FACTORS INFLUENCING THE COMBINATION OF INSULIN WITH MUSCLE FROM NORMAL RATS*

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We have previously reported (1, 2) a new phenomenon which is demonstrated as follows: A hemidiaphragm from a rat is equilibrated for a short period of time (termed the fixation period) in a medium containing insulin. It is next thoroughly washed, and then in an assay period equilibrated in a medium containing glucose but no added insulin. Invariably, the insulin-treated hemidiaphragm utilizes more glucose and synthesizes more glycogen than its paired untreated control. We attributed this to a chemical combination of insulin with structural elements of the muscle, the combined insulin exerting its characteristic effects on muscle metabolism.

In our original studies the selection of the concentration of insulin, the length of the fixation period, and the concentration of glucose during the assay period was somewhat haphazard. We discovered, however, that certain effects, such as the unusual ability of the diaphragm of the hypophysectomized rat to combine with insulin (3), could best be demonstrated when the above factors were selected with some care. In order to extend our knowledge of the phenomenon, therefore, we designed a series of experiments in which the factors enumerated above were systematically varied. The results of these experiments constitute the substance of this paper.

Methods

Normal rats of the Wistar strain were used following 18 to 24 hours fasting. The rat was killed by decapitation and the diaphragm removed and divided into approximately equal halves and used without washing. After weighing on the torsion balance, the two hemidiaphragms were placed in separate vessels containing medium (25°) and medium plus insulin respectively. The vessels were agitated for the time specified, removed, and drained for 30 seconds. The hanging drop was touched off with filter paper. Control experiments showed that about 0.02 ml. of medium was

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retained on the surface of the hemidiaphragm. The hemidiaphragms were then washed by agitation for 30 seconds in 25 ml. of medium and drained as before. This washing was repeated once. The hemidiaphragms were then transferred to separate vessels containing medium plus glucose as indicated. After gassing with 100 per cent oxygen, the vessels were equilibrated at 38° for 90 minutes. The hemidiaphragms were then analyzed for glycogen by a slight modification (3) of the Good, Kramer, and Somogyi method (4). The composition of the phosphate-saline medium was 0.040 M sodium phosphate, 0.087 M NaCl, 0.005 M MgCl₂, pH 6.8.

Glycogen is expressed as micromoles of glucose equivalents per gm. of wet tissue. The results are given in two ways: (1) as the insulin effect, i.e., the final glycogen of the insulin-treated hemidiaphragm less the final glycogen of the non-treated paired control; (2) as glycogen synthesis, i.e., the final glycogen less the initial glycogen. The latter was determined in a separate series of twenty hemidiaphragms. The mean ± s.e.m. was 14.9 ± 0.78 μM (glucose equivalents) per gm. of wet tissue. All of the results are given in the form of spot diagrams, each spot representing mean values obtained on a series of rats, usually five in number.

Results

Effect of Varying Time of Fixation and Insulin Concentration on Insulin Effect

These effects are shown in Figs. 1 to 3. The rapidity of combination of the insulin with the diaphragm is particularly well shown in Fig. 1. As little as 12 seconds exposure are sufficient to give significant insulin effects, even when relatively low concentrations of glucose are used during the assay period. Increase of glucose concentration during the assay period increases the insulin effects for a given amount of insulin bound during the fixation period. The amount of insulin bound appears to be the limiting factor, since increase of glucose from 0.4 to 1.0 per cent did not result in any increase of insulin effect. As the insulin concentration is increased, the amount of insulin bound, as indicated by the insulin effect at any given concentration of glucose, tends to reach a saturation level, as shown by the leveling off of the insulin effect. This is particularly well shown in Fig. 3. Here also increase of glucose concentration from 0.1 to 1.0 per cent causes a striking increase in the insulin effect on glycogen synthesis.

Mass Action Effect of Insulin and Glucose

Two types of mass action effect appear to be operative in the phenomenon being studied. The first concerns itself with the time and concentration of insulin during the preliminary fixation period. The second mani-
fests itself when the concentration of glucose during the assay period is varied, the amount of bound insulin being constant.

The first effect can be shown by the data given in Fig. 4. Assume that the bound insulin, denoted by $I$, is proportional to the extra glycogen synthesized, i.e., the insulin effect. In general, the maximal amount of insulin capable of being bound will depend upon the nature of the tissue, the nutritional and endocrine status of the animal, etc. Denote it by $I_{\text{max}}$. Then the rate of combination of insulin, $dI/dt$, will be proportional to the number of tissue loci capable of binding insulin which have not already combined with insulin; i.e., $I - I$. At any given constant concentration of insulin, $C$, we have the equation

$$\frac{dI}{dt} = kC(I_{\text{max}} - I) \quad (1)$$

From this by integration we obtain

$$I = I_{\text{max}} \left(1 - e^{-kCt}\right) \quad (2)$$

Such an equation will give curves of the general form shown in Fig. 4;
i.e., rising more or less steeply to an asymptotic maximal value. It also explains why the curves with 0.5 and 1.0 unit per ml. are essentially the same. For if saturation is practically obtained in 1 minute with 0.5 unit per ml., further increase of insulin concentration will not significantly alter the course of the curve.

The general form of the curves shown in Figs. 1 to 3 may also be expressed by an equation derived on the basis of similar assumptions used to derive Equation 2. In this case, the time of the fixation period remains constant, while the concentration of insulin is varied. The differential equation is then

\[
d\frac{I}{dC} = kt(I_{\text{max}} - I)
\]

from which is obtained \( I = I_{\text{max}} (1 - e^{-ktC}) \), which is the same as Equation 2 except that \( C \) is the variable rather than \( t \). Such an equation adequately represents the general form of the curves shown in Figs. 1 to 3. The level of the asymptote depends upon the value of \( I_{\text{max}} \) and the steepness with which the curve attains this level depends upon \( kt \).
Fig. 5 shows the mass action effect of glucose. In this case hemidiasphragms with constant amounts of bound insulin were obtained by keeping the concentration of insulin and the time of exposure during the fixation period constant. The diaphragms are then equilibrated with varying concentrations of glucose. In the lower curve, the insulin appears to be the limiting factor, since increase of glucose from 0.4 to 1.0 per cent did not cause any further increase of the insulin effect. When the bound insulin is maximal, as in the upper curve, the mass effect of the glucose is manifested by the undiminished increase of the insulin effect throughout the entire experimental range. However, the possibility of saturating the enzyme systems concerned with both insulin and glucose is shown by the data of Fig. 6. In this case, the glucose concentration was carried up to 3 per cent. The results are expressed in terms of glycogen synthesis rather than insulin effect. With maximal bound insulin, synthesis levels off at about 30 μM per gm. or 0.6 per cent. This is approximately twice the initial value of glycogen.

We believe that the data and the method of interpretation here presented show for the first time the possibility of applying physicochemical concepts to the interaction of a hormone with tissue and its resulting effects on meta-
bolic processes. The data also illustrate the completion of a reaction of insulin in a finite time interval. There is no intention of giving the mathematical method chosen any primacy over other conceivable approaches. As is well known in the field of catalysis, several methods of analysis are capable of formulating experimental data equally well. Nor have the symbols selected any rigid physicochemical meaning. The analysis presented here is intended to be illustrative of a general interpretation. At present the method of measuring bound insulin by means of the insulin effect is too indirect and inexact. What is needed is an independent method of measuring bound insulin directly. This might be achieved in the future by the use of radioactive insulin. Until such a method is available, we must be content with a semiquantitative but nevertheless illuminating concept of hormonal-tissue interaction on metabolic processes.

SUMMARY

1. The phenomenon of the binding of insulin by rat diaphragm has been studied by systematically varying the concentration and time of exposure to insulin during the fixation period and concentration of glucose during the assay period.

2. The data obtained can be satisfactorily formulated by means of a general equation based on assumptions involving the concept of mass action.

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