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EFFECTS OF INSULIN ON MONOSACCHARIDE TRANSPORT AND INCORPORATION OF AMINO ACIDS INTO PROTEIN IN DIAPHRAGM DIFFERENTIATED WITH PHLORIZIN

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(Received February 8th, 1960)

SUMMARY

The effects of phlorizin on monosaccharide transport, amino acid transport and amino acid incorporation into protein have been compared in isolated rat diaphragm. Conditions have been found under which phlorizin inhibits the uptake of glucose and membrane transport of D-xylose and D-galactose and the effect of insulin on these processes without affecting incorporation of [^{14}C]glycine into protein and the stimulation of this process by insulin. The results provide further evidence for the view that effects of insulin on amino acid incorporation are not dependent upon an effect of the hormone on carbohydrate metabolism.

INTRODUCTION

Insulin *in vitro* increases both uptake of glucose and incorporation of [^{14}C]amino acids into protein by isolated rat diaphragm muscle. There are points of evidence to

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suggest that the influence of insulin on amino acid incorporation is direct and not brought about through an effect on uptake of glucose[1-3]. In the present investigation further evidence in support of this view has been obtained by comparing effects of phlorizin on uptake of glucose and membrane transport of D-xylose and D-galactose and on incorporation of [^{14}C]glycine into protein and membrane transport of α-[^{14}C]aminoisobutyric acid and [^{14}C]glycine in isolated rat diaphragm.

METHODS

The methods and procedure for the preparation and incubation of intact and cut diaphragm preparations and for measurement of glucose uptake and xylose and galactose spaces are described elsewhere[4,5]. For measurement of xylose and galactose spaces intact diaphragms were incubated for 30 min at 37° in a bicarbonate buffered salt solution[6] pH 7.4 gassed with $O_2 + CO_2$ (95:5), containing either D-xylose or D-galactose at concentrations of 8.3 m*M* and 25 m*M* respectively. Uptake of glucose by the cut diaphragm preparation was measured by the disappearance of glucose from the medium (originally 2.5 mg/ml). Amino acid incorporation was measured by the method of MANCHESTER AND YOUNG[1], diaphragm being incubated for 2 h in the presence of [1-^{14}C]glycine (1 m*M* and specific activity of about 400 μC/mmole). Amino acid accumulation, *i.e.* the ratio of the concentration of the free labelled amino acid in the tissue to that in the medium, was measured as described by MANCHESTER AND YOUNG[7]. Phlorizin (British Drug Houses, Ltd.) was recrystallized twice from hot water before use, and dissolved directly in the medium. A five-times recrystallized sample of insulin (Boots Pure Drug Co., Ltd.) was dissolved in *N*/300 HCl to yield a stock solution containing 20 units/ml which was diluted to 0.1 unit/ml with buffer shortly before each experiment.

RESULTS

The effects of phlorizin on uptake of glucose, incorporation of [^{14}C]glycine into diaphragm protein and intracellular accumulation of xylose and galactose are shown in Tables I and II. The intracellular accumulations of D-xylose and D-galactose by the

TABLE I

EFFECT OF PHLORIZIN ON THE ACCUMULATION OF D-XYLOSE AND D-GALACTOSE BY THE INTACT DIAPHRAGM PREPARATION

Each figure is the mean ± S.E. of the mean of six observations. The value of P for a difference which is significant is indicated. Insulin was present at a concentration of 0.1 unit/ml in each instance.

Sugar added to the medium	*Phlorizin (3 mM)*	*Intracellular xylose or galactose space (ml/100 g of wet muscle)*	
D-xylose	—	28 ± 2.4	
	+	15 ± 1.8	
		Difference — 13 ± 3.0	(P < 0.01)
D-galactose	—	28 ± 2.6	
	+	18 ± 2.0	
		Difference — 10 ± 3.3	(P < 0.01)

TABLE II

EFFECT OF PHLORIZIN ON THE UPTAKE OF GLUCOSE AND INCORPORATION OF [^{14}C]GLYCINE INTO PROTEIN BY ISOLATED RAT DIAPHRAGM, BOTH IN THE PRESENCE AND ABSENCE OF INSULIN

Each figure is the mean ± S.E. of the mean of six observations. The value of P for a difference which is significant is indicated. The effect of insulin is significant in each instance ($P < 0.001$).

Addition to medium	*Glucose uptake (mg/g wet wt/h)*		*Radioactivity in diaphragm protein (counts/min/disc)*	
	No insulin added	*Insulin added (0.1 unit/ml)*	*No insulin added*	*Insulin added (0.1 unit/ml)*
Intact diaphragm				
No addition				505 ± 12
Phlorizin (3 m*M*)				493 ± 19
Cut diaphragm				
No addition	2.75 ± 0.10	6.30 ± 0.25	383 ± 12	582 ± 16
Phlorizin (3 m*M*)	2.06 ± 0.09	3.60 ± 0.09	338 ± 9	484 ± 17
	$P < 0.001$	$P < 0.001$	$P < 0.02$	$P < 0.01$
No addition	1.78 ± 0.06	4.45 ± 0.17	404 ± 26	622 ± 30
Phlorizin (1 m*M*)	1.14 ± 0.08	3.21 ± 0.11	424 ± 30	646 ± 19
	$P < 0.001$	$P < 0.001$		

intact diaphragm (measured in the presence of insulin) were reduced by 50 % and 30 % respectively in the presence of phlorizin (3 m*M*) (Table I). The incorporation of [^{14}C]glycine into protein in the intact diaphragm preparation (measured in the presence of insulin) was unaffected by phlorizin (3 m*M*) (Table II). Uptake of glucose by the cut diaphragm preparation was inhibited by phlorizin (3 m*M*) 25 % in the absence and 40 % in the presence of insulin. The insulin effect on glucose uptake was thus inhibited 60 % by 3 m*M* phlorizin (Table II). Incorporation of [^{14}C]glycine by the cut diaphragm preparation was inhibited 12 % by 3 m*M* phlorizin in the absence and 17 % in the presence of insulin. The insulin effect on glycine incorporation was thus inhibited 25 % (Table II). Phlorizin at 1 m*M* concentration inhibited uptake of glucose by the cut diaphragm preparation 30 % in the absence and 25 % in the presence of insulin (22 % inhibition of the insulin effect) (Table II). At this concentration phlorizin had no effect on glycine incorporation in the cut diaphragm preparation in the presence or absence of insulin. These results show that phlorizin can inhibit uptake of glucose and membrane transport of D-xylose and D-galactose in isolated diaphragm, and also effects of insulin thereon, under conditions in which it has no demonstrable effect on incorporation of [^{14}C]glycine into protein.

The incorporation of labelled amino acids into diaphragm protein involves both transport of the amino acid across the cell membrane and its participation in peptide bond formation. The rate of entry of labelled amino acids from the medium into diaphragm appears to be a rapid process[7] and it appears not to be the rate-limiting step in the overall process of transfer of labelled amino acids from the medium into diaphragm protein[7–9]. Because it was therefore possible that phlorizin might inhibit amino acid transport in diaphragm without inhibiting the overall process of amino acid incorporation into protein, the effects of phlorizin on the accumulation of glycine and α-aminoisobutyric acid in diaphragm were also investigated.

Phlorizin (3 m*M*) was found to depress the accumulation both of [^{14}C]glycine and of α-[^{14}C]aminoisobutyric acid by the cut diaphragm preparation (Table III),

TABLE III

EFFECT OF PHLORIZIN (3 m*M*) ON THE ACCUMULATION OF [^{14}C]AMINO ACIDS BY INTACT AND CUT RAT DIAPHRAGM PREPARATIONS

Each figure is the mean ± S.E. of the mean of six observations.

Amino acid added to the medium	*Ratio:* $\frac{\text{Concentration of free labelled amino acid in tissue water}}{\text{Concentration of free labelled amino acid in incubation medium}}$		*Significance of difference (P)*
	No phlorizin added	*Phlorizin added*	
Intact diaphragm preparation			
α-[1-^{14}C]aminoisobutyric acid	0.77 ± 0.021	0.76 ± 0.026	N.S.*
[1-^{14}C]glycine	1.61 ± 0.11	1.56 ± 0.063	N.S.*
Cut diaphragm preparation			
α-[1-^{14}C]aminoisobutyric acid	2.35 ± 0.065	1.92 ± 0.068	= 0.001
[1-^{14}C]glycine	3.83 ± 0.099	3.34 ± 0.075	< 0.001

* Not significant ($P > 0.1$).

but it had no significant effect on the accumulation of these amino acids by the intact preparation (Table III). Thus under conditions in which incorporation of glycine into protein is unaffected by phlorizin, there appears equally to be no effect of phlorizin on amino acid transport; on the other hand, under comparable conditions phlorizin markedly depresses the transport of glucose, xylose and galactose.

DISCUSSION

The present results show that, whereas phlorizin inhibits monosaccharide transport in both the cut and the intact diaphragm preparation, it only inhibits amino acid transport and incorporation into diaphragm protein in the cut preparation. An important difference between the cut and intact diaphragm is that substances which are confined to extracellular water in the latter preparation appear to penetrate muscle cells in the former, possibly through the cut ends of muscle fibres[4,10]. In the intact diaphragm phlorizin appears to be confined to extracellular water[5]. This would suggest that its effects on monosaccharide transport are exerted at the surface of the muscle cell and, conversely, that it has no effect on amino acid transport or incorporation at this locus. If, as seems probable, phlorizin penetrates the cut diaphragm preparation, then its effects on amino acid transport and incorporation in this preparation may result from an intracellular action. KELLER AND LOTSPEICH[11] have claimed that phlorizin inhibits oxidative phosphorylation in isolated mitochondria. Because amino acid transport and incorporation into protein are dependent upon energy-rich phosphate, effects of phlorizin on these metabolic processes could be secondary to an effect of phlorizin on oxidative phosphorylation in mitochondria in the cut diaphragm preparation.

The results presented here provide a clear demonstration of the separate nature of the effects of insulin on monosaccharide transport and on amino acid incorporation. They support the view previously advocated[1-3] that stimulation by insulin of amino acid incorporation into diaphragm is not dependent upon an effect of the hormone on glucose uptake. The evidence for this view may be briefly summarized:

1. Insulin *in vitro* stimulates incorporation of labelled amino acids into the protein

of isolated diaphragm from the normal[1,2], hypophysectomized[3], adrenalectomized[3] or alloxan-diabetic[12] rat. Such an effect is equally visible whether glucose is present in the medium or not. Addition of glucose or pyruvate does not produce an observable augmentation of incorporation, nor does the presence of these substances modify the size of the insulin effect[1,2,13].

2. Hypophysectomy augments, and treatment of the hypophysectomized or intact rat with pituitary growth hormone diminishes, the magnitude of the stimulation of glucose uptake by isolated diaphragm produced by insulin, whereas these same operations either have no effect or produce slightly the reverse effect on the sensitivity of isolated diaphragm to an effect of insulin on amino acid incorporation —that is, hypophysectomy may decrease, and treatment with growth hormone increase, the size of the effect of insulin on this process[3].

At first sight acceptance of these views implies that insulin has two separate and distinct effects on muscle metabolism—on protein synthesis and on monosaccharide transport respectively. However, if, as has been suggested by RANDLE AND SMITH[4,14], insulin accelerates transport of glucose in diaphragm by restricting the access of energy-rich phosphate to a process concerned with the regulation of glucose transport, it may well be that this same action of insulin makes energy-rich phosphate more freely available to processes involved in protein synthesis. But at the present time such an hypothesis is purely conjectural.

ACKNOWLEDGEMENTS

We are grateful to Professor F. G. YOUNG, F.R.S., for much helpful advice and encouragement.

This work has been aided by grants from the Medical Research Council (to P.J.R) and from the U.S. Public Health Service (to F.C.B). The first author is a U.S. Public Health Service Post-doctoral Fellow.

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