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Effect of glucagon and its 1–23 peptide fragment on lipolysis in isolated rat and human fat cells

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Glucagon has been shown markedly to stimulate the *in vitro* release of glycerol and free fatty acids from adipocytes of several species including rat [1, 2, 3] and chicken [4] while the sensitivity of human adipose tissue is said to be relatively poor [5, 6, 7]. Recent *in vivo* studies on structure–function relationships of glucagon and its peptide fragments have suggested that for man the structure responsible for lipolysis is contained within the 1–23 amino acid sequence of glucagon [8].

The present experiments were performed in order to compare the *in vitro* lipolytic activity of glucagon and its 1–23 peptide fragment on rat and human adipocytes. For this purpose isolated fat cells were prepared either from the epididymal adipose tissue of male Wistar rats (150–200 g wt.) or from human subcutaneous adipose tissue (operation material) according to the procedure by Rodbell [9], essentially as modified by Schwabe *et al.* [10]. Suspensions of fat cells made of 50,000 cells/ml in Krebs–Ringer bicarbonate buffer, pH 7·4, containing 2% bovine serum albumin (Behringwerke, Marburg/Lahn, Germany) and 0·1% glucose were incubated in a metabolic shaker at 37 C in the presence of 0, 1, 10, 100, and 1,000 ng/ml of natural crystalline porcine glucagon—molecular weight 3485 (Eli Lilly Co., Indianapolis, USA)—or the 1–23 peptide fragment—molecular weight 2,706—of glucagon as synthesized by the conventional method described in detail by Wünsch and Weinges [11]. Gas phase was 95% O_2 + 5% CO_2. Epinephrine (10^{-5}M) was used as control in each experimental series to check the sensitivity of the cells. Incubations were stopped after 1 hr (rat fat cells) or 4 hr (human fat cells), respectively, by cooling to 0 C followed by centrifugation at 4°C. In the aqueous phase, glycerol was determined enzymatically according to Eggstein and Kuhlmann [12]. The results were analysed statistically by means of Student's *t*-test, and differences were regarded significant if P was 0·05 or less.

Epinephrine (10^{-5}M) enhanced glycerol release from rat and human isolated fat cells from 0·18 ± 0·01 to 1·74 ± 0·23 μmoles of glycerol/10^6 cells, hr (966% increase) and from 0·12 ± 0·01 to 0·25 ± 0·03 μmoles of glycerol/10^6 cells, 4 hr (211% increase), respectively: (P < 0·001). In human adipocytes the lipolytic effect of glucagon and its 1–23 peptide fragment was weak. The glycerol release significantly exceeded the control values only at the high concentration of 1,000 ng/ml. There was no significant difference between the lipolytic effectiveness of both compounds (Fig. 1, right). On rat adipocytes both glucagon and its 1–23 fragment were much more effective than on human fat cells. Maximum lipolytic response was achieved at a concentration of 10 ng/ml. The 1–23 fragment was, however, considerably less potent than the whole molecule (Fig. 1, left).

These *in vitro* experiments support the hypothesis—as originally suggested by Assan and Slusher [8] due to their *in vivo* studies—that in man the lipolytic potency of glucagon can be fully mimicked by the 1–23 peptide fragment of the glucagon molecule. In rats, in which glucagon exhibits a much greater lipolytic activity than in man, the full effect can be achieved only with the complete molecule.

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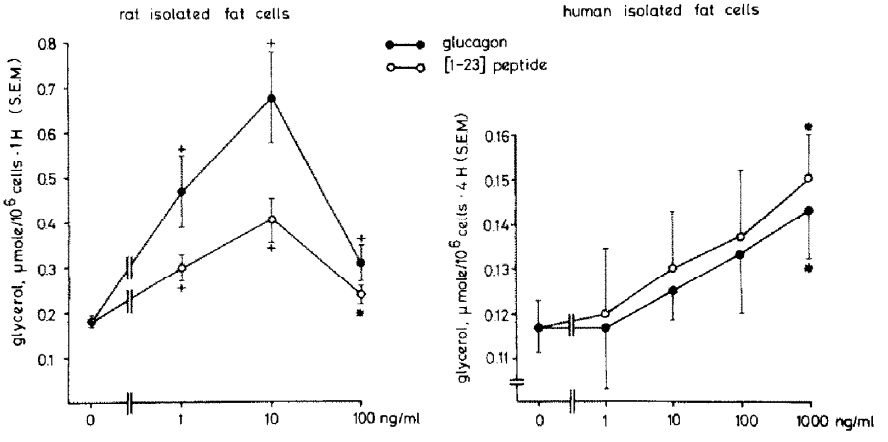

Fig. 1. Effect of glucagon and its 1–23 peptide fragment, respectively, on glycerol release (means ± S.E.M.) from rat (left) and human (right: isolated fat cells. 1H = 1 hour, 4H = 4 hours incubation time. *: P < 0·05, + *g* P < 0·001 compared to controls.

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Metabolic *N*-oxidation of secondary and primary aromatic amines as a route to ring hydroxylation, to various *N*-oxygenated products, and to dealkylation of secondary amines

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We now propose a general metabolic *N*-oxidation complex leading to the nitroso compound and to *p*- and *o*-ring hydroxy derivatives of aromatic amines and also by further metabolism to the hydroxylamine from the amine. The involvement of a similar complex and scheme explains the metabolism of *N*-alkyl-aromatic amines to yield secondary and primary hydroxylamines, nitrones and ring hydroxy amines.

The schemes are analogous to those proposed by us [1] for the metabolism of primary and secondary aliphatic amines but with some differences because of the involvement of the aromatic ring for aromatic amines.

It is probable that the same enzymes are involved in the oxidation of both the aromatic and aliphatic primary and secondary amines; the evidence is as follows:

(a) Aromatic primary and secondary amines of various types e.g. *p*-chloroaniline, phenothiazine, 2-chlorophenothiazine, 1-naphthylamine and iminodibenzyl inhibited the microsomal *N*-oxidation of phentermine (Beckett *et al.* unpublished) and the latter inhibited the microsomal oxidation of a range of diverse aliphatic primary and secondary aliphatic amines [1].

(b) The secondary aromatic amines, *N*-methyl- and *N*-ethyl-aniline are metabolised by microsomes to the primary hydroxylamine, phenyl-hydroxylamine, faster than is their parent primary amine, aniline [2]; similarly the secondary aliphatic amine, mephentermine, is metabolised faster to the primary hydroxylamine than is the primary aliphatic amine, phentermine [1].

(c) The inhibitor of oxidation of C located α to basic N atoms or of ring oxidation i.e. SKF 525A did not inhibit the microsomal formation of nitrosobenzene from *N*-alkylanilines [3], nor did it inhibit greatly the *N*-oxidation of the aliphatic amines, phentermine or mephentermine [1].

The proposed scheme for the *N*-oxidation for primary amines and the subsequent reactions is shown in Fig. 1. One of the electrons from the N lone pair is transferred to convert oxygen in the triplet state to the singlet state via the mediation of a flavoprotein; the complex of the nitrogen radical cation with the flavoprotein/oxygen (II) is formed. Two routes for change of II are available. One involves reduction of the complex to form the complex III containing the anion radical with the reduced flavoprotein. This complex (III) then produces the zwitterion-reduced flavoprotein complex (IV); reduction within the complex of the zwitterion directly to VI or after proton rearrangement to give the *N*-hydroperoxide complex (V), yields the primary hydroxylamine (VII) with regeneration of the flavoprotein and elimination of water. The oxygen atom of the primary hydroxylamine (VII) is then derived from atmospheric oxygen.

The other route from II involves dissociation of the complex to give flavoprotein and the zwitterion (VIII) which upon proton rearrangement yields the *N*-hydroperoxide (IX) which can change chemically in neutral aqueous solution by three different routes. By route a, electron attraction towards the oxygen atoms of the *N*-hydroperoxide facilitates attack from the OH^- ion from water (or X^- ion from negative ions in solution) in the *p*-position of the ring to yield the quinoid-type structure (X) which upon proton rearrangement yields the *p*-hydroxy primary amine (XI). Similarly route b yields the *o*-hydroxy amine (XIII) by a similar mechanism. Route c will yield the nitroso compound (XIV) and the oxygen atom of this compound is derived from air. Obviously substitution on the ring will alter the importance of routes a and b to each other and to route c; electron attracting groups on the ring will be expected to facilitate a or b at the expense of c. The oxygen atom of the ring OH groups is derived from the water.

Subsequent to these changes, the metabolic products XI, XIII, XIV and VII may be further metabolised e.g. the nitroso compound (XIV) can be reduced metabolically to VII which can be both oxidised further by oxidation of the OH group and rearrangement to yield *p*- and *o*-hydroxy-*N*-hydroxy compounds or by reduction back to the parent amine (1).

It is postulated that, in a similar manner, (see Fig. 2) a secondary aromatic amine (XV) is oxidised to the free radical ion complex (XVI) which can be reduced to other complexes XVII, XVIII and XIX, which then yield the secondary hydroxylamine (XXI), the oxygen in this compound being derived from the air. Also dissociation of the complex (XVI) gives the zwitterion (XXII) which upon proton rearrangement yields the unstable *N*-hydroperoxide (XXIII). This hydroperoxide then is attacked by OH^- ions (or other X^- ions from solution) to yield *p*- and *o*-ring hydroxy secondary amines XXV and XXVII as indicated, the oxygen atoms of the hydroxy groups thus being derived from water.