Effect of Glucagon on the Metabolism of Adipose Tissue*

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In previous studies (1) it was found that adrenaline had a marked stimulatory action upon the respiration of rat epididymal adipose tissue in vitro. In view of the many similarities between the metabolic actions of adrenaline and of glucagon it was of interest to study the action of glucagon upon the metabolism of adipose tissue in vitro. Glucagon is now known to cause the release of free fatty acids from adipose tissue in vitro (2) and to activate phosphorylase in adipose tissue (2, 3); it has been suggested that its lipolytic activity is related to its action on phosphorylase (3, 4). In addition, glucagon increases glucose uptake by adipose tissue (2, 5), although there is still some doubt whether this effect is characteristic of glucagon or is an effect of insulin contamination (5). Also, glucagon has been reported to increase the oxygen uptake of adipose tissue incubated with glucose, sucinate, and acetate (6) and to decrease the incorporation of acetate-1-C14 into adipose tissue lipid (6). Glucagon is known to influence both carbohydrate and fat metabolism in other tissues in vitro. Fatty acid synthesis from acetate (7) and from glucose (8, 9) by liver slices is inhibited by glucagon, and fatty acid oxidation is increased, as indicated by increased ketone body formation (8, 10).

In the experiments to be reported it is shown that glucagon increases the oxygen consumption of adipose tissue. Glucose is required for this effect of glucagon and the stimulation of glucose uptake parallels the stimulation of oxygen consumption. In addition glucagon causes a simultaneous release of free fatty acids and glycerol from tissue incubated in the absence of glucose. A preliminary report of some of this work has been made (11).

EXPERIMENTAL PROCEDURE

Methods

Rat epididymal adipose tissue, prepared as described previously (1), was used. Male rats (100 to 260 g) from the Holtzman Rat Company, maintained on a diet of Victor Fox pellets ad libitum, were used in all experiments. Rats were killed by decapitation. The technique of incubation and measurement of oxygen consumption will be described elsewhere. The incubation medium was a phosphate-buffered Ringer's solution lacking calcium (12) and containing albumin (Nutritional Biochemicals Corporation, Bovine albumin Fraction V) at a concentration of 3 g per 100 ml. The albumin was dissolved in the phosphate Ringer's solution and then dialyzed overnight at +2°C against two changes of the Ringer's solution. When glucose was added, its concentration in the incubation medium was 10 mM.

A stock solution of glucagon2 (Lilly lot No. 258-234B-167-1) was made in 0.1 M glycine buffer, pH 9.0; this solution was diluted in the albumin-containing medium to give the concentration required. The glucagon solution was always initially placed in the side bulb of the Warburg flask. In those experiments in which glucagon at a final concentration of 100 μg per ml was used, the glucagon was suspended in 0.1 M glycine buffer, pH 9.0, to give a final concentration of 3 μg per ml and 0.1 ml of this suspension was used. Adrenaline and insulin3 solutions were prepared as described before (13).

At the end of the incubation, aliquots of the incubation medium were deproteinized with zinc sulfate and barium hydroxide and analyzed for their glucose or glycerol content. Glucose was measured by the glucose oxidase method (Glucostat reagent, Worthington Biochemical Corporation) according to the method of Sutherland (17). Phosphate was estimated by the method of Saifer (14). In some experiments glycerol was measured as described by Korn (15). The FFA contents of aliquots of the incubation medium and of homogenates of the tissue were measured by the method of Dole (16).

In the experiments in which phosphorylase activation was measured, paired tissues were incubated, one with hormone and one without hormone, for 30 minutes. The tissues were then removed from the flasks, washed, homogenized in 0.25 M sucrose-0.1 M sodium fluoride solution, and the phosphorylase activity of the homogenates measured according to the method of Sutherland (17). Phosphorylase was estimated by the method of Weil-Malherbe and Green (18). Activation of phosphorylase is expressed as the difference between the phosphorylase activity of the tissue incubated with hormone and the phosphorylase activity of the tissue incubated without hormone; it is measured as μeq of inorganic phosphorus liberated per 100 mg of tissue. FFA were also measured in aliquots of the incubation medium.

In most of the experiments reported, paired tissues were incubated for 3 hours, one with hormone and one without hormone. The effect of the hormone is calculated from the difference between the glucose uptake, oxygen uptake, glycerol release or FFA release by the two tissues. Glucose uptake, oxygen uptake, and glycerol release are expressed as μmoles per 100 mg of tissue (wet weight) in 3 hours. FFA release is measured as μeq per 100 ml of tissue in 3 hours; it is the sum of the FFA recovered from the medium and the tissue.

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2 I am grateful to Dr. O. K. Behrens of Eli Lilly and Company for gifts of crystalline glucagon and of crystalline zinc insulin.
3 The abbreviations used are: FFA, free fatty acids or nonesterified fatty acids; S.E., standard error of the mean.
RESULTS

Dose-Response Curves for Effect of Glucagon on Oxygen Uptake, Glucose Uptake, and FFA Release

In the absence of glucose, glucagon, at concentrations of 0.1 to 100 μg per ml, causes the release of between 2 and 3 μeq of FFA per 100 mg of tissue in 3 hours (Fig. 1). A concentration of 0.01 μg per ml still has an effect on FFA release and a concentration of 0.001 μg per ml has a very small and barely significant effect (P < 0.10). The oxygen uptake of adipose tissue incubated in the absence of glucose is increased only slightly by glucagon at concentrations from 0.01 to 100 μg per ml. This slight stimulation of oxygen uptake occurs in the first hour of incubation and thereafter no stimulation is observed (Fig. 3). In contrast, in the presence of glucose, glucagon at concentrations of 10 or 100 μg per ml causes a marked stimulation of oxygen uptake (Fig. 2). This stimulation is maintained during the whole 3-hour incubation period (Fig. 3). Smaller concentrations (0.01 to 1.0 μg per ml) also increase oxygen uptake but not to the same extent. Glucose uptake is also increased by glucagon. The stimulation of glucose uptake parallels the stimulation of oxygen uptake. In the presence of glucose, higher concentrations of glucagon (10 and 100 μg per ml) tend to have smaller effects on FFA release than lower concentrations of glucagon (0.1 and 1.0 μg per ml). This trend is not apparent in the dose-response curve for the stimulation of FFA release by glucagon in the absence of glucose. Unfortunately, the wide variations in the glucagon effect on FFA release from day to day and from animal to animal, which are reflected in the large standard errors of the means shown in Fig. 2, make it impossible to demonstrate a statistically significant difference between the effect on FFA release of 100 μg per ml of glucagon and of 1 μg per ml of glucagon. Nevertheless, in each experiment in which all six concentrations of glucagon were compared, the same trend was noted.

Effect of Insulin on Action of Glucagon

Since higher concentrations of glucagon (10 and 100 μg per ml) stimulated glucose uptake it seemed possible that this effect might be partly caused by insulin contamination. It was previously shown that the addition of insulin markedly enhanced the action of adrenaline on the oxygen consumption of adipose tissue incubated with glucose and it seemed likely that a...
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medium, medium glycerol was measured at the end of the incubation. Glycerol release caused by glucagon was 0.681 ± 0.051 (S.E) pmoles per 100 mg of tissue in 3 hours: the simultaneous FFA release caused by glucagon (into the medium and into the tissue) was 1.619 ± 0.201 (S.E) μeq per 100 mg of tissue in 3 hours.

The time course of glycerol and of FFA release in the presence and absence of glucagon is shown in Fig. 6. Glucagon had a

similar enhancement of the glucagon effect might be occurring when the higher concentrations of glucagon were used. Since a concentration of glucagon of 10 μg per ml already has a maximal effect on glucose uptake, it follows that this concentration should contain an amount of insulin capable of causing maximal stimulation of glucose uptake, i.e. between 10⁸ and 10⁹ units. An experiment was, therefore, carried out to see whether the addition of higher concentrations of insulin (0.1 unit per ml) to tissue incubated with glucagon, 10 μg per ml, would have any further effect on glucose uptake. The results of these experiments are shown in Fig. 4. It is clear that the separate effects of glucagon and of insulin on glucose uptake summate when the two are added together. In addition, the effect of glucagon on oxygen uptake is enhanced by insulin which itself has very little effect on oxygen uptake.

**Effect of Glucagon on FFA Release and on Phosphorylase Activity**

The dose-response curves for glucagon indicated that its FFA-releasing activity was not directly related to its action on glucose uptake and oxygen uptake. In view of the suggestion that the lipolytic action of glucagon might be related to its action on carbohydrate metabolism (3, 4), the effect of glucagon on phosphorylase activity was measured and compared with that of adrenaline. The results of these experiments are shown in Fig. 5. Adrenaline, 10 μg per ml, and glucagon, 10 μg per ml, increase phosphorylase activity to a similar degree over a 30-minute incubation period. However, adrenaline is almost 7 times more active than glucagon in causing release of FFA over this same period.

**Effect of Glucagon on Glycerol Release**

It is known that adrenaline causes the release from adipose tissue not only of FFA but also of glycerol (19-21). In five experiments, in which no glucose was present in the incubation medium, medium glycerol was measured at the end of the incubation. Glycerol release caused by glucagon was 0.681 ± 0.051 (S.E) pmoles per 100 mg of tissue in 3 hours: the simultaneous FFA release caused by glucagon (into the medium and into the tissue) was 1.619 ± 0.201 (S.E) μeq per 100 mg of tissue in 3 hours.

The time course of glycerol and of FFA release in the presence and absence of glucagon is shown in Fig. 6. Glucagon had a

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marked and immediate effect on glycerol release which could be seen as early as 15 minutes after the addition of glucagon. The curves for glucagon release and FFA release in the presence of glucagon do not parallel each other except during the first hour of incubation. This suggests that some of the FFA liberated from triglycerides are retained within the tissue. The FFA in the tissue were measured at the end of the 4-hour incubation. The sum of the FFA released into the medium and retained in the tissue as FFA was still not equal to the amount of FFA expected as calculated from the amount of glycerol released.

**DISCUSSION**

The marked effect of glucagon on the oxygen consumption of adipose tissue resembles the action of adrenaline in that both are enhanced by insulin and both require glucose. The small brief stimulation observed in the absence of glucose is possibly due to the presence of glycogen in the tissue. The meaning of the striking mole for mole relationship between the increase in glucose uptake and the increase in oxygen uptake which was observed in each individual experiment is not clear; it would indicate the oxidation of only one-sixth of the extra glucose taken up and imply the trapping of a considerable percentage of the glucose carbon in other tissue constituents, possibly glyceride glycerol. It has been shown that this is the fate of much of the additional glucose taken up under the influence of adrenaline by adipose tissue (20).

The cause of the variability of the response of adipose tissue to glucagon from day to day and from animal to animal is not known. The response of adipose tissue in vitro to insulin is known to be dependent on the hormonal balance of the animal; it is decreased by increased adrenal cortical activity (22) and increased by hyperthyroidism (23). Possibly the response to glucagon is similarly influenced by other factors.

How much of the glucagon effect on glucose uptake can be attributed to insulin contamination cannot be conclusively settled by the present results. However, the fact that the effect of a concentration of glucagon (10 μg per ml), which has a maximal effect on glucose uptake by itself, is further increased by the addition of insulin suggests that the action of glucagon by itself cannot be caused by the presence of contaminating insulin. The separate effects of glucagon (10 μg per ml) and of insulin (0.1 unit per ml) on glucose uptake summate when the two are added together, but the addition of 10 times more glucagon instead of the insulin causes no greater glucose uptake than that seen with glucagon (10 μg per ml) alone.

The effect of glucagon on FFA release does not appear to be directly related to its effect on oxygen uptake and glucose uptake. Thus, a concentration of glucagon which has a maximal effect on FFA release (1 μg per ml) does not have a very great effect on glucose uptake or oxygen uptake, whereas a higher concentration (100 μg per ml) which has a much greater effect on glucose uptake and oxygen uptake has only the same effect on FFA release (in the absence of glucose) or a smaller effect on FFA release (in the presence of glucose). This depression of FFA release by glucose in the presence of higher concentrations of glucagon (10 or 100 μg per ml) is probably associated with re-esterification of the liberated FFA with glycerol precursors provided by the increased glucose metabolism.

The effect of glucagon on FFA release also does not appear to be closely associated with its effect on activation of phosphorylase. During a 30-minute incubation adrenaline and glucagon are almost equally active in activating phosphorylase, whereas adrenaline causes the release of about 7 times more FFA than glucagon. This does not exclude the possibility that 3',5'- (cyclic)-AMP might be an intermediate in both actions of the hormones. If the concentration required to activate phosphorylase were much lower than the concentration required to cause release of FFA and, further, if adrenaline caused the formation of a greater amount of 3',5'- (cyclic)-AMP than glucagon, the following might obtain: in such a situation adrenaline would both activate phosphorylase and cause release of FFA whereas glucagon might only activate phosphorylase. However, the results do suggest that activation of phosphorylase is not itself associated with increased release of FFA.

Like adrenaline (19–21), glucagon causes the release of glycerol from adipose tissue. In the absence of glucose in the incubation medium about 80% of the FFA, which must have been associated with the released glycerol in the tissue triglycerides, are released from the tissue together with the glycerol or retained in the tissue as FFA. The failure to account for all of the FFA expected from the amount of glycerol released may be due to the re-esterification in the tissue of some of the liberated FFA. The extent to which this could occur would be limited by the small endogenous supply of glycerol precursors.

It has been proposed (24) that adrenaline and adrenocorticotrophic hormone influence the rate of FFA production by inhibiting the synthesis of triglycerides. Since glycerol cannot be used by adipose tissue as a source of α-glycerophosphate (21, 25, 26), an increase in the rate of glycerol release necessarily implies an increase in the rate of triglyceride breakdown. Both adrenaline (21) and glucagon increase the rate of glycerol release and, therefore, probably also increase the rate of triglyceride breakdown. This does not exclude an additional action on synthesis of triglyceride but it does suggest that it is not the only explanation.

It has been suggested (20) that the accumulation of FFA in the tissue under the influence of adrenaline may be responsible for the observed effects of adrenaline on glucose metabolism in adipose tissue. This suggestion might also apply to glucagon and the observed differences between the effects of the two hormones might be ascribable to the differences in the concentrations of FFA which they cause to accumulate in the tissue. In another paper it is shown that adrenaline inhibited the oxygen uptake of adipose tissue incubated in the absence of albumin in the medium and that this inhibition was related to the high concentration of FFA in the tissue under these conditions. In early experiments in which glucagon was added to tissue incubated in the absence of albumin no inhibition of oxygen uptake was ever observed during a 3-hour incubation period. This difference in the actions of adrenaline and of glucagon on oxygen uptake under these particular conditions is probably related to the difference in their ability to cause accumulation of FFA in the tissue. Clearly the action of the hormones on phosphorylase is not related to their action on FFA release. Also it is unlikely that their action on phosphorylase is related to their action on glucose metabolism. As was pointed out by Vaughan (2) there are quantitative and possibly qualitative differences between the actions of glucagon and the actions of adrenaline and of adrenocorticotrophic hormone on adipose tissue metabolism. Further experiments will show whether these differences may be ascribed solely to differences in FFA metab-
olism or whether other effects, independent of FFA, are also involved.

**SUMMARY**

1. Glucagon, in concentrations as low as 0.01 µg per ml, increases the release of free fatty acids by adipose tissue *in vitro*. A maximal effect is obtained with a concentration of 0.1 µg per ml.

2. Glucagon increases the oxygen uptake and glucose uptake by adipose tissue *in vitro*. The presence of glucose is essential for this stimulation of oxygen uptake. A maximal effect on both glucose uptake and oxygen uptake is obtained with a concentration of 10 µg per ml. There is a mole for mole relationship between the increase in glucose uptake and the increase in oxygen uptake caused by glucagon.

3. The addition of insulin further increases the glucose uptake by tissue incubated with glucagon, 10 µg per ml, whereas the addition of 10 times more glucagon has no further effect. It is concluded that the increase in glucose uptake caused by glucagon is not due to insulin contamination.

4. Glucagon causes the release of glycerol from adipose tissue.

5. Comparison of the actions of adrenaline and of glucagon on activation of phosphorylase and release of free fatty acids shows that, over a 30-minute incubation period, the effects of the hormones on phosphorylase activation are almost equal but the effect of adrenaline on free fatty acid release is about 7 times greater than that of glucagon. It is concluded that these two effects of the hormones are not directly related.

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