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FUNCTION OF PHOSPHOLIPIDS IN RECECPTORS

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Abstract—The rectus abdominis muscle of *Bufo viridis* loses its responsiveness to acetylcholine when extracted for 2 min with 25% acetone. Washing with frog Ringer solution slowly restores contractions. Addition of phosphatidylethanolamine or phosphatidylserine enormously speeds up the recovery of sensitivity towards acetylcholine. Lecithin is less active, while heparin, alpha-glycerophosphate and tween-20 are inactive. Qualitatively, the same holds true for the noradrenaline-induced contraction of rabbit-uterus.

This finding is quite similar to the acetone-induced inactivation and the phospholipid-borne reactivation of electron transport particles within the mitochondria. We, therefore, assume that neurohormones and other pharmacological agents exert their activity by modifying a transducer system present in the cell membrane.

THE NATURE of pharmacological receptors is still unknown. The action of neurohormones on various muscles is essentially a particular case of a stimulus-reaction-recovery phenomenon. Nobody has yet succeeded in reestablishing the above sequence by reconstituting a destroyed or extracted organic molecule of the cell, except McIlwain,[1] who showed that various phospholipids are capable of restoring the electrical excitation response of protamine-extracted brain slices. The present paper presents evidence that phospholipids are essential for the functioning of neurohormones.

MATERIALS AND METHODS

Dipalmitoyl phosphatidylethanolamine, phosphatidylserine and dipalmitoyl lecithin were obtained from Mann Research Lab. Inc. N.Y. (Catalog Nos. 4380, 5669, 2959). The phospholipid solutions were prepared by either sonication or dialysis.[2]

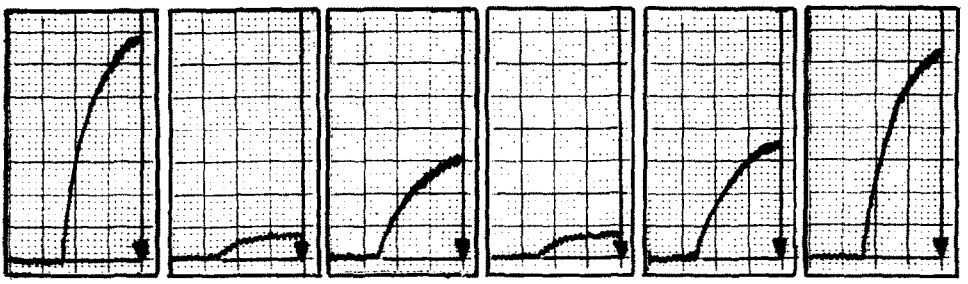
The experiments were carried out at room temperature in oxygenated frog Ringer on the rectus abdominis muscle of *Bufo viridis* toads. For the rabbit uterus, oxygenated Krebs-bicarbonate solution containing 1/3 the normal concentration of Ca^{++} (1 mM) and 3 times the usual quantity of K^+ (18 mM) was used. The rabbit donors should be adult (2·5 kg).

RESULTS

A. Acetylcholine

The rectus abdominis muscle of *Bufo viridis* was suspended in oxygenated frog Ringer solution at room temperature. After testing its responsiveness to a supramaximal dose (40 μg/ml) of acetylcholine (Fig. 1), the muscle was extracted for 2 min with 25 per cent acetone by replacing the bathing fluid by an acetone-water mixture

25/75 v/v. After 2 min the muscle was washed three times with frog Ringer solution to remove the acetone. These treatments eliminated the acetylcholine response (Fig. 2). Even after 1 hr during which the frog Ringer was changed every 10 min, only 50 per cent of the response was recovered (Fig. 3).



FIGS. 1–6. Reaction of rectus abdominis muscle of *Bufo viridis* to the following treatments in an organ bath:

1. 40 μg/ml acetylcholine.
2. The same 2 min after acetone treatment, followed by washing and immersion in frog Ringer solution.
3. The same one hr later.
4. Another preparation treated as under (1) and (2), 2 min after adding 2 μg/ml phosphatidylserine, washing with frog Ringer solution and repeated application of acetylcholine as under (1).
5. Another preparation treated as under (1) and (2), 2 min after adding 5 μg/ml phosphatidylserine, washing with frog Ringer solution and repeated application of acetylcholine as under (1).
6. Another preparation treated as under (1) and (2), 2 min after adding 20 μg/ml phosphatidylserine, washing with frog Ringer solution and repeated application of acetylcholine as under (1).

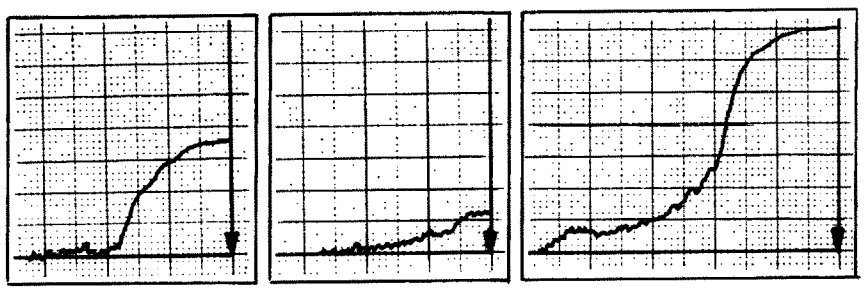
After removing the acetylcholine and washing, phosphatidylserine solution prepared by dialysis was added to the water bath for 2 min in various concentrations. Each time the phospholipid was washed out by frog Ringer solution. Figs. 4, 5 and 6 show that the acetylcholine response was immediately restored in full, depending on the dosage of phosphatidylserine added. Phosphatidylethanolamine, too, is able to restore the responsiveness of the receptor. Lecithin is somewhat less active, and up to 20 μg/ml never gave full recovery. On the other hand, glycerophosphate, heparin, tween-20 and olive oil emulsion are inactive up to 1 mg/ml.

It is intriguing that phospholipid solutions prepared by sonication are inactive even when tried 25 times the concentration (0·5 mg/ml) of the full activity of the solution prepared by dialysis. We attribute this to differences in micellar structure.

B. Noradrenaline

Next we tried to repeat these experiments with noradrenaline which allows examination in warm-blooded animals. We found that the rabbit uterus responds to noradrenaline quite evenly and reproducibly, provided the concentration of K^+ in the organ bath is elevated and the concentration of Ca^{++} is lowered, as described under Materials and Methods. Under these conditions, 20–40 μg/ml noradrenaline evoke maximal contraction of the rabbit uterus.

Figure 7 shows the response to 100 μg/ml noradrenaline—a supramaximal dose. Figure 8 represents the action of the same concentration of noradrenaline after treatment with 25/75 acetone-water as described before. After 3 times washing with Krebs-bicarbonate solution, a phosphatidylethanolamine solution, prepared by dialysis, was added to obtain a final concentration of 20 μg/ml. After 2 min the phos-



FIGS. 7–9. Reaction of adult rabbit uterus to the following treatment in an organ bath:

7. 100 μg/ml noradrenaline.
8. The same after acetone treatment, followed by washing and immersion in Krebs-bicarbonate solution (see Methods).
9. The same after adding 20 μg/ml phosphatidylethanolamine, washing with Krebs-bicarbonate solution and challenge by 100 μg/ml noradrenaline.

pholipid was washed out with Krebs-bicarbonate solution and the preparation was again challenged by 100 μg/ml noradrenaline (Fig. 9). The response was more than 100 per cent of the control. We must remark here that it is very difficult to repeat the experiment on the same uterus, and only 1 or 2 experiments succeed out of ten trials, whereas with acetylcholine and frog rectus abdominis half the trials are successful. This is probably due to the great endurance of the tissue taken from cold-blooded animals.

DISCUSSION

Phospholipids are required for the action of all units of the electron transport system and for oxidative phosphorylation. These lipids can be extracted by acetone-water mixture resulting in inactivation. By reconstituting the lipids, irrespective of whether they are the same ones or of different origin, activity is restored.[3–5] The uptake of ions in the mitochondria—especially that of calcium which in the muscle is supposed to be the link between the membrane, excited by a pharmacological agent and the resulting contraction—is strongly dependent upon the function of an electron transport chain.[6] Vice versa, calcium is known to modify the electron transport and oxidative phosphorylation. The presence of an electron transport chain and of chemical-osmotic transducers is by no means unique for the mitochondria. The cell membranes themselves, just as the microsomes, contain such systems.[7]

The main problem concerns the role of the phospholipid. We cannot assume that it is just a barrier for the ions, since during the short acetone-water treatment we did not obtain contraction of the muscles, as was to be expected when a barrier for the

calcium entry is destroyed. It is equally difficult to explain its role as a diffusible carrier. Most probably it provides the necessary hydrophobic region for the electron transport and transducer effect[7] or it is required for some kind of structural change in the membrane protein.

Our working hypothesis is that drug action modifying or causing a stimulus-reaction-recovery sequence is dependent primarily on the function of some kind of an electron transport-transducer system present in the cell membrane which could be, but need not be, an ATP-ase. Such "receptor" is not necessarily a certain target or site, but could be simulated by a multiple pattern of interaction, promoting quantitative modifications of coupling, substrate supply, state of oxidation, etc. Different patterns represent different drugs and different actions of the same drug would then be dependent on the initial state of the system.

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Note added in proof: D. W. WOOLLEY and B. W. GOMMI, *Nature* **202**, 1074 (1964), have recently shown the decisive importance of phospholipids for the action of serotonin on its receptor. They approached the problem by enzymatic destruction of the lipid structure.

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