EFFECT OF INSULIN AND GLUCOSE ON THE GLUCOSE CONSUMPTION AND GLYCOGEN SYNTHESIS BY THE RAT DIAPHRAGM

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Insulin activated both the uptake of glucose-C^{14} from the medium and its incorporation into glycogen by the incubated diaphragm: the percentage of glucose-C^{14} incorporated into glycogen increased with an increase in the dose of insulin. An increase in the concentration of glucose in the medium also caused this more rapid assimilation by the muscle tissue. However, significant stimulation of glycogen synthesis was observed only in the presence of a high (unphysiological) glucose concentration in the incubation fluid; the percentage of glucose-C^{14} incorporated into glycogen remained small and constant. It is postulated that the glucose-C^{14} found in the case of substrate control is the result of exchange of the glucose residues of glycogen with intracellular glucose.

KEY WORDS: insulin; glycogen synthesis; glucose utilization; rat diaphragm.

The effect of insulin on carbohydrate metabolism in muscle tissue is manifested primarily as an increase in the assimilation of sugars by the tissue and in the rate of glycogen formation [9, 10]. An increase in the concentration of exogenous glucose also leads to an increase in its absorption by muscle tissue, but the effect of this factor on glycogen formation has not been adequately studied [4, 6].

The purpose of this investigation was to make a comparative analysis of the effect of insulin (hormonal control) and glucose (substrate control) on the assimilation of glucose and glycogen synthesis by the rat diaphragm.

EXPERIMENTAL METHOD

Female Wistar rats weighing 120-150 g were used. The rats were kept under ordinary conditions and deprived of food for 18-24 h before sacrifice.

Preparations of the diaphragm were obtained by the method of Kipnis and Cori [3]. Krebs-Ringer-bicarbonate buffer solution, saturated with carbogen gas mixture (4.2% CO_2 and 95.8% O_2) at 37°C, the pH of which was adjusted to 7.4 by means of 1.3% $NaHCO_3$, was used to isolate and incubate the diaphragms. The isolated diaphragms were washed for 5-7 min in cold buffer, divided into half-diaphragms, each of which was placed in a separate 50-ml flask containing 2 ml of incubation medium, and incubated for 90 min at 37°C. In the tests with insulin the medium contained glucose in a concentration of 250 mg% and uniformly labeled glucose-C^{14} (0.2 μCi/ml). In the tests with different glucose concentrations in the medium the quantity of glucose-C^{14} was changed so that the specific radioactivity remained constant in all the samples.

Glycogen was isolated from the diaphragm by alcoholic precipitation and hydrolyzed in 1 N H_2SO_4 [11]. The total quantity of glucose in the glycogen digest was determined by the glucose oxidase method [1]. The absorption of glucose by the diaphragm was determined from the difference between the concentrations of

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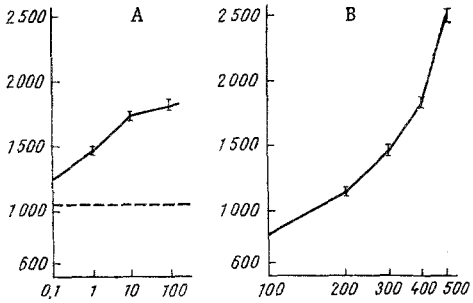

Fig. 1. Effect of insulin (A) and glucose (B) on uptake of glucose by the rat diaphragm. Mean values (M ± m) of 6-8 experiments. Abscissa, A: insulin concentration (in milliunits/ml on a logarithmic scale), in B: glucose concentration in medium (in mg% on a logarithmic scale); ordinate for both graphs: uptake of glucose (pulses/min/mg wet weight of tissue). Broken line marks basal level of glucose uptake (in samples without hormone).

TABLE 1. Effect of Various Concentrations of Insulin and Glucose in the Medium on Glycogen Synthesis by the Rat Diaphragm*

Composition of incubation medium	Quantity of glucose in glycogen digest (in μg/100 mg wet wt. of tissue)	Quantity of glucose-C^{14} in glycogen digest (in pulses/min/mg wet wt. of tissue)
Glucose 250 mg %		
Insulin (in milliunits/ml)		
0	179±17,6	116±13,1
1	470±38,2 †	328±23,8 †
10	618±38,4 †	466±27,4 †
Glucose (in mg %)		
100	162±21,0	52±3,6
200	195±15,2	66±2,2 ‡
300	173±22,4	115±12 ‡
400	238±25,2	134±11 ‡
500	288±18,5 **	179±2,14 ‡

*Here and in Table 2, mean values (M ± m) from 6-8 experiments are given.
† $P < 0.001$ compared with values in the absence of insulin.
‡ and ** denote $P < 0.01$ and $P < 0.05$, respectively, compared with values for a glucose concentration of 100 mg%.

glucose-C^{14} in the medium before and after incubation. Radioactivity was measured by means of a Packard liquid spectrometer, using Bray's scintillation fluid. The results were analyzed by Student's method with a level of significance $P < 0.05$.

EXPERIMENTAL RESULTS AND DISCUSSION

The absorption of glucose-C^{14} by the diaphragm muscle increased proportionally to the logarithm of the insulin concentration in the incubation medium within the dose range of 0.1-10 milliunits/ml (Fig. 1A). The greatest absorption took place with insulin in a concentration of 10 milliunits/ml and remained virtually unchanged with a further increase in its concentration. In the different experiments this maximal absorption amounted to 160-200% of the absorption in the control samples (without insulin).

With an increase in the concentration of exogenous glucose (from 100 to 500 mg%) its uptake by the diaphragm also increased (Fig. 1B). However, the magnitude and character of the two controlling factors – hormonal and substrate – differed, evidently reflecting differences in the mechanism of their effect on glucose utilization by the muscle.

Quantitative estimation of glucose in the glycogen digest carried out on the same samples (Table 1) showed that insulin stimulates glycogen formation; both the total quantity of glucose (endogenous and exogenous) in the glycogen digest and the quantity of glucose-C^{14} incorporated into glycogen (exogenous glucose) were increased. Insulin evidently does not change the relative proportions of metabolites of endogenous and exogenous glucose incorporated into glycogen. This occurs for two reasons: first, with a high concentration of exogenous glucose (250 mg%) the transport processes do not limit the velocity of glycogen synthesis from exogenous glucose; second, glycogen formation from both endogenous and exogenous sources under these conditions depends on glycogen synthesis activity. Insulin is known to stimulate the activity of this enzyme, as was first shown on homogenates of the diaphragm incubated with insulin [7, 8].

By contrast with insulin, an increase in the glucose concentration in the incubation medium did not give rise to a proportional increase in glycogen formation. The total quantity of glucose in the glycogen digest was increased only in the presence of the highest glucose concentration (500 mg%) in the medium. The incorporation of glucose-C^{14} into glycogen, it is interesting to note, was proportional to and increased significantly with an increase in the concentration of exogenous glucose. This fact can be explained on the assumption that the change in the quantity of glucose-C^{14} in the glycogen digest reflects an increase not only in the rate of glycogen synthesis but also in the rate of the subsequent exchange of the glucose residues in the glycogen with the intracellular glucose. It has been shown that amylo-1,6-glucosidase, an enzyme catalyzing the hydrolysis of glycogen at the branching points of the polysaccharide chain, can increase the incorporation of glucose-C^{14} into glycogen either through the partial reversal of hydrolysis in the presence of a high concentration of glucose-C^{14} or through an exchange reaction taking place between glucose-C^{14} and the glucose in the substrate-enzyme complex [2, 5].

TABLE 2. Effect of Various Concentrations of Insulin and Glucose on the Conversion of Assimilated Glucose into Glycogen

Composition of incubation medium	Quant. of glucose-C^{14} assimilated (in μg/g wet wt. of tissue)	Quant. of glucose-C^{14} in glycogen digest (in pulses/min/mg wet wt. of tissue)	Ratio II/I (in %)
	I	II	
Glucose 250 mg % Insulin (in milliunits/ml)			
0	9,1±0,14	0,79±0,098	9
1	12,2±0,32	2,42±0,19	20
10	14,3±0,34	3,8±0,24	27
Glucose (in mg %)			
100	6,1±0,13	0,42±0,01	6,7
200	9,9±0,18	0,56±0,012	5,8
300	11,7±0,06	0,98±0,09	8,4
400	14,5±0,05	1,15±0,1	7,9
500	21,4±0,08	1,53±0,03	7,1

In the present experiments increased glucose concentrations in the medium led to marked stimulation of glucose utilization by the tissue, with a consequent increase in the intracellular content of glucose-C^{14} and also, as a possible result of this, an increase in the incorporation of glucose-C^{14} into the glycogen fraction under the influence of amylo-1,6-glucosidase.

Calculated values of the quantity of glucose-C^{14} utilized under the influence of insulin and glucose and the relative proportion of the assimilated glucose metabolized into glycogen under these conditions are given in Table 2. The increase in the relative proportion of assimilated glucose incorporated into glycogen takes place only under the influence of specific hormonal control, but in the case of substrate control, despite marked stimulation of the assimilation of exogenous glucose by the tissue, the relative proportion of this glucose incorporated into glycogen remains small and virtually unchanged whatever the glucose concentration used.

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