ABSENCE OF SECRETION OF ACETYLCHOLINE ON STIMULATION OF NERVES IN AN UNSTRIATED MUSCLE

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The views about chemical transmission at neuromuscular junctions are well known. It is generally believed that such transmission takes place by secretion of acetylcholine, adrenaline or noradrenaline. This idea of neurohumoral transmission has appeared unsatisfactory to some and acetylcholine has been assigned other roles besides its possible action at neuromuscular junctions. According to Nachmansohn (1955), the mechanisms of conduction and transmission are essentially similar. The rhythmic activity of the heart is in some way linked with the enzymatic synthesis of acetylcholine (Burn, 1950). Jullien, Ripplinger and Cardot (1954) believe that acetylcholine is just a product of metabolism which must be removed. They believe that acetylcholine has no motor function.

During estimations of acetylcholine in the stomach muscle of the frog, Rana tigrina, it was noticed that though the muscle contained considerable quantities of acetylcholine, none was detected in the eserinised saline in which the muscles were immersed and oxygenated at room temperature. It was therefore decided to investigate the liberation of acetylcholine in this muscle.

METHODS

These experiments were performed on the stomach muscle of the frog Rana tigrina. The animal was stunned with a blow on the head and rapidly pithed. The stomach was removed along with its nerves and its mucous membrane was gently removed avoiding any injury as far as possible. It was then aerated for 15 minutes in ordinary Ringer's solution and then in eserinised Ringer's solution for two hours. The muscles were removed and the acetylcholine extracted. The Ringer's solution in which the muscles were immersed was tested for acetylcholine.
For testing the effect of stimulation, the muscle along with its nerves was laid in a petri dish on a pair of electrodes and stimulated with strong induction shocks. It was then immersed in 12 c.c. of eserinised Ringer's solution in a beaker and aerated for one hour. Any fluid sticking to the petri dish was washed into the beaker. The aeration was done to allow any acetylcholine liberated to diffuse into the Ringer's solution.

To test the effect of potassium, the muscle was aerated as above and then immersed in a strong solution of potassium chloride, 0·15 M, for 10 minutes and then quickly washed to remove the excess of potassium chloride sticking to the muscle, so that it may not affect the frog's rectus or leech muscle, and then aerated for one hour in eserinised Ringer's solution. Such a strong solution of potassium chloride is a powerful stimulant of the muscle. The Ringer's solution was then tested for acetylcholine.

The effect of calcium was similarly tested, the muscle being immersed in 0·1 M calcium chloride for 15 minutes.

Acetylcholine in the muscle was estimated, using the frog's rectus by the method similar to that used by Richter and Crossland (1945) for brain tissue. After experimentation, the muscle was immediately transferred to a stoppered bottle kept in a freezing mixture, and was allowed to freeze. Eserinised Ringer's solution was prepared and brought to pH 4 by adding N/10 HCl. Twenty c.c. of this was heated to a temperature of 95–100° C. in a water-bath and the weighed muscle transferred to it. It was cut into very small pieces while it was kept at this temperature for 5 minutes. The mixture was decanted, allowed to cool and then centrifuged for 10 minutes. The deposit was stirred in a few c.c. of pH 4 eserinised Ringer's solution and centrifuged again. The supernatant fluid was collected, brought to pH 4, and kept in a refrigerator.

Before testing, the extract was brought to pH 7 to 7·4 with N/10 NaOH. The extract was further diluted with eserinised Ringer's solution, so that 1 c.c. of the extract was equivalent to 100 mg. of the tissue. The assay of acetylcholine was carried out on eserinised frog rectus and sensitive leech preparation against solutions of known acetylcholine concentrations, avoiding errors of sensitisation as suggested by Feldberg (1948).

**RESULTS**

The acetylcholine content of 10 muscles respectively was 5·9, 5·2, 5·1, 5·3, 4·7, 4·6, 6·7, 5·2 and 4·2 μg./g. of tissue. This is less than that found in mammalian smooth muscle. All of this was intracellular, as shown by immersion in Ringer's solution.