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# Transport of calcium into muscles in response to serotonin and other hormones

Earlier work from this laboratory[1-5] on the mechanisms of action of serotonin and of acetylcholine on smooth muscles has indicated that these hormones cause contraction because they cause the active transport of $Ca^{2+}$ into the cells. The hormone in the extracellular fluid combines reversibly and specifically with a special lipid in the cell membrane, forming a complex. Calcium also forms a complex with this lipid (the hormonal receptor substance), and competes with serotonin for it. On the intracellular side of the membrane is an enzyme for which the complex is the substrate. It attacks the lipoidal part of the complex, degrading it, and thus liberating the calcium and serotonin, which pass again into the aqueous phase but inside the cell. Resynthesis of the lipid by an energy-requiring enzymic reaction is believed to complete the cycle so that more calcium can be transported with the next sequence of degradation and resynthesis. In this way it was pictured that the calcium ions are able to pass across the permeability barrier in the cell membrane. The contraction of the muscle is then caused by the interaction of the calcium with actomyosin and ATP inside the cell.

Although the lipid and the enzyme have been extracted from muscles and nerves and purified[2,6] and their ability to cause transport of calcium has been demonstrated *in vitro* in cell-free systems, there has not been an experiment to show that when a muscle is caused to contract by application of the hormone, calcium actually is carried into the cells. The purpose of this paper is to describe such experiments. The hormones found to cause uptake of calcium were serotonin, acetylcholine and epinephrine.

The technique was very similar to that used by BIANCHI AND SHANES[7] to show that voluntary muscle (sartorius of the frog) picked up calcium when stimulated to contract by an electrical impulse. The method was adapted in the present work for use with a smooth muscle (rat uterus) stimulated hormonally. The uterus from an estrogenized rat[1] was removed and divided into the 2 horns. Each horn was slit longitudinally, blotted, weighed, and washed 10 times (10 min each time) with the modified Ringer's solution previously described[1]. One horn was then placed in 10 ml of the Ringer's solution in which the $CaCl_2$ was radioactive ($10^6$ counts/min of $^{45}CaCl_2$; total $CaCl_2$ 0.2 mg) and serotonin (0.3 $\mu$g) was then added. The other horn was treated similarly except that serotonin was omitted. 3 min later each tissue was washed 10 times with non-radioactive Ringer's solution and dry ashed with $HNO_3$. The ash was dissolved in HCl and its radioactivity was then determined in a Packard scintillation counter. The difference in radioactivity showed the amount taken up by the tissue in response to the hormone. The experiment was repeated 4 times.

The data in Table I show that both stimulated and unstimulated tissues picked up calcium from the solution, but that the stimulated ones took up more. The extra calcium carried into the tissue during a contraction induced by serotonin was, on the average, 2000 counts/min/g. This represented the transport of 0.14 $\mu$g $Ca^{2+}$/g of tissue per contraction.

The reason why the quiescent tissue as well as the stimulated one adsorbed calcium is easy to understand. The calcium was present in the lipids of the tissues (extractable by chloroform–methanol). As was shown previously[1] these lipids act as

ion exchangers which rapidly equilibrate with metallic ions in the extracellular fluid. The calcium was probably held as a lipid-soluble salt of the acidic lipids in which the metallic elements were in equilibrium with ions in the aqueous phase. The amount of calcium bound by the tissues of different rats varied, probably because of differences in lipid content, but for a single animal the two uterine horns were equal in this respect.

TABLE I

UPTAKE OF $^{45}Ca^{2+}$ BY ISOLATED UTERINE HORNS IN RESPONSE TO VARIOUS HORMONES

| *Hormone addition* | *Uptake by control tissue (counts/min/g)* | *Uptake by treated tissue (counts/min/g)* | *Difference due to hormone (counts/min/g)* |
|---|---|---|---|
| Serotonin | 15 500 | 17 500 | 2000 |
| Acetylcholine | 9 800 | 11 500 | 1700 |
| Epinephrine | 14 300 | 15 700 | 1400 |

To investigate whether other hormones likewise caused increased uptake of calcium, the experiments described above were repeated, but with acetylcholine (3 $\mu$g/10 ml) or epinephrine (0.3 $\mu$g) instead of serotonin. The data in Table I show that both of these hormones were effective.

The results of the experiments with serotonin and acetylcholine are compatible with the mechanism of action of the hormones described earlier. They are of course not sufficient to prove the hypothesis. The present findings also correspond well with the observations of GARATTINI *et al.*[8] who after the findings of WOOLLEY[1] showed that injection of serotonin into living rats resulted in increased uptake of calcium by certain tissues.

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