Serotonin receptors
II. Calcium transport by crude and purified receptor

Recent evidence indicates that the probable mechanism of action of serotonin is to bring about the passage of calcium ions through the cell membrane[1]. This passage of Ca^{++} and its reaction with actomyosin + adenosine triphosphate would explain why the hormone causes smooth muscles to contract. The chemical reactions involved are the combination of serotonin with a specific lipid in the cell membrane (the serotonin receptor), followed by the binding of Ca^{++} by the receptor-serotonin compound. The resulting calcium-containing complex is soluble in fat, and is thus able to diffuse through the lipoidal cell membrane. It is in this way that the lipid-insoluble Ca^{++} ion is made to penetrate the lipoidal membrane of the cell. Inside the membrane there seems to be an enzyme which attacks this complex, degrading it, and thereby liberating sertonin + Ca^{++} + degraded receptor[2].

The serotonin receptor has been extracted from smooth muscles and nerves[2], and has been recognized to be a lipid which combines specifically with serotonin, thereby causing it to become soluble in fat solvents. However, the actual transport of calcium in a cell-free system has not yet been demonstrated. The difficulty was that the relatively crude concentrates of the receptor lipid contained considerable calcium combined with the phosphatides so that the actual transport of calcium in response to additions of serotonin could not be clearly established[2]. By the use of radioactive Ca^{++} it has now been possible to show that the lipoidal receptor when combined with serotonin does actually pick up Ca^{++} and make it fat-soluble. When the complex is subsequently decomposed by addition of acid, Ca^{++} is liberated and becomes again water-soluble. Thus, *in vitro* in the absence of cells or cell particles the transport through a lipid phase of Ca^{++} as well as serotonin can be demonstrated. This can be done either with rather crude preparations of the receptor lipid, or with material purified by chromatographic fractionation of the crude extract.

To make these demonstrations, crude receptor was extracted from fresh hog duodenum with chloroform–methanol as described earlier[2]. The extract was concentrated rapidly at low temperature[2], and the resulting residue was suspended in dry chloroform. Anhydrous Na_2SO_4 was added, and the soluble portion was separated, and freed of chloroform under reduced pressure. The lipid mixture so obtained was called crude receptor. This material was purified by a series of adsorptions on columns of silicic acid according to a method about to be published. Purified receptor was thus obtained. It was not, however, a completely pure substance.

The cell-free, *in vitro* system previously used for demonstration of the transport of serotonin through a lipid solvent was used[2]. It consisted of a centrifuge tube containing 2.5 ml benzene, 2.5 ml *n*-butanol, and 4 ml water. The organic layer contained the receptor preparation, and the aqueous phase contained the salts (36 mg NaCl, 1.68 mg KCl, 7.0 mg $NaHCO_3$, 2.0 mg $CaCl_2$, and 100,000 counts (0.04 mg) $^{45}CaCl_2$). One such tube was used as control and a second which contained, in addition, 1 mg serotonin acted as the experimental system. The amount of receptor used in each experiment varied from 4 to 75 units. Additional controls were of course run, from which receptor or serotonin, or both were omitted. After each mixture had been shaken and thoroughly cleared by centrifugation, an aliquot of the organic layer was removed and shaken with 5 ml 0.2 *N* HCl. The aqueous acid extract was

washed with benzene to remove the last traces of lipid, evaporated, and the radioactivity counted. Results of a typical experiment are shown in Table I.

It can be seen that the presence of serotonin in this system resulted in the transport through the organic layer of considerable calcium in excess of that which was carried in the absence of the hormone. Note that in the absence of receptor the serotonin caused no transport of calcium. The calcium carried over in the presence of receptor, but in the absence of serotonin, probably was the result of nonspecific impurities in the receptor preparations. Thus, an impurity such as phosphatidic acid

TABLE I

^{45}Ca TRANSPORTED BY SEROTONIN AND ITS RECEPTOR

Serotonin mg	*Crude receptor units*	*Purified receptor units*	*Total counts/min*
0	0	0	0
1	0	0	0
0	28	0	4420
1	28	0	6330
0	0	5	1630
1	0	5	5740

would be expected to pick up Ca^{++} from the aqueous phase. The calcium phosphatidate would probably be soluble in the organic layer, and, when this was shaken with aqueous acid, would be converted to phosphatidic acid, and $CaCl_2$. The latter would extract into the final aqueous layer and be counted. Such nonspecific transport of calcium made it essential to show that the addition of serotonin actually brought about a decided increase in the calcium transported in this *in vitro* system. In the living cell, on the other hand, this problem probably does not arise because instead of acid, the cell uses the internal enzyme to decompose the calcium–serotonin–receptor complex. This enzyme presumably has specificity for its substrate, and would not be expected to attack such substrates as calcium phosphatidate.

The nonspecific binding of calcium by impurities in the receptor preparations made it essential to use the large amount of non-radioactive calcium specified in the directions. When the total calcium was reduced to less than 1 mg the differences in counts transported with and without serotonin were diminished. It was necessary to have enough total calcium present to saturate all of the nonspecific binding sites before a satisfactory demonstration of the action of serotonin could be made.

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