SHORT COMMUNICATIONS

Lipolytic Effects of Serum and Plasma on Isolated Fat Cells of the Rat *in vitro*

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Summary. Serum and plasma obtained from fasted rats were examined for their capacity to promote glycerol release from isolated fat cells of the rat, *in vitro*. Dose-response curves indicated that the lipolytic potencies of serum and plasma were very similar. Plasma comparison experiments suggested that lipolysis promoted by this circulating agonist had a requirement for calcium ions. Since adrenaline can enhance lipolysis in a calcium-free

medium, the lipolytic capacity of serum and plasma could not be simply explained in terms of circulating catecholamines. The concentrations of glycerol or free fatty acids did not increase when serum or plasma were incubated in the absence of fat cells.

Key words: Serum, plasma, lipolysis, isolated fat cells, dose-response curve.

Introduction

Carlson [1] has emphasized the importance of lipolysis of adipose tissue triglycerides, by humoral agents, for the normal function of the body, and Froesch [2] has investigated pharmacologic inhibition of lipolysis in order to adjust the metabolic situation in diabetes mellitus. Both these studies provoke the question as to whether there is a specific fat-mobilising substance circulating in blood, as discussed by Friesen (1964) [3]. Recant *et al.* (1963) [4] in a study designed primarily to elucidate the reasons for elevated free fatty acid (FFA) levels in diabetes, observed that serum had "non-esterified fatty acid releasing activity". This was shown by increased release of FFA from rat epididymal fat pad (EFP) tissue of young animals when incubated in serum. Burns *et al.* (1967) [5] reported that when rat EFP or isolated fat cells, were incubated in human serum, a high glycerol release was induced; recently these findings have been confirmed and extended [6, 7, 21]. Barrett [8] showed that rat plasma promoted the release of FFA and glycerol from EFP. However, he reported that serum had a relatively trivial action as compared with plasma, in its capacity to promote lipolysis, and suggested that the lipolytic component was lost during the clotting process. Whilst it has been reported that "spontaneous lipolysis" occurred when plasma was incubated in the absence of tissue [8], Burns *et al.* [5] found no increase in glycerol concentration of human serum incubated alone. The object of the present study was to investigate the lipolytic effects of serum and plasma on isolated rat fat cells *in vitro*, and to elucidate the question of "spontaneous lipolysis".

Material and Methods

Male albino Wistar rats weighing 190–210 g were fasted overnight but allowed water *ad libitum*. The animals were sacrificed by carbon dioxide inhalation. Isolated fat cells were prepared in Krebs-Ringer bicarbonate buffer (containing 3.5 g/100 ml human albumin and 45 mg/100 ml glucose) by a modified Rodbell [9] scheme [6]. Blood obtained from the same rats was either allowed to clot for the preparation of serum, or anticoagulated with lithium/heparin, liquid or solid sodium citrate, or the potassium salt of ethylene diamine tetraacetic acid (EDTA), for the preparation of plasma. No special precautions were taken to prevent access of air to serum or plasma. The concentration of endogenous glucose of serum and plasma ranged between 100–120 mg/100 ml when samples were estimated by the automated glucose oxidase micro-method of Faulkner [10]. The potency of endogenous insulin was approximately 55 micro-units/ml of serum or plasma using an ox insulin standard range.

Dose-response curves were defined by using serum and plasma doses ranged in arithmetic progression from 0.10 ml – 1.50 ml per incubation vial. The total volume of the liquid content of each vial was maintained at 2.0 ml by addition of 500 microlitres of adipose cell suspension and appropriate addition of buffer.

Previous investigations [6, 7] had shown the maximally effective dose of serum to be 1.10 ml or 55% by volume. Therefore plasma comparison studies were performed using a volume of 1.10 ml per vial. Where samples of serum or plasma were incubated alone, a volume of 2.0 ml was used.

All experimental incubations were carried out for 90 min in a shaking water bath at 37°C in an atmosphere of 75% nitrogen, 20% oxygen and 5% carbon dioxide.

Glycerol release was employed as an index of lipolysis. Estimation was performed before and at the end of incubation using the technique of Wieland [11]. The increase in glycerol concentration of the medium was related to the weight of adipose cell lipid present. Lipid determination was performed on a 1.0 ml aliquot of fat cell suspension by a modified Folch [12] system described previously [13].

Results

Serum and plasma dose-response curves

It was found that adipose cells incubated in buffer alone released 2.6 ± 0.5 nanomoles of glycerol/mg lipid/90 min whilst the corresponding values when 1.10 ml of serum or plasma were present, were: 25.3 ± 1.9 and 20.8 ± 1.2 respectively. The dose-response data were converted to percentages of the control value and plotted graphically (Fig. 1). The several dosages, of both serum and plasma, of 0.10 ml–1.10 ml produced conspicuous rises, in glycerol output, above the control value and there was a positive correlation between glycerol release provoked by the same dose of serum or plasma.

Fig. 1. Comparison of the lipolytic activity of fasted rat serum (■—■) and lithium/heparin anti-coagulated plasma (●—●) dose-response curves for glycerol release from isolated fat cells, expressed as a percentage of the control (nil serum, nil plasma) value. Each point is the mean of 4–6 observations $\pm$ SEM

Plasma comparison experiments

The histogram in Fig. 2 shows the glycerol release provoked by serum and variously anti-coagulated plasma. Whilst serum was the most potent promoter of lipolysis in isolated fat cells, the least potent was potassium EDTA anti-coagulated plasma which was highly significantly different ($P < 0.001$).

Plasma produced by employing solid sodium citrate as anti-coagulant had a similar effect on fat cells to plasma produced by the more usual, liquid sodium citrate.

"Spontaneous lipolysis"

The results in Table 1 show that when serum or plasma was incubated in the absence of adipose cells, no significant ($P > 0.05$) increase in either FFA or glycerol concentration was observed.

Table 1. The effect on glycerol and FFA concentrations of serum and plasma incubated in the absence of adipose cells

Component	Pre-incubation values		Post-incubation values	
	FFA	Glycerol	FFA	Glycerol
Serum	0.83 ± 0.01	0.143 ± 0.004	0.78 ± 0.06	0.152 ± 0.004
Plasma	0.73 ± 0.07	0.156 ± 0.004	0.65 ± 0.09	0.142 ± 0.015

Results expressed as mean $\pm$ SEM of 2–5 observations FFA concentration in milli-equivalents per litre and glycerol in millimoles per litre. Students t-test for paired comparisons of pre-incubation and post incubation values, showed $P > 0.05$ in all cases.

Fig. 2. Histogram showing glycerol released (as a percentage of the control value) from isolated fat cells in response to 1.10 ml of either fasted rat serum (A), or variously anticoagulated plasma: lithium/heparin (B), liquid sodium citrate (C), solid sodium citrate (D) or the potassium salt of ethylene diamine tetra-acetic acid (E). Each column represents the mean value of 3–6 observations $\pm$ SEM

Discussion

The findings reported here concerning the relative fat-mobilizing capacities of serum and plasma indicate comparable lipolytic potencies. If it is assumed that lipolysis promoted by serum and plasma is due to the same agonist then the results are at variance with a previous report [8], but confirm the observations of Recant *et al.* (1963) [4] and Burns *et al.* (1967) [5] on the lipid-mobilizing capacity of serum.

The possible importance of endogenous insulin and glucose has been considered since both these metabolites increased in concentration in incubation vials with increased dosage of serum or plasma. Exogenous insulin has been shown to be without effect on the rate of serum stimulated lipolysis up to doses of 20000

micro-units/ml [14]. Although glucose may enhance glycerol release provoked by caffeine, adrenaline or ACTH, though not that resulting from dibutyryl cyclic-AMP administration [15, 16], glucose up to 250 mM has been shown not to affect glycerol release from isolated adipose cells stimulated by the serum agonist [17].

The plasma comparison experiments using citrate and EDTA to bind calcium, indicate that the availability of calcium ions may be important in the action of the circulating lipolytic agonist. When EDTA is employed to eliminate calcium, a permanent bond is formed; but with citrate, some cations are still available, though obviously insufficient to permit blood clotting to proceed. The importance of calcium ions in the lipolytic action of adrenocorticotrophic hormone was first recognised by Lopez *et al.* (1959) [18], but it is known that adrenaline will induce lipolysis in rat adipose tissue in a calcium-free medium [19]. It is suggested, therefore, that the observed lipolytic activity of serum and plasma must be explained in terms more complex than being due simply to circulating catecholamines.

The lack of change of the concentration of glycerol or FFA in serum or plasma during incubation alone corroborates the observations of Burns *et al.* [5]. No doubt the differences between the data reported here and those of Barrett [8] lie in the fact that this latter used plasma from fed rats when lipoprotein lipase activity is maximal [20] whilst in this study, animals were fasted prior to experimentation.

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References

- Carlson, L. A.: Lipolysis of adipose tissue triglycerides. Regulation, physiological and clinical significance. Excerpt. Med. Int. Cong. Ser. No. **161**, 143—149 (1969).
- Froesch, E.R.: The physiology and pharmacology of adipose tissue lipolysis: its inhibition and implications for the treatment of diabetes. *Diabetologia* **3**, 475—487 (1967).
- Friesen, H.: Pituitary peptides and fat mobilization. *Metabolism* **13**, 1214—1229 (1964).
- Recant, L., Alp, H., Koch, M.B., Eggeman, J.: Non-esterified fatty-acid-releasing activity of diabetic serum. *Lancet* **1963 II**, 614—616.
- Burns, T.W., Hales, C.N., Hartree, A.S.: Observations on the lipolytic activity of human serum and pituitary fractions in vitro. *J. Endocr.* **39**, 213—225 (1967).
- Curtis-Prior, P.B.: Preparation and sensitivity of isolated fat cells of the rat, to a serum lipolytic agonist. Guy's Hospital Reports (1972) accepted for publication.
- Curtis-Prior, P.B., Hanley, T.: A fat-mobilizing factor in serum (1972) in preparation.
- Barrett, A.M.: The effect of chlorophenoxyisobutyric acid on the release of free fatty acids from isolated adipose tissue in vitro. *Brit. J. Pharmacol.* **26**, 363—371 (1966).
- Rodbell, M.: Metabolism of isolated fat cells. *J. Biol. Chem.* **239**, 375—380 (1964).
- Faulkner, D.E.: An automated micro-determination of blood glucose with the Auto-Analyser. *Analyst.* **90**, 736—744 (1965).
- Wieland, O.: In: Methods of Enzymatic analysis by Bergmeyer, H.U., p. 211. London: Academic Press 1963.
- Folch, J., Lees, M., Sloane-Stanley, G.H.: A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226**, 497—509 (1957).
- Curtis-Prior, P.B., Trethewey, J., Stewart, G.A., Hanley, T.: The contribution of different organs and tissues of the rat to assimilation of glucose. *Diabetologia* **5**, 384—391 (1969).
- Curtis-Prior, P.B.: Insulin and anti-insulin effects on serum lipolytic activity in isolated fat cells of the rat. (1972). Submitted for publication.
- Bally, P.R., Kappeler, H., Froesch, E.R., Labhart, A.: Effect of glucose on spontaneous limitation of lipolysis in isolated adipose tissue: a potential regulatory mechanism. *Ann. N.Y. Acad. Sci.* **131**, 143—156 (1965).
- Ho, R.J., England, R., Meng, H.C.: Effect of glucose on lipolysis and energy metabolism in fat cells. *Life Sci.* **9**, Part I, 137—150 (1970).
- Curtis-Prior, P.B.: Unpublished observations (1971).
- Lopez, E., White, J.E., Engel, F.L.: Contrasting requirements for the lipolytic action of corticotropin and epinephrine on adipose tissue in vitro. *J. Biol. Chem.* **234**, 2254—2258 (1959).
- Mosinger, B., Braun, T., Kujelooa, V., Wenkeova, J.: Fatty acid metabolism and fat reserve mobilisation. *CS. gastroenterol. a vyziva* **16**, 206—213 (1962).
- Hollenberg, C.H.: Effect of nutrition on activity and release of lipase from rat adipose tissue. *Am. J. Physiol.* **197**, 667 (1959).
- Curtis-Prior, P.B.: Ethanol inhibition of serum stimulated lipolysis in isolated fat cells of the rat. *Experientia* (1972) accepted for publication.

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