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In vitro Stimulation by Insulin of α-Amino-isobutyric Acid Transport in the Absence of Protein Synthesis

Since the demonstration by Kipnis and Noall[1] that insulin stimulates the accumulation of the non-utilizable amino-acid α-amino-isobutyric acid (AIB) by cells of the intact rat diaphragm *in vitro*, attempts have been made to relate this phenomenon to the action of insulin on protein synthesis. A review of the available evidence led Manchester and Young[2] to the conclusion that the action of insulin on accumulation of amino-acid by the diaphragm of the rat is probably limited to non-utilizable amino-acids and that it may represent an effect secondary to a direct stimulation of protein synthesis. In their view, AIB accumulation "can be regarded as filling the gap left by amino-acids used up in the synthesis of extra protein which occurs in the presence of insulin". The more recent observation[3] that insulin increases the transport of a number of naturally occurring amino-acids into muscle cells prompted us to re-examine the possibility that the effect of insulin on AIB accumulation may be a direct action of the hormone on an amino-acid transport system.

The conditions used for the investigation of AIB-^{14}C transport and amino-acid incorporation into protein in the 'intact' hemidiaphragm of the rat have been described in detail[4,5]. Briefly, normal female rats (Charles River strain) weighing 60–70 g were killed by dislocation of the cervical vertebræ, their diaphragms excised with rib cage attached and bisected through the central ligament and through the insertions to the xiphoid process and spine. The 'intact' hemidiaphragms thus obtained were incubated in Krebs bicarbonate buffer gassed with 5 per cent carbon dioxide and 95 per cent oxygen and containing glucose (10 mM). At the termination of incubation the diaphragms were dissected from surrounding tissue, blotted, weighed and the radioactivity of the tissue water and the purified proteins determined by liquid scintillation spectrometry. The extracellular space was measured by the distribution of insulin-1-^{14}C. Extracellular space of the tissue was determined for each control and experimental situation utilized in this investigation and the results expressed as the concentration ratio of AIB-^{14}C

$$\left(\frac{\text{c.p.m./ml. intracellular water}}{\text{c.p.m./ml. incubation medium}}\right).$$

Bovine insulin (Lilly *PJ*-4609, 23·8 u/mg) was added to the incubation medium at a concentration of 0·1 u/ml. Protein synthesis in the isolated diaphragm was blocked by the addition of puromycin[6] (500 μg/ml.). This concentration was chosen because it maximally depressed the incorporation of uniformly labelled ^{14}C-valine into diaphragm protein (Table 1). Similar findings have been obtained with ^{14}C-histidine, ^{14}C-alanine and ^{14}C-leucine in diaphragms of normal and hypophysectomized rats.

The results are presented in Table 2. It may be seen that puromycin at a concentration which essentially abolishes amino-acid incorporation into protein did not depress the accumulation of AIB in the intracellular compartment of control diaphragm muscle nor did it influence in any way the marked stimulation of uptake of AIB effected by the simultaneous addition of insulin to the medium.

Table 1. Effect of Puromycin on the Incorporation of ^{14}C-Valine* into Protein of 'Intact' Rat Hemidiaphragm *in vitro*

	Time of incubation (min)			
	45	90	120	180
	Specific activity (c.p.m./mg protein)			
Control	189·1 ± 25·9 (3)†	229·3 ± 7·1 (11)	331·0 ± 22·3 (3)	482·0 ± 81·0 (3)
Puromycin (500 μg/ml.)	3·5 ± 0·1 (6)	3·5 ± 0·2 (12)	3·3 ± 0·2 (6)	3·6 ± 0·2 (6)

* 0·1 mM (0·05 μc./ml.)
† Mean ± *S.E.* No. of observations in parentheses.

Table 2. Effect of Insulin and Puromycin on the Accumulation of ^{14}C-AIB* in the Intracellular Water of 'Intact' Rat Hemidiaphragm *in vitro*

Additions		Time of incubation (min)		
Insulin	Puromycin	90	120	180
		Concentration ratio		
–	–	1·48 ± 0·10 (12)†	1·60 ± 0·09 (13)	2·10 ± 0·20 (12)
–	+	1·53 ± 0·09 (6)	1·62 ± 0·19 (7)	1·81 ± 0·05 (6)
+	–	2·63 ± 0·09 (12)	3·55 ± 0·16 (12)	4·16 ± 0·17 (12)
+	+	2·50 ± 0·09 (6)	3·34 ± 0·22 (6)	4·11 ± 0·15 (6)

* 0·05 mM, 0·025 μc./ml.
† Mean ± *S.E.* No. of observations in parentheses.

These experiments clearly demonstrate that the stimulation by insulin of AIB accumulation in muscle cells of the isolated intact rat diaphragm is not secondary to the stimulation of protein synthesis and that it may represent a primary action of the hormone. Whether this conclusion is also applicable to naturally occurring amino-acids remains to be determined. It may be of interest that the stimulation of AIB transport by growth hormone in diaphragms of hypophysectomized rats is similarly not abolished by the action of puromycin[7].

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Precocious Organic Iodine Secretion in the Blood by the Thyroid Gland

While examining the variation in time of plasmatic PB^{131}I (normal human subject) after ingestion of an Na^{131}I as a tracer, we have found, in some cases, the existence of a radioactivity 'peak' during the first few hours after ingestion. The phenomenon is repeated, whatever the method of separation of the plasmatic proteins: precipitation with trichloroacetic acid; fractioning by ion-exchanger resin[1], or in a 'Sephadex' column[2]. Fig. 1 illustrates a typical case.

Assuming that the occurrence is not due to some form of artefact, it would appear logical to assume that it indicates the secretion of iodinated organic substances of