THE EFFECT OF GLUCAGON ON THE STATE OF LYSOSOMAL ENZYMES IN ISOLATED PERFUSED RAT LIVER

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1. Introduction

The participation of lysosomal enzymes in glucagon induced autophagocytosis was first suggested by Ashford and Porter [1] who demonstrated histochemically the existence of acid phosphatase in autophagosomes formed after perfusion of the liver with glucagon. Deter and De Duve [2] found that the formation of autophagic vacuoles after glucagon treatment *in vivo* was accompanied by an increased fragility and osmotic sensitivity of lysosomes. This could be determined by measuring lysosomal enzyme activity under isotonic conditions ("free" activity). In comparing the results with "total" enzyme activity, obtained after disruption of the lysosomes, the degree of lysosomal integrity could be determined.

In order to determine whether the well known ketogenic and ureogenic action of glucagon in the isolated perfused rat liver could be related to the formation of autophagic vacuoles [1, 2] we measured lysosomal enzymes in the isolated perfused rat liver before and after hormone administration. This system enabled simultaneous measurement of products of liver catabolism in the perfusion medium. Because of the possible relationship to the ketogenic action of glucagon, lysosomal lipase was chosen and measured together with another lysosomal enzyme, *N*-acetyl-β,D-glucosaminidase.

2. Methods

2.1. *Perfusion technique and metabolite determinations*

Livers from rats (100–150 g body weight) starved for 24 hr were perfused with washed human red cells in a buffered albumin (Behringwerke, Marburg) solution as described before [3]. After 30 min of preperfusion, a small tissue sample (300–400 mg) was removed for determination of basal "free" enzyme activities. After addition of a single dose of 200 μg of glucagon (E.Lilly, Indianapolis, USA) two further tissue pieces were removed at the times indicated in fig. 1. Ketogenesis and urea production was calculated from metabolite changes in the medium throughout the experimental period [3].

2.2. *Determination of lysosomal enzymes*

Lysosomal enzymes were determined as "free" and "total" activity (as defined in [2]). "Free" activity was determined in the 1000 *g* supernatant of isotonic homogenates prepared by standardized homogenization (1:10 w/v) in 0.25 M sucrose [4]. "Total" enzyme activity was obtained after homogenization of 0.5 g tissue in 25 ml distilled water [4]. Lysosomal triglyceride lipase was measured by determination of glycerol release from triolein at pH 6.0 [5]. *N*-Acetyl-β,D-glucoaminidase was tested according to a modification [4] of the method of Findlay et al. [6].

3. Results

In experiments where glucagon was injected *in vivo*, it affected lysosomal enzymes only in starved animals [4]. We therefore took livers from 24 hr starved rats to test whether glucagon would have the same effect in the isolated perfused liver. As table 1

Table 1
Activity of lysosomal enzymes in perfused livers from starved rats.

	N-Acetyl-β,D-glucosaminidase (μmoles/g liver/10 min)			Triglyceride lipase (μmoles/g liver/30 min)		
	"Free" activity		"Total" activity	"Free" activity		"Total" activity
	After pre-perfusion	Difference 45 min later	At the end of experiment	After pre-perfusion	Difference 45 min later	At the end of experiment
Controls	5.23 ± 0.49 (13)	−0.02 ± 0.22 (11)	44.18 ± 1.97 (13)	1.62 ± 0.23 (8)	−0.13 ± 0.28 (4)	16.37 ± 1.53 (7)
+Glucagon	4.53 ± 0.41 (14)	+ 1.88 ± 0.32 (10)	44.7 ± 1.52 (14)	1.44 ± 0.24 (9)	+ 0.58 ± 0.15 (6)	15.95 ± 1.81 (8)
Significance $p <$	n.s.	0.001	n.s.	n.s.	0.05	n.s.

Tissue samples were taken for determination of "free" activity from perfused livers after 30 and 75 min of perfusion. Glucagon was added as a single dose of 200 μg after the first samples had been taken. Solvent was given to the control perfusions. "Total" activity of lysosomal enzyme activity was measured at the end of the experiment. All results are given as mean ± S.E.M. with the number of experiments in parantheses.

shows "free" activity of both enzymes tested increased after glucagon, whereas "total" activity, which was measured at the end of the perfusion, showed no difference between the control and the glucagon treated livers. This is in good agreement with experiments *in vivo* [2, 4] where "free" activity was increased without any change in "total" activity. The time sequence of "free" *N*-acetyl-β,D-glucosaminidase and lipase activity after glucagon is shown in fig. 1., where the individual experiments have been normalized. "Free" activity of both enzymes increased by about 40% after addition of glucagon to the perfusion system. No significant changes were obtained in the control perfusions. The change in the state of lysosomal enzymes seems to be extremely rapid; significant differences were observed after 15 min (first sample).

In parallel with the observed changes in lysosomal enzyme activity, a rapid constant increase in ketone body and urea production was observed (table 2), which corresponded with rates seen in previous experiments where no biopsies had been taken [3].

Table 2
Effect of glucagon on urea production and ketogenesis in perfused livers from starved rats.

	Urea production (μmoles/g/min)	Ketogenesis (μmoles/g/min)
Control (C)	0.159 ± 0.033 (6)	0.384 ± 0.074 (9)
Glucagon (G)	0.275 ± 0.027 (6)	0.525 ± 0.063 (9)
G − C	0.116 ± 0.03 (6)	0.141 ± 0.049 (9)
Significance of difference $p <$	0.02	0.025

The rate of urea and ketone body (acetoacetate + β-hydroxybutyrate) formation was calculated from metabolite concentration, determined every 15 min after a preperfusion period. Glucagon was added as a single dose of 200 μg at 30 min. All results are given as means ± S.E.M. with the number of experiments in parentheses. Significance of the differences was calculated with the paired data *t* test [3].

4. Discussion

The catabolic action of glucagon on lipid and protein in rat liver has been demonstrated by several workers (see ref. in [3]) *in vivo* and in perfused rat liver. Since the concentrations of free fatty acids and amino acids present in the isolated liver seem too low to account for the observed rise in ketogenesis and urea formation, endogenous lipid and protein was thought to be the source of ketone bodies and urea released into the medium after glucagon. Struck et al. [7] and Bewsher and Ashmore [8] postulated a glucagon sensitive lipase in rat liver in analogy to the

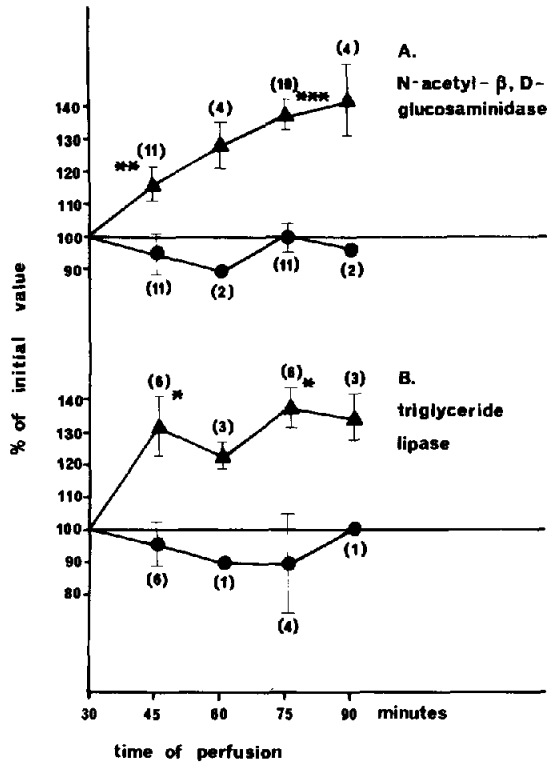

Fig. 1. Effect of glucagon on "free" lysosomal enzyme activity in perfused livers from starved rats. "Free" lysosomal enzyme activity was determined in tissue samples taken from perfused liver after a preperfusion period of 30 min. The results from following samples were corrected for the initial value of the same liver. ●—● control perfusions; ▲—▲ + 200 μg glucagon. Significance against control group: * $p < 0.02$; ** $p < 0.01$; *** $p < 0.001$.

enzyme described in adipose tissue. So far, only indirect evidence for the existence of such an enzyme could be obtained [9]. Of the three lipases demonstrated in rat liver [5] the lysosomal enzyme seemed the one most likely responsible for intracellular lipid breakdown, since in general lysosomal enzymes are known to be involved in glucagon induced autophagocytosis [1, 2]. The observed increase in "free" activity of *N*-acetyl-β,D-glucosaminidase and lipase after glucagon administration indicates an increased fragility of lysosomes during homogenization in isotonic sucrose. This has been shown by Deter and DeDuve [2], Arstila and Trump [10, 11] and Ericson [12] to be caused by the formation of autophagic vacuoles from pre-existing material. Enzyme synthesis does not seem to be involved in the observed increase of "free" activity, since "total" activity of the same enzymes was the same in the controls and the glucagon treated livers. Also the results of Arstila and Trump [11], who were unable to prevent the appearance of autophagic vacuoles after glucagon by cycloheximide, indicate that *de novo* synthesis of protein is not required for the changes in the state of lysosomal enzymes.

The increase in urea and ketone body production concommitant with the changes in the state of lysosomal enzymes could be further evidence for a causal relationship between the occurrence of autophagic vacuoles and cell catabolism. The extent to which increase in "free" lipase activity observed in the present studies is involved in the stimulation of gluconeogenesis by glucagon [7] awaits further investigation. In the intact animal, only extended starvation lead to changes in the state of lysosomal enzymes similar to those observed in the present experiments [4]. Possibly insulin, which has been repeatedly shown to inhibit the ketogenic and ureogenic action of glucagon [cf. 3], prevents glucagon induced autophagocytosis *in vivo*. Recently, Mortimore and Mondon [13] have shown that insulin can almost completely prevent proteolysis in the isolated liver.

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