Decrease by glucagon in peroxide generation by isolated hepatocytes

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Exposure of isolated rat liver cells to glucagon or dibutyryl cyclic AMP leads to a prompt decrease in the rate of cellular peroxide generation as evidenced by (i) a reduced rate of [^{14}C]formate oxidation and (ii) a lowered steady-state concentration of catalase Compound I.

Hepatocyte *Peroxide* *Formate* *Catalase Compound I* *Glucagon*

1. INTRODUCTION

Glucagon treatment of intact rats or isolated hepatocytes leads to an apparent activation of a number of functions of the subsequently isolated mitochondria (overviews see [1,2]). With respect to some of these, evidence has been presented, indicating that glucagon exerts a stabilizing rather than a true stimulatory effect [3,4]. From the observation that chloroquine and dibucaine likewise stabilize mitochondrial functions such as state-3 respiration and succinate dehydrogenase (EC 1.3.99.1) activity, it was inferred that glucagon might lead to an inhibition of phospholipase A (EC 3.1.1.4) activity. Evidence, however, has been obtained that the hormone increased rather than decreased the lysophospholipid content of liver cell fractions [5]. Another clue possibly is provided by the finding that superoxide exposure of isolated mitochondria lowers their ability to retain Ca^{2+} [6], as glucagon treatment has been shown to prolong Ca^{2+} retention time [7–10]. Therefore, the question has been addressed as to whether glucagon might decrease the rate of H_2O_2 production in isolated liver cells.

2. MATERIALS AND METHODS

[^{14}C]Formate was from New England Nuclear (Dreieich), Worthington collagenase type CLS came from Seromed (München). Hepes, gelatin, glucagon, isoproterenol, [8-lysine]vasopressin and 2-phenethylamine were purchased from Serva (Heidelberg). Phenylephrine and Scintigel came from Roth (Karlsruhe). Insulin was obtained from Novo (Copenhagen); dibutyryl cyclic AMP (Bt_2cAMP) was supplied by Boehringer (Mannheim). All other chemicals were purchased from Merck (Darmstadt).

Male Sprague-Dawley rats (A. Ivanovas, Kisslegg) weighing about 220 g after starvation for 48 h were used for liver cell isolation [11]. If not stated otherwise the incubation mixture contained 0.6 ml of Ca^{2+}-free Krebs–Henseleit bicarbonate buffer [12], pH 7.4, containing 1.5% gelatin, 0.4 ml rat serum, 0.1 ml of defatted [13] bovine serum albumin in 0.9% NaCl, 0.01 ml of 110 mM $CaCl_2$, 0.05 ml of 220 mM L-lactate and 0.2 ml of the liver cell suspension (corresponding to about 25 mg dry wt). Incubations were performed in a shaking water bath at 37°C in stoppered plastic

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tubes (10 × 1.9 cm) equipped with a center well, with 95% O_2/5% CO_2 as the gas phase.

For formate oxidation measurements 10 μl of a 0.16 M [^{14}C]formate solution were added after 30 min preincubation and incubation continued for the indicated times. The reaction was stopped by injection of 0.5 ml of 67% (v/v) acetic acid and the incubation vessel put on ice. Shortly after, 0.25 ml of phenethylamine was injected into the center well, which was removed 90 min later for liquid scintillation counting. For the determination of catalase Compound I the incubation mixture was prepared with bicarbonate buffer containing 25 mM Hepes, pH 7.4; the incubation volume was doubled. After 20 min preincubation 10 μl glucagon (2 mg/ml) or solvent only was added and incubation continued for 10 min before 2.2 ml of the incubation mixture and 0.5 ml of the Hepes–bicarbonate buffer were transferred into a cuvette (d = 1 cm) and kept at 37°C under continuous stirring and carbogen gassing in a Shimadzu UV 300 double beam spectrophotometer. The absorbance changes at 660–630 nm after the addition of 10 μl of 2.2 M methanol and 10 μl of 0.5 M KCN, respectively, were recorded. Absorption coefficients of 3.9 and 5.4 $mM^{-1} \cdot cm^{-1}$ were used with regard to the catalase Compound I and the catalase–cyanide complex, respectively [14].

3. RESULTS AND DISCUSSION

Insight into peroxidative metabolism of hormonally stimulated hepatocytes was approached by following the formation of $^{14}CO_2$ from labelled formate and by measuring the catalase Compound I content (review [15]). With respect to the former method the possibility had to be examined as to whether under the conditions employed appreciable formate degradation occurred via the consecutive action of N^{10}-formyltetrahydrofolate synthetase (EC 6.3.4.3) and dehydrogenase (EC 1.5.1.6). For this purpose the effect of added serine and histidine (final concentration 7 mM) on formate oxidation was studied; no effect of serine (n = 3) and only a 12% decrease by histidine was noticed. A marked increase, however, was caused by methionine (n = 5; not shown) in agreement with [16]. It appears, therefore, that in the absence of added methionine the rate of $^{14}CO_2$ formation from [^{14}C]formate can be taken as an indicator for peroxide metabolism in isolated liver cells.

With lactate as the main substrate hepatocytes oxidized [^{14}C]formate in a manner linear with cell number (fig.1) and incubation time (fig.2). In the

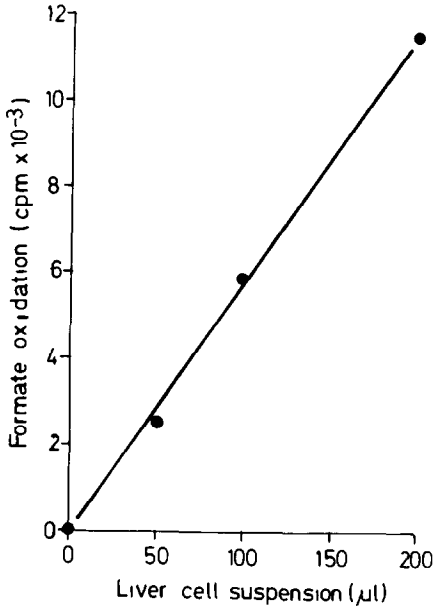

Fig.1. Relationship between hepatocyte concentration and the rate of [^{14}C]formate oxidation. For experimental details see section 2.

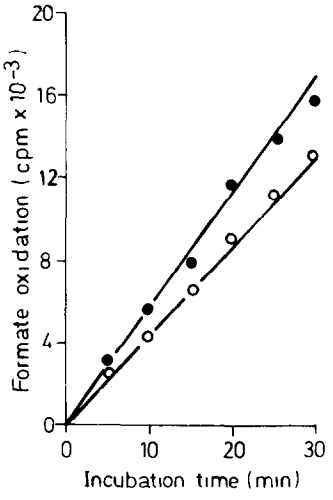

Fig.2. Decrease by Bt_2cAMP in the rate of [^{14}C]formate oxidation by isolated hepatocytes. Final concentration of Bt_2cAMP was 0.3 mM. Further experimental details are given in section 2.

presence of Bt_2cAMP the rate of $^{14}CO_2$ release was decreased (fig.2). This effect was apparent at different formate concentrations up to substrate saturation (fig.3). The maximal rate of formate oxidation was determined to be 0.47 ± 0.06 μmol/min per g dry wt (n = 7), which is well comparable with the value reported for rat liver homogenate [17]. This rate, in addition to Bt_2cAMP, was decreased by glucagon, and to a smaller extent by isoproterenol and phenylephrine, whereas vasopressin and insulin remained ineffectual (table 1). The inhibitory effect of glucagon was found to be more pronounced (32 ± 2%, n = 3) when serine was present in the incubation mixture (7 mM). Preincubation of hepatocytes in the presence of glucagon or Bt_2cAMP up to 20 min did not influence their effect on formate oxidation (table 2). With regard to the hormone sensitivity, fig.4 shows that the half-maximal glucagon effect was obtained at 1–2 nM. Note that the incorporation of ^{14}C radioactivity into acid-stable products (e.g., glucose, citrate, malate, aspartate) was not significantly influenced by glucagon. Therefore, the results seem to indicate that formate degradation in isolated hepatocytes is decreased by glucagon. Moreover, on the basis of the findings

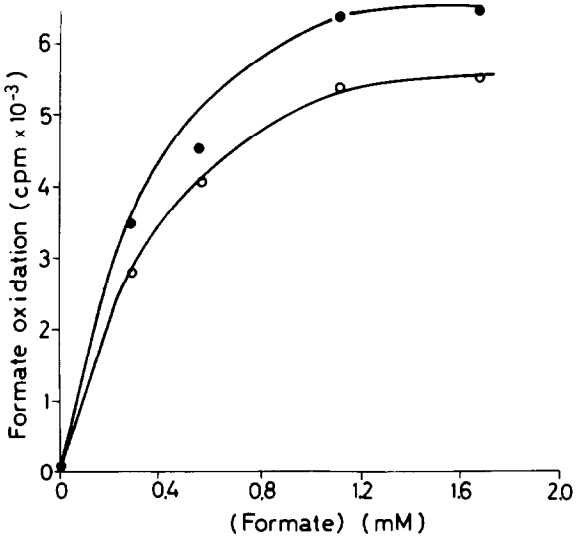

Fig.3. Effect of Bt_2cAMP (○) on the rate of [^{14}C]formate oxidation by isolated hepatocytes at different formate concentrations. Incubation time was 10 min. Further details are described in section 2. Very similar effects were obtained with glucagon (7 μg/ml).

Table 1

Effect of hormonal agents on [^{14}C]formate oxidation by isolated hepatocytes

Condition	[^{14}C]Formate oxidation (μmol/g dry wt per 10 min)
Control	4.74 ± 0.64 (7)
Glucagon (7 μg/ml)	3.94 ± 0.58[a] (7)
Bt_2cAMP (0.4 mM)	4.04 ± 0.52[a] (7)
Isoproterenol (5 μM)	4.21 ± 0.61[a] (7)
Phenylephrine (10 μM)	4.38 ± 0.59[a] (7)
Vasopressin (250 mU/ml)	4.63 ± 0.65 (6)
Insulin (500 μU/ml)	4.85 ± 0.65 (6)

[a] Statistical significance against control $P < 0.005$ was calculated according to Student's t-test for paired data

Mean values ± SE are given for the number of different cell preparations in parentheses. For experimental details see section 2

reported above it appears reasonable to assume that this hormone effect was due to a decrease in the rate of peroxide formation. This view was strengthened by the results obtained by the spectrophotometric determination of that proportion of cellular catalase being present as the catalase–H_2O_2 complex (i.e., Compound I). This was accomplished with methanol as the hydrogen donor. Pilot experiments revealed that the concentration required for 50% titration of catalase Com-

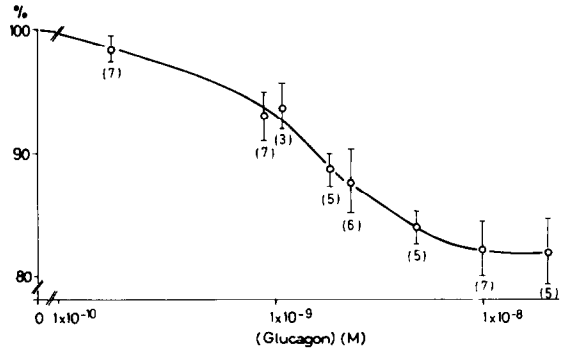

Fig.4. Decrease in [^{14}C]formate oxidation by isolated hepatocytes as a function of glucagon concentration. Mean values ± SE are given for the numbers of different cell preparations in parentheses. Incubation time with glucagon was 10 min. Further details are given in section 2.

Table 2

[^{14}C]Formate oxidation by isolated hepatocytes after preincubation with glucagon (7 μg/ml) or Bt_2cAMP (0.4 mM)

Preincubation (min)	[^{14}C]Formate oxidation (μmol/g dry wt per 10 min)		
	Control	Glucagon	Bt_2cAMP
0	1.99 ± 0.10 (7)[a]	1.64 ± 0.04 (6)	1.63 ± 0.06 (7)
10	1.95 ± 0.05 (5)[a]	1.69 ± 0.08 (5)	1.49 ± 0.08 (5)
20	2.02 ± 0.04 (5)[a]	1.63 ± 0.04 (5)	1.49 ± 0.07 (5)

[a] According to Student's t-test for paired data the control values are significantly higher ($P < 0.01$ or better) than those of the hormonally stimulated cells

Formate concentration in the incubation mixture was 0.28 mM. For further experimental details see section 2. Mean values ± SE are given for the number of different cell preparations in parentheses

pound I of control liver cell preparations amounted to 0.119 ± 0.007 mM ($n = 7$), being well in the range reported for the isolated liver with lactate as the substrate [18,19]. Therefore, only a single methanol dose has been employed in the determination of the proportion of catalase Compound I of hepatocytes incubated in the absence and presence of glucagon. The data recorded in table 3 indicate that the decrease by glucagon in cellular peroxide formation as indicated by the reduced formate oxidation was also reflected by a decrease in the steady-state level of catalase Compound I. Unfortunately, information is lacking with regard to the subcellular compartment(s) involved and the mechanism(s) underlying this glucagon effect, which has also been shown with adipocytes [20]. Possibly it is related to the observation that glucagon pretreatment of rats enhances mitochondrial and microsomal Ca^{2+} uptake and retention [7–10, 21–23] as these processes in vitro were shown to be adversely affected by peroxide [24,25]. Furthermore, it is conceivable that glucagon might lower thiol group or/and lipid peroxidation and thus contribute to the maintenance of membrane integrity in hepatocytes.

Table 3

Effect of glucagon on the proportion of catalase Compound I in isolated hepatocytes

Condition	% Compound I
Control	62.3 ± 2.8
Glucagon	50.8 ± 3.1[a]

[a] $P < 0.005$ calculated according to Student's t-test for paired data

Mean values ± SE for 9 different cell preparations are given. For further details see section 2

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