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Phosphoproteins and ion transport of cerebral cortex slices

HEALD[1] has recently discussed the possibility that phosphoproteins of cerebral cortex may play an important role in sodium transport of that tissue, basing his views on the observation that application of electrical stimulation to the slices for brief periods resulted in marked stimulation of ^{32}P turnover in the phosphoprotein fraction without any significant change in the level of this fraction. In the present communication we present results which support this view.

The general procedure of electrical stimulation was essentially that of HEALD except that rat-brain-cortex slices were used instead of guinea-pig cortex, and glycylglycine substituted for Tris buffer in the medium which consisted of 134 mM NaCl, 6.74 mM KCl, 1.34 mM $MgSO_4$, 2.8 mM $CaCl_2$, 50 mM glycylglycine (pH 7.4) and 6 mM glucose. The gas phase was oxygen. The cortex slices were allowed to incubate for 30 min at 37.5° in this medium. At the end of this incubation, carrier-free $^{32}P_i$ was added to the flasks and a contact of exactly 90 sec was allowed. The slices were then removed, rinsed and transferred to fresh incubation medium for 60 sec after which pulses were applied for 15 sec when the experiment was ended.

In Table I we show the effects of 10 and 20 μM ouabain, a well-known inhibitor of ion transport. The drug was introduced after the incubation with $^{32}P_i$ and rinsing, and was thus in contact with the slices for only 60 sec before application of electrical stimulation. The slices were pre-labeled with ^{32}P before they were allowed to come into contact with the drug. It is clear from the table that ouabain prevents the effect of electrical pulses on phosphoprotein radioactivity at both 10 and 20 μM. On the other hand the control shows a very well marked increase.

CUMMINS AND MCILWAIN[2] have demonstrated that increased flux of sodium and potassium on the passage of electrical pulses through brain cortex slices is a primary effect and not secondary to increased respiratory activity of the tissue under these conditions. Thus glutamate, when used as substrate, can maintain ionic gradient in the unstimulated tissue and permits increased flux in the stimulated tissue although

TABLE I

EFFECT OF OUABAIN ON PHOSPHOPROTEINS OF ELECTRICALLY STIMULATED CORTEX SLICES

The phosphoprotein specific activity is corrected to an ATP specific activity of 10^5 counts/min per μmole P in order to correct for small variations in radioactivity. In Expt. 1 the figures are based on alkali-labile P and Expt. 2 on phosphorylserine derived from the proteins after addition of 10 μmoles of carrier phosphorylserine[5]. The experimental conditions have been described in the text and the equipment for applying pulses was essentially the same as described by WALLGREN AND KULONEN[9].

Expt.	*Conditions*	*Ouabain (M)*	*Counts/min/μmole P in phosphoprotein*	*Increase (counts/min/μmole P)*	*Inhibition by ouabain (%)*
1	Unstimulated	—	7600	—	—
	Unstimulated	10^{-5}	7200	—	5.0
	Stimulated	—	20 200	12 600	—
	Stimulated	10^{-5}	13 000	5800	56.0
2	Unstimulated	—	3670	—	—
	Unstimulated	$2 \cdot 10^{-5}$	4700	—	0
	Stimulated	—	7500	3830	—
	Stimulated	$2 \cdot 10^{-5}$	4900	200	95.0

no change in respiration is observed on application of electrical pulses. We used this system to study the response of the phosphoprotein fraction and the effect of ouabain on it. Glucose in the medium was replaced by 20 mM glutamate, all other experimental conditions being as before. It was found that application of electrical pulses still increased the phosphoprotein turnover and again the increase in turnover was abolished by 20 μM ouabain used exactly as described earlier in the text.

TABLE II

EFFECT OF SUBSTITUTION OF Na^+ BY Li^+ ON PHOSPHOPROTEINS

Conditions as in the text.

Medium	*Stimulation*	*Counts/min/μmole P in phosphoprotein*	*Increase (counts/min/μmole P)*	*Increase (%)*
Na^+	−	8350	—	
Li^+	−	8500	—	
Na^+	+	12 350	4000	48.0
Li^+	+	9000	500	6.0

Table II shows the effect of replacing Na^+ by Li^+ on phosphoprotein turnover. The experiment was performed by equilibrating the slices in the glycylglycine–glucose medium (described above) for 45 min at 37.5°. $^{32}P_i$ was then introduced for 90 sec as before, after which slices were rinsed briefly and transferred either to the usual medium or to the medium in which Na^+ had been replaced by Li^+ (on equimolar basis) for a further period of 60 sec. As before, stimulation was applied for 15 sec and the experiment ended. It is plain that substitution of Li^+ for Na^+ in the medium abolishes the response of phosphoproteins to electrical stimulation.

The following conclusions emerge from these experiments. Firstly, the increase in phosphoprotein turnover specifically depends on the presence of Na^+ in the medium, as shown by the Li^+ experiment. The effect of Li^+ is unlikely to be due to secondary factors (*e.g.*, inhibition of O_2 uptake) since the slices were pre-labelled with tracer and the contact with Li^+ was very brief. Secondly, the experiment when glutamate is substituted for glucose indicates that phosphoprotein response to electrical stimulation is independent of respiration. Thirdly, the effect of ouabain at low concentrations on phosphoprotein turnover supports the view that phosphoprotein turnover and sodium transport are related. In our experiments the drug was in contact with the tissue very briefly. This is important since WERMAN[3] has shown that ouabain (10 μM) inhibits respiration of brain-cortex slices, but the drug was in contact with the slices for a long time. On the other hand, SCHWARTZ[4] has shown that if the slices are preincubated for 30 min the addition of ouabain causes an increase of respiration rather than an inhibition. It is important to note that the drug is as effective when glutamate is used as substrate instead of glucose. It is, therefore, to be concluded that the inhibition of electrically stimulated phosphoprotein turnover by ouabain is a consequence of the effect of this drug on sodium transport.

Recently it has been claimed that ouabain has no effect on phosphoproteins of cerebral cortex slices[4]. However, the experiments were of long duration (60 min incubation) and there was no attempt to correlate the results with the rate of ion transport. Our experiments emphasize the fact that a proper choice of conditions is essential

to demonstrate an action of ouabain on phosphoprotein metabolism. Since it has been found[7,8] that phosphorylation and dephosphorylation of these molecules appear to be dependent upon Na^+ or K^+, respectively, and since both are blocked by ouabain, it is obvious that under certain conditions no effect of ouabain would be observed. This might be due to the complex interactions of phosphoprotein, Na^+, K^+ and drug.

It is to be noted that the results of the present work have been obtained both by the assay of alkali-labile P as a measure of the phosphoprotein P and also by chromatographic separation of radioactive phosphorylserine from the protein residues of the tissue. Our previous conclusions that phosphoproteins are concerned with sodium transport[5-8] are thus further strengthened.

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The effect of urea and guanidine on the helix content of poly-N^5-(3-hydroxypropyl)-L-glutamine in aqueous-solvent systems

The remarkable changes in optical-rotatory properties of proteins during denaturation, brought about by agents such as acid, alkali, urea and guanidine, have been interpreted in terms of decrease in helical content of the protein[1]. It was, therefore, of interest to investigate whether the above denaturing agents are capable of influencing synthetic polypeptides in a similar way. Only few data on the effect of urea on optical-rotatory properties of polyamino acids have been published[1-3]. DOTY AND GRATZER[4] have reported recently that the helicity of a poly-L-alanine segment in a block copolymer in which the central block consisted of L-alanine residues and the two flanking blocks of DL-glutamic acid residues, dropped from 92% in water to 70% in 8 M urea. KULKARNI AND BLOUT[5] have shown that a copolymer of L-alanine