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LETTERS TO THE EDITOR

The Effects of Insulin and Glucose on Mitochondrial-Bound Hexokinase Activity of Rat Epididymal Adipose Tissue

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Bessman [1] has previously suggested that several of the known effects of insulin on cell metabolism could be explained if this hormone caused binding of hexokinase (ATP: D-hexose 6-phosphotransferase; EC 2.7.1.1.) to mitochondria. Such a bound hexokinase would act as an ATP-acceptor, stimulating energy metabolism and anabolic processes (1). The results of the present experiments lend some support to the hypothesis of Bessman [1].

We have observed that part of the hexokinase activity of rat epididymal adipose tissue was associated with the precipitate obtained when the supernatant resulting from centrifugation of the adipose tissue homogenate at 1000 × g for 5 min was further centrifuged at 10000 × g for 10 min. Electron microscopy showed that this precipitate consisted mainly of mitochondria [7]. In addition, mitochondria were further identified by treatment with calcium phosphate and gradient centrifugation according to Greenawalt et al [4]. These results are published elsewhere [7].

The preparation of homogenates and the assay of hexokinase used in these experiments have been described previously [2].

In rats (100—180 g body weight) that had been fasted for 48 h, 26 ± 2 (SE) per cent of the total hexokinase activity of epididymal adipose tissue was associated with the mitochondrial fraction. The other 74% was found in the supernatant after centrifugation at 10000 × g for 10 min. On the other hand, 37 ± 3% hexokinase activity of the adipose tissue was observed to be mitochondrial-bound in rats fed a high carbohydrate diet (+ glucose (10% w/v) in the drinking water) *ad libitum* for 48 h. As blood-insulin concentrations are higher in carbohydrate-fed rats than in fasted rats, it could be hypothesized that the difference in per cent of hexokinase activity that was mitochondrial-bound was related to this hormone. This led us to perform incubations of whole epididymal fat pads (Krebs-Ringer bicarbonate medium) in the absence and in the presence of glucose and insulin, singly or combined. The results are presented in Table 1. Increased mitochondrial-bound hexokinase activity was observed in fat pads incubated in the presence of either glucose or insulin. A further increase was observed when glucose and insulin were added together in the incubation medium. It should be emphasized that the data shown in Table 1 represent the amount of the hexokinase activity that was found to be associated with the mitochondria, and that no difference in total hexokinase activity was observed.

Table 1. *The increase in mitochondrial-bound hexokinase activity in epididymal fat pads from 48 h fasted rats, incubated in the presence of glucose and insulin*

Addition to incubation medium	No. of observations	Per cent mitochondrial-bound hexokinase activity			
		Without the addition	With the addition	Difference	P*
Insulin	22	26.5	34.3	7.8 ± 1.6	0.001
Glucose	15	27.4	34.6	7.2 ± 2.4	0.01
Glucose + Insulin	14	26.2	37.9	11.7 ± 3.2	0.005

* Probability for no difference

Whole epididymal fat pads were incubated for 20 min at 37°C in Krebs-Ringer-bicarbonate medium (containing 1% bovine serum albumin), and then homogenized. The amount of mitochondrial-bound hexokinase activity was estimated from the difference between the activities in the supernatants after centrifugation of the homogenate at 1000 g for 5 min and at 10000 g for 10 min. From each rat, the one fat pad was incubated as the control with no addition to the incubation medium while the other fat pad was incubated in the presence of glucose or insulin or both. Glucose was present in a concentration of 5.6 mM and insulin in a concentration of 5 mU/ml. The differences in per cent mitochondrial-bound hexokinase activity between fat pads incubated with and without addition to the incubation medium are presented together with their S.E. values.

Preliminary experiments in our laboratory with suspensions of mitochondria isolated from adipose tissue have indicated that the binding of hexokinase

activity is more stable in the presence than in the absence of glucose. On the other hand, no such effect in the cell-free system was observed with insulin. This suggests that the increase in mitochondrial hexokinase activity observed with glucose in the incubation medium (Table 1) was brought about by the appearance of intracellular glucose. The further increase in particle-bound enzyme activity observed when insulin was added to the glucose-containing incubation medium (Table 1) could then be explained by the well known stimulatory effect of insulin upon glucose transport into the cells of adipose tissue, resulting in increased intracellular glucose [3]. Still, at least in addition to this, there must be another mechanism (direct or indirect) by which insulin increases the mitochondrial-bound hexokinase activity, since such an increase was also observed in the absence of glucose (Table 1).

As proposed by SIEKEVITZ [6], association of enzymes with subcellular structures may play an important role in the regulation of metabolic pathways. More recently, MARGRETH et al. [5] have emphasized the possible importance of this concept when considering the regulation of glycolysis in muscle tissue. This suggests that an influence on the localization of hexokinase may well be of significance with regard to the role of insulin as a regulator of carbohydrate metabolism.

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References

1. BESSMAN, S.P.: A molecular basis for the mechanism of insulin action. Amer. J. Med. **40**, 740—749 (1966).
2. BORREBAEK, B.: Adaptable hexokinase activity in epididymal adipose tissue studied in vivo and in vitro. Biochim. biophys. Acta **141**, 221—230 (1967).
3. CROFFORD, O.B., and A.E. RENOLD: Glucose uptake by incubated rat epididymal adipose tissue. J. biol. Chem. **240**, 3273—3244 (1965).
4. GREENAWALT, J.W., C.S. ROSSI, and A.L. LEHNINGER: Effect of active accumulation of calcium and phosphate ions on the structure of rat liver mitochondria. J. cell. Biol. **23**, 21—38 (1964).
5. MARGRETH, A., U. MUSCATELLO, and E. ANDERSON-CEDERGREN: A morphological and biochemical study on the regulation of carbohydrate metabolism in the muscle cell. Exp. Cell Res. **32**, 484—509 (1963).
6. SIEKEVITZ, P.: On the meaning of intracellular structure for metabolic regulation. Ciba Found. Symp. Regulation Cell Metab. **17**, 17—45 (1959).
7. SPYDEVOLD, Ø., and B. BORREBAEK: Association of epididymal adipose tissue hexokinase with subcellular structure. Biochim. biophys. Acta **167**, 291—301 (1968).

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