INCREASED SENSITIVITY OF RAT ADIPOSE TISSUE TO THE LIPOLYTIC ACTION OF EPINEPHRINE DURING FASTING AND ITS REVERSAL DURING RE-FEEDING

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1. Introduction

We have recently reported that adipose tissue from streptozotocin-diabetic rats is 5–10-times more sensitive to the lipolytic action of epinephrine than adipose tissue from normal rats and that this hypersensitivity is reversed to normal by insulin treatment [1]. These observations suggested that the sensitivity of rat adipose tissue to catecholamines might be modulated by the plasma insulin level. In order to test this hypothesis we have measured the lipolytic response of rat adipose tissue to epinephrine in the fasted state and during refeeding. These nutritional conditions bear close similarities to diabetes (fasting) and to insulin treatment (refeeding). Fasting for 72 h increased the sensitivity of adipose tissue to epinephrine 5–6-fold. Re-feeding for 48 h restored the sensitivity of the lipolytic response to normal. However, the basal lipolytic rate in the re-fed state was considerably increased compared to the normal or fasted state.

2. Materials and methods

Male Zbz Cara (formerly Osborne-Mendel) rats weighing 130–160 g were fasted for 72 h. Loss of body weight during that time was between 23% and 26%. During refeeding the animals received NAFAG chow (No. 890, NAFAG, Gossau, Switzerland) consisting of 65% cereals, 20% protein and 5% fat. They had free access to drinking water to which 20 g/100 ml of sucrose had been added. After 24 h re-feeding, body weight increased again to 90% of the initial weight and after 48 h rose further to 95%. The fat-pad weights varied from 110–150 mg/pad in normal rats and 60–100 mg/pad in fasted rats. In the 24 h re-fed animals pads weighed from 65–120 mg and after 48 h re-feeding from 75–130 mg. Incubation of pooled adipose tissue was carried out at 37°C in 3 ml Krebs-Ringer bicarbonate buffer gassed for 10 min with 95% O_2/5% CO_2 and containing 30 mg/ml human serum albumin (HSA, from the Swiss Red Cross, Bern) and 2 mg/ml glucose. HSA was dialyzed extensively against several changes of distilled water and filtered by sterile filtration. One gram contained 6–12 μequiv. FFA. Epinephrine was added after a 1 h preincubation period, and the incubation was continued for another 30 min. Adipose tissue from fasted re-fed rats was incubated in the presence of 10 μl guinea pig anti-insulin serum (neutralizing 10 mU of insulin) to eliminate possible interference of traces of endogenous insulin adhering to the tissue. This amount of anti-insulin serum had no effect on the lipolytic response of normal tissue to any of the epinephrine concentrations tested [1].

Tissue free fatty acids (FFA) and FFA in the medium were determined according to the method of Duncombe [2] with slight modifications, as described in detail previously [1]. Glycerol in the medium was measured by the method of Eggstein and Kreutz [3]. The medium was deproteinized with perchloric acid (5% final concentration), neutralized with solid $KHCO_3$ and the $KClO_4$, which had precipitated during 30 min at 0°C, was removed by centrifugation.

All results are expressed as μmol FFA or glycerol formed in 30 min fat-pad. At maximal hormone

concentrations these indices reflect the total lipid-mobilizing capacity of the pad. For the calculation of hormone concentrations which cause half-maximal stimulation of the lipolytic indices the reference point (pad weight, etc.) is irrelevant.

3. Results and discussion

As shown in fig.1, glycerol and FFA release and the rise in tissue FFA elicited by epinephrine concentrations below 10 ng/ml are significantly higher in adipose tissue from 72 h fasted than in adipose tissue from normal rats. At hormone concentrations to which normal tissue barely responds (< 1 ng/ml) considerable amounts of glycerol and FFA are released from the fasted pads with a concomitant elevation of tissue FFA. Epinephrine concentrations required for half-maximal stimulation of glycerol release are ~ 2.5 ng/ml in fasted and ~ 15 ng/ml in normal adipose tissue. Half-maximal stimulation of FFA release occurs at ~ 1.6 ng/ml and ~ 15 ng/ml, respectively and tissue FFA increase half-maximally at ~ 5 ng/ml and ~ 35 ng/ml. Maximal stimulation of glycerol release appears to be somewhat lower after fasting, maximal FFA-release is identical in both conditions. However, in the fasted tissue maximal stimulation of all lipolytic indices is achieved at hormone concentrations far below those required by normal tissue. At hormone concentrations above 100 ng/ml less FFA accumulates in fasted than in normal pads. Basal glycerol release and basal tissue FFA-levels for either tissue are not significantly different, if expressed fat-pad. In contrast, basal FFA-release from fasted tissue is significantly higher than from normal tissue, probably due to a decreased re-esterification capacity of the fasted tissue [4].

In fig.2 the responses to different epinephrine concentrations of adipose tissue from fasted re-fed and normal rats are compared. After 24 h re-feeding the sensitivity of the tissue to epinephrine is still increased, although somewhat higher concentrations than in fasted tissue are required for half-maximal stimulation of lipolysis. However, compared to the normal and fasted tissue, a marked increase

Dose-response curves of epinephrine-stimulated lipolysis in adipose tissue of normal and 72h fasted rats.

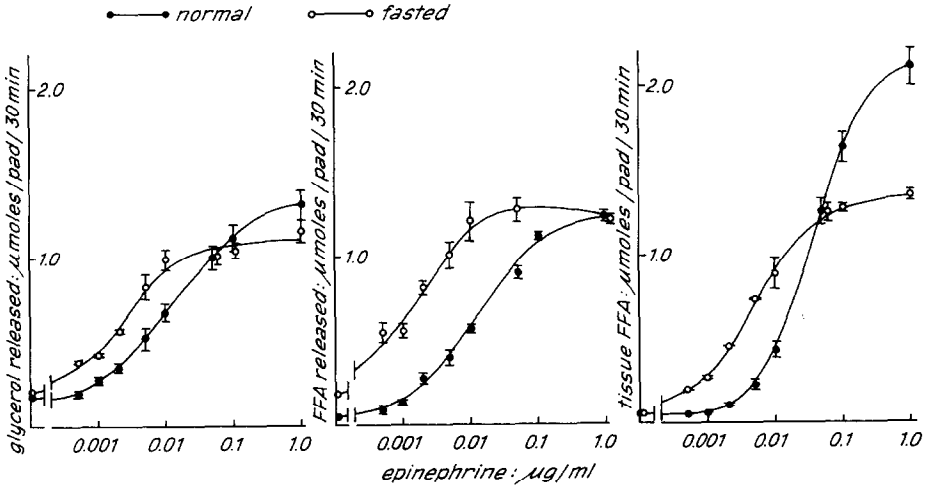

Fig.1. Dose–response curves of epinephrine-stimulated lipolysis in adipose tissue of normal and 72 h fasted rats: After 1 h preincubation at 37°C in the absence of hormone, epinephrine was added and the incubation continued for another 30 min. The points represent the mean of 8 incubations (4 experiments) in normal and of 4 incubations (2 experiments) in fasted animals, the bars give the SEM.

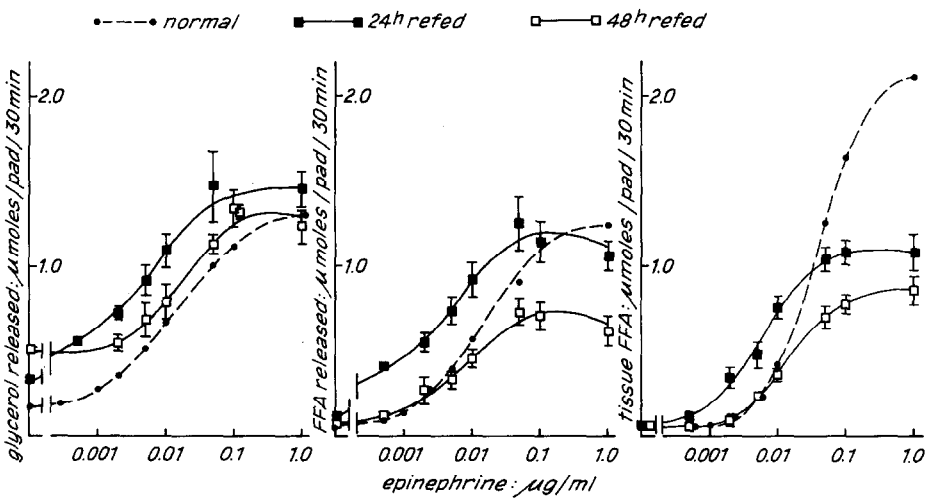

Fig.2. Dose–response curves of epinephrine-stimulated lipolysis in adipose tissue of normal, 24 h re-fed and 48 h re-fed rats. Incubation conditions were the same as described in fig.1. Each point represents the mean of 6 incubations (3 experiments) and the bars give the SEM. The dose–response curves for normal adipose tissue are the same as in fig.1, except SEM-values were omitted for graphic-clarity.

in the basal lipolytic rate (glycerol release, fig.2) has occurred. Re-feeding for 48 h causes a further increase in the basal lipolytic rate (fig.2), but the responsiveness of the lipolytic rate to epinephrine is reduced. It is similar to that in normal pads. Half-maximal stimulation of glycerol release occurs at ~ 16 ng/ml, of FFA-release at ~ 9 ng/ml and of the tissue FFA at ~ 12 ng/ml. The latter two values still lie ~ 1.5- and ~ 3-times below the hormone concentrations required for half-maximal stimulation of normal tissue, but they are ~ 5- and ~ 2-times higher than in the case of fasted tissue. Maximal stimulation of glycerol release is comparable to that of normal tissue, whereas maximal FFA-release and the maximal increase in tissue-FFA lie significantly below the normal values. Overall re-esterification of FFA appears to be considerably increased after 48 h re-feeding, both in the basal and in the hormone-stimulated state. This high rate of re-esterification by tissue of fasted re-fed rats is explained by the high glycogen content of the tissue [5,6].

The increased sensitivity of adipose tissue during fasting to the lipolytic action of epinephrine constitutes a further mechanism which, besides the decreased re-esterification capacity of the tissue [4], explains increased FFA-mobilization and the elevation of plasma FFA-levels in the fasting state [7]. In this respect, the fasted is similar to the diabetic state [1]. Since both re-feeding of fasted rats and insulin treatment of diabetic rats [1] are capable of reversing the increased lipolytic sensitivity of adipose tissue, circulating insulin levels are likely to be involved in the regulation of the tissue-sensitivity to epinephrine.

Acknowledgements

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References

[1] Zapf, J., Feuerlein, D., Waldvogel, M. and Froesch, E. R. (1975) Diabetologia 11, 509.

[2] Duncombe, W. G. (1963) Biochem. J. 88, 7.
[3] Eggstein, M. and Kreutz, F. H. (1966) Klin. Wschr. 44, 262.
[4] Vaughan, M. (1962) J. Biol. Chem. 237, 3354.
[5] Frerichs, H. and Ball, E. G. (1962) Biochemistry 1, 501.
[6] Froesch, E. R., Bürgi, H., Bally, P. and Labhart, A. (1965) Mol. Pharmacol. 1, 280.
[7] Havel, R. J. (1975) in: Handbook of Physiology, Section V, adipose tissue (Renold, A. E. and Cahill, G. F., Jr. eds) p. 577, American Physiological Society, Washington DC.