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Effect of Insulin on Glucagon Enhanced Lipolysis in Vitro*

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Summary. Glucagon in concentrations similar to those found in human plasma markedly stimulates lipolysis in rat adipose tissue *in vitro*. The effects of these "physiological" concentrations of glucagon are reduced or abolished by insulin at concentrations of 25 and 100 μU/ml. Considering the marked insulinogenic effect of glucagon these observations may provide an explanation for the delayed increase of blood FFA observed after glucagon injection *in vivo*.

Effet de l'insuline sur la lipolyse induite par le glucagon in vitro

Résumé. A des concentrations proches de celles qui sont rencontrées dans le plasma humain, le glucagon stimule fortement la lipolyse au niveau de la graisse épididymaire du rat, étudiée *in vitro*. Les effets de telles concentrations de glucagon sont réduits, voire abolis par l'insuline aux concentrations de 25 et 100 μU/ml. Rapprochées de l'effet insulinogénique puissant du glucagon, ces observations peuvent fournir une explication quant au caractère retardé de l'élévation du taux sanguin des acides gras libres observée après injection de glucagon *in vivo*.

Wirkung von Insulin auf die glucagon-induzierte Lipolyse in vitro

Zusammenfassung. Glucagon stimuliert in Konzentrationen, wie sie auch im menschlichen Plasma vorkommen, die Lipolyse im Ratten-Nebenhodenfettgewebe *in vitro* stark. Die Effekte derartiger Glucagonkonzentrationen werden durch Insulin (25—100 μE/ml) verringert bis aufgehoben. Unter Berücksichtigung der ausgeprägten Wirkung von Glucagon auf die Insulinfreisetzung können diese Beobachtungen eine Erklärung für die Verzögerung des Anstiegs der freien Fettsäuren im Serum liefern, die man nach Glucagoninjektionen *in vivo* beobachtet.

Keys words: Insulin, glucagon, adipose tissue, lipolysis, FFA.

Recently, attention has been drawn to the interactions between glucagon and insulin : glucagon is a potent stimulus of insulin release both *in vivo* [16, 1, 9] and *in vitro* [22, 20], and since the oral ingestion of glucose increases the level of glucagon or a "glucagon-like material" in the blood [17, 6], it has been suggested that glucagon may be one of the mediators of insulin secretion after glucose feeding.

On the other hand, glucagon and insulin have clearly opposite effects on adipose tissue metabolism : glucagon is strongly lipolytic [3, 23, 8], whereas insulin reduces lipolysis even in the absence of glucose [5, 2].

It has been claimed that insulin does not reduce the lipolytic action of glucagon on isolated fat cells of the rat [2], but in these experiments the concentrations of glucagon tested were 10 to 150 times greater than those found in normal peripheral plasma by immunoassay [17, 6, 21, 15, 11]. On the other hand, using submaximal but still high concentrations of glucagon, Rodbell and Jones [14] demonstrated an inhibitory action of small concentrations of insulin on the release of fatty acids and glycerol by isolated fat cells. Since glucagon enhances lipolysis at very low concentrations, the present experiments were designed to study the antilipolytic effect of "physiological" concentrations of insulin on the stimulation of lipolysis by "physiological" concentrations of glucagon.

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Material and Methods

Male albino rats of the Wistar strain, weighing 160—180 g and fasted 12—14 h were used. The animals were killed by decapitation, and the epididymal fad pads were removed rapidly according to the technique of Renold et al. [13]. Pieces of tissue weighing 120—140 mg were incubated for 4 h in 4 ml of a modified [13] Krebs-Ringer bicarbonate buffer containing 3% human albumin (Fraction V, Armour Co). Glucose was present at the concentration of 100 or 300 mg%. Ten times recrystallized, bovine insulin and cystein-treated glucagon (insulin contamination 0.002%) were obtained through the courtesy of Dr. Schlichtkrull, Novo Industri A.S. Copenhagen, Denmark, and used at the concentrations of 0, 25, and 100 μU/ml, and of 0, 0.002, 0.005 and 0.010 μg/ml, respectively. Aliquots of the medium were taken for glycerol analysis by an enzymatic procedure [4].

Results

The results are summarized in Tables 1 and 2.

1. In the presence of glucose at a concentration of 100 mg% and in the absence of insulin, concentrations as small as 0.002 μg/ml of glucagon stimulated lipolysis as tested by glycerol release. This stimulating effect of glucagon was reduced by insulin at a concentration of 25 μU/ml. At this concentration the effect of 0.002 μg/ml of glucagon was abolished; the effects of 0.005 and 0.010 μg/ml, although reduced, remained statisti-

cally significant. At the concentration of 100 μU/ml, insulin abolished the effect of glucagon at all concentrations tested.

Table 1. *Effects of insulin on the lipolytic action of glucagon in the presence of 100 mg% glucose*

Insulin μU/ml	Glucagon μg/ml	Glycerol release μM/g/4 h[a]	*p*
0	0	5.19 ± 0.39 (22)	—
0	0.002	11.01 ± 1.00 (12)	< 0.01
0	0.005	22.84 ± 3.35 (6)	< 0.01
0	0.010	20.25 ± 2.37 (6)	< 0.01
25	0	3.87 ± 0.87 (6)	—
25	0.002	3.86 ± 0.44 (6)	N.S.
25	0.005	6.26 ± 0.46 (6)	< 0.01
25	0.010	5.83 ± 0.47 (6)	< 0.01
100	0	4.86 ± 0.28 (6)	—
100	0.002	4.73 ± 0.21 (6)	N.S.
100	0.005	4.54 ± 0.18 (6)	N.S.
100	0.010	4.90 ± 0.14 (6)	N.S.

[a] Mean ± Standard Error of the Mean, the number of determinations is indicated in parentheses.

Table 2. *Effects of insulin on the lipolytic action of glucagon in the presence of 300 mg% glucose*

Insulin μU/ml	Glucagon μg/ml	Glycerol release μM/g/4 h[a]	*p*
0	0	5.98 ± 0.42 (11)	—
0	0.002	7.15 ± 1.41 (12)	N.S.
0	0.005	9.09 ± 1.28 (12)	< 0.05
0	0.010	12.20 ± 2.20 (12)	< 0.02
25	0	4.82 ± 0.30 (6)	—
25	0.002	5.39 ± 0.30 (6)	N.S.
25	0.005	7.52 ± 0.99 (6)	< 0.05
25	0.010	8.26 ± 0.76 (6)	< 0.01
100	0	4.34 ± 0.20 (12)	—
100	0.002	4.40 ± 0.20 (12)	N.S.
100	0.005	5.45 ± 0.32 (12)	< 0.02
100	0.010	6.99 ± 0.57 (12)	< 0.01

[a] Mean ± Standard Error of the Mean, the number of determinations is indicated in parentheses.

2. In the presence of glucose at a concentration of 300 mg%, glucagon enhanced lipolysis when added in concentrations of 0.005 and 0.010 μg/ml. These concentrations remained effective even in the presence of 25 or 100 μU/ml of insulin, although the effect was reduced.

Discussion

It is still hazardous to define the normal range of plasma glucagon concentration levels on the basis of the available data obtained by radioimmunoassay [19]. It has been reported that basal levels in man range from 200 μμg Eq/ml to 8000 μμg Eq/ml [17, 6, 21, 15, 11, 18], with most values between 500 and 2000 μμg Eq/ml. Values up to 20000 μμg Eq/ml have been reported in various clinical conditions [6], and very high values of 36000 and 55000 μμg Eq/ml have been observed in a case of glucagonoma [12].

The present data indicate that concentrations of glucagon comparable with the concentrations of the hormone found in human plasma [recent data obtained using an antiserum "discriminating" pancreatic glucagon and gut glucagon-like immunoreactivity indicate that true concentrations of pancreatic glucagon in peripheral human plasma could be in the range of 200 to 300 μgEq/ml (UNGER, 1968, and HEDING, 1968, personal communications)] markedly stimulate lipolysis in incubated rat adipose tissue. The data also show that "physiological" concentrations of insulin (i.e. 25 and 100 μU/ml) are able to reduce or abolish the lipolytic effects of these concentrations of glucagon. These results are not consistent with those obtained by other workers [2] using much higher concentrations of glucagon. A reduction of the lipolytic effect of glucagon has also been observed by increasing the concentration of glucose in the incubation medium from 100 to 300 mg per cent.

These observations, correlated with the potent insulinogenic effect of glucagon, may provide an explanation for the delayed increase of blood free fatty acids observed after glucagon injection in man [10, 7], as well as in the dog [8]. It is possible that the lipolytic properties of glucagon injected *in vivo* can only be observed after the maximum hyperglycaemia and hyperinsulinaemia have subsided. However, it remains to be demonstrated that glucagon injected intravenously or intramuscularely, still exerts a lipolytic effect 2 to 4 h after its injection, when the increase in blood FFA is observed.

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