is it that only the perfusate from the stimulated veratrinized preparation can liberate a large amount of potassium ions? This may be explained as follows:

Cell membrane is usually impermeable to potassium ions. Veratrine increases the permeability of this membrane, making it possible for potassium ions to pass through it. This is the cause of the sensitizing action of veratrine. It may be concluded, therefore, that the chemical agent is potassium ions. The action of veratrine is to increase the permeability of the muscle cell membrane to potassium ions, which are liberated profusely by muscular contraction. All the phenomena involved in the above experiments are clearly explained on the assumption that veratrine increases the permeability of muscle cell membrane to potassium ions.

Summary.

1. Veratrine greatly sensitizes the action of potassium ions on frog's rectus muscle. Quinine inhibits the sensitizing action of veratrine.

2. Perfusate of the preparation from the stimulated veratrinized hind limbs evokes a contraction of the veratrinized test muscle.

3. The other purfusates of the preparations perfused with *Ringer*'s solution or with veratrine-*Ringer*'s solution do not affect the test muscle. But a similar contraction is observable if potassium chloride of a certain strength, is added to the perfusates.

4. It can be shown that the chemical agent causing the muscular contraction is potassium ions.

5. The above experimental results may be wholly explained by the assumption that veratrine increases the permeability of muscle cell membrane to potassium ions.

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