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On the lack of effect of acetylcholine on phosphoprotein metabolism in the salt gland of the sea gull

HEALD and his collaborators (see review by HEALD[1]) found that after brief periods of stimulation of brain-cortex slices with electrical pulses the incorporation of ^{32}P into phosphoprotein (measured as *O*-phosphoserine after acid hydrolysis) was increased about 30%. AHMED AND JUDAH[2] reported that omission of Na^+ from the incubation medium inhibited the incorporation of ^{32}P into the phosphoprotein of liver slices before it inhibited the incorporation of ^{32}P into A.T.P. On the basis of their respective findings both groups of workers suggested that phosphoproteins may play a role in sodium transport. The salt gland secretes NaCl in response to acetylcholine[3]. HOKIN AND HOKIN[4] showed that if slices of the albatross salt gland were stimulated with acetylcholine there was no increase in the incorporation of ^{32}P into a phosphoprotein fraction under conditions in which there was a marked increase in the

TABLE I

EFFECTS OF ACETYLCHOLINE (WITH ESERINE) ON THE INCORPORATION OF ^{32}P INTO VARIOUS PHOSPHATE COMPOUNDS IN THE GOOSE SALT GLAND

Sea-gull salt-gland slices were incubated for 65 min in 1.0 ml of bicarbonate saline[8] containing 0.1% glucose. 0.1 ml of a mixture of acetylcholine and eserine was then added to give the final indicated concentrations, and 0.1 ml of water was added to the controls. The slices were incubated for a further 20 min, then removed from the medium, blotted, and frozen. The radioactivities in the various substances were determined as described elsewhere[4]. All counts were corrected to a specific of 10^6 counts/min/μg of orthophosphate P in the medium. All values are averages of quadruplicate slice incubations.

Compound	Radioactivity: Control	Radioactivity: With 3 μM acetylcholine + 0.1 mM eserine	Increase (%)
	(counts/min/μg of phosphoserine P)		
Phosphoprotein	863	876	+ 2
	(counts/min/mg of fresh tissue)		
Phosphatidic acid	209	1 160	+457
Phosphatidyl inositol	194	582	+200
Phosphatidyl choline	344	354	+ 3
Phosphatidyl ethanolamine	105	131	+ 25
Total lipids	1 120	2 620	+135
Orthophosphate	28 000	25 000	− 7
7-min acid-hydrolyzable phosphate esters	6 520	4 340	− 33
Stable organic phosphate esters	53 200	54 100	+ 2

incorporation of ^{32}P into phosphatidic acid and a lesser increase in the incorporation of ^{32}P and [2-^{3}H]inositol into phosphatidyl inositol. The phosphoprotein fraction was measured as the inorganic phosphorus released on alkaline hydrolysis of the protein residue which remained after extraction of the phospholipids and "phosphatidopeptides". Since protein-bound phosphoserine is a more accurate measure of phosphoprotein, in the present investigation phosphoserine was isolated from the protein.

Sea-gull salt-gland slices were incubated with $^{32}P_i$ for 65 min, acetylcholine (3 μM) and eserine (0.1 mM) were then added, and the incubation was continued for a further 20 min. The protein residue obtained after extraction of the phospholipids

and the "phosphatido-peptides"[4] was mixed with *O*-phosphoserine (1 μg phosphoserine-P/mg of the original tissue) and hydrolyzed in 2.0 ml of 2 N HCl in a sealed ampcule in the autoclave for 10 h. The hydrolysate was centrifuged, the residue was washed twice with water, and the combined supernatant fluids were dried *in vacuo*. The residues were redissolved in water and submitted to electrophoresis on paper in pyridine–acetic acid[5] on Whatman No. 3 MM paper for 2 h at 1000 V. The electropherograms were autoradiographed and then stained by the method of WADE AND MORGAN[6]. The most intensely labelled spot was one which ran with the mobility of orthophosphate. The second most intensely labelled spot was one which ran with the same mobility as a sample of phosphoserine which was run alongside. The phosphoserine spot revealed by autoradiography coincided with that revealed by staining. Based on a mobility of 1.00 for orthophosphate the phosphoserine spot had a relative mobility of 0.76. Five other more faintly labelled spots were also detected which had relative mobilities of 0.63, 0.44, 0.35, 0.19 and 0.10. The phosphoserine spot was cut out, eluted in 1 N NH_4OH overnight, and radioactivity and total phosphorus[7] were determined on appropriate aliquots. Although the usual type of stimulation of incorporation of ^{32}P into phosphatidic acid and phosphatidyl inositol occurred in response to acetylcholine, there was no significant change in the specific activity of the phosphoprotein fraction, measured as phosphoserine (Table I). The amount of ^{32}P found in intracellular orthophosphate and in the stable organic phosphate fraction in the salt gland was not affected by acetylcholine. The amount of radioactivity in the 7-min acid-hydrolyzable phosphate fraction was diminished. At the time intervals used here the 7-min hydrolyzable phosphate of ATP is in isotopic equilibrium with the tissue orthophosphate; this fall in total radioactivity is due therefore to a fall in the level of ATP and not to a change in specific activity of the ATP. These results indicate that in the salt gland stimulated to secrete NaCl, phosphoproteins do not undergo a type of reaction which leads to increased incorporation of ^{32}P into the fraction, in contrast to phosphatidic acid and phosphatidyl inositol. This does not rule out the possibility that phosphoproteins may participate in sodium transport in the salt gland. But one might have expected some change on stimulating the salt gland tissue if the explanations advanced by HEALD[1] and by JUDAH AND AHMED[2] to explain the changes in phosphoprotein labelling which they observed are correct.

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[1] P. J. HEALD, *Nature*, 193 (1962) 451.
[2] K. AHMED, AND J. D. JUDAH, *Biochim. Biophys. Acta*, 57 (1962) 245.
[3] K. SCHMIDT-NIELSEN, *Circulation*, 21 (1960) 955.
[4] L. E. HOKIN, AND M. R. HOKIN, *J. Gen. Physiol.*, 44 (1960) 61.
[5] R. M. C. DAWSON, *Biochem. J.*, 75 (1960) 45.
[6] H. E. WADE AND D. M. MORGAN, *Nature*, 171 (1953) 529.
[7] G. R. BARTLETT, *J. Biol. Chem.*, 234 (1959) 466.
[8] H. A. KREBS AND K. HENSELEIT, *Z. Physiol. Chem.*, 210 (1932) 33.

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