Control of energy metabolism by glucagon and adrenaline in perfused rat liver

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Changes in subcellular distribution of adenine nucleotides, mitochondrial/cytosolic proton gradients, rates of respiration, gluconeogenesis (fasted state) and glycogenolysis (fed state) were studied in isolated perfused rat livers following addition of glucagon (10^{-8} M) or adrenaline (10^{-7} M). Glucagon increased the gradient in all states. The cytosolic ATP/ADP ratio was increased in the fasted but decreased in the fed state which is consistent with a diminished futile cycling in gluconeogenesis (fasted state) or a decreased glycolytic rate (fed state). Adrenaline caused an increase in the proton gradient and the mitochondrial ATP/ADP ratio. The two effects are attributed to increased calcium entry into the matrix space.

Subcellular distribution *Adenine nucleotide* *Proton gradient* *(Perfused rat liver)* *Glucagon* *Adrenaline* *Ca^{2+} effect*

1. INTRODUCTION

The energetic state of a cell is reflected by the subcellular distribution of adenine nucleotides and the protonmotive force across the mitochondrial membrane and is determined by the balance between the various ATP-generating and -consuming processes. It was shown that in liver the cytosolic phosphorylation state of the ATP-ADP system depends upon the rate of glycolysis [1]. When glycolysis is absent, i.e. in the fasted state, rates of respiration and gluconeogenesis are the main determining factors.

Glucagon and adrenaline, though acting via different signalling systems (reviews [2–4]), have in common a stimulation of mitochondrial respiration [2,5–7] and gluconeogenesis [2,8,9] in the fasted state. Their effects are different in the fed state, where glycolysis is inhibited by glucagon but stimulated by adrenaline [5,10]. The different metabolic effects should influence the energetic state of the liver cell. To obtain more insight into hormonal control of energy metabolism, we studied the changes in subcellular distribution of adenine nucleotide and proton gradients in perfused livers following administration of glucagon or adrenaline.

2. MATERIALS AND METHODS

Male albino rats (Wistar strain; Thomae Co., Biberach, FRG) weighing 180–220 g received a standard laboratory diet (Altromin; Lage, FRG) and water ad libitum prior to the isolation of the liver under pentobarbital anaesthesia. Livers were perfused with Krebs-Henseleit bicarbonate buffer, pH 7.4, saturated with O_2/CO_2 (95:5) in a non-recirculating system [10]. In experiments with livers from fed rats, the buffer was substrate-free, whereas with livers from 24 h fasted rats, it was supplemented with L-lactate (3 mM) and pyruvate (0.3 mM). All experiments were terminated by freeze-fixation of the livers after 50 min perfusion. For determination of subcellular pH gradients, the buffer contained 0.3 mM 5,5-dimethyl-2,4-oxazolidinedione (DMO). 5 min prior to freeze-fixation of the liver, [^{14}C]DMO was infused at rates of 5 μCi/min. Rates of oxygen consumption and pro-

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duction of glucose, lactate and pyruvate were calculated from the flow rate and arterio-venous concentration differences in the perfusate which were monitored continuously by platinium electrodes and determined by enzymatic analyses of perfusate samples taken at 1-min intervals, respectively.

For determination of mitochondrial and cytosolic contents of adenine nucleotides and [^{14}C]DMO, the freeze-fixated livers were ground in liquid nitrogen and freeze-dried at 0.25 Pa at −40°C. About 0.3 g of the freeze-dried powder was sonicated in a mixture of heptane and CCl_4 and fractionated in the same media by density gradient centrifugation. The organic media were used to prevent metabolic disturbances during the fractionation [11]. Heating of the sample above 4°C was strictly avoided. Specific activities of mitochondrial and cytosolic marker enzymes, citrate synthetase ans phosphoglycerate kinase, respectively, contents of adenine nucleotides and specific activities [^{14}C]DMO were determined in each fraction of the density gradient. Based on the marker enzyme distribution in the fractions, metabolite contents and radioactivities in pure mitochondrial and cytosolic fractions were extrapolated according to [12]. Glucose, lactate and pyruvate concentrations in the perfusate, marker enzyme activities and adenine nucleotide contents in the fractions were determined as in [13].

All chemicals were of reagent grade and were purchased from Merck (Darmstadt) or Roth (Karlsruhe). Enzymes and coenzymes used for enzymatic analyses were from Boehringer (Mannheim) or Sigma (München). Glucagon was purchased from Novo Industrie (Mainz) and [2-^{14}C]DMO from NEN (Boston, MA).

RESULTS AND DISCUSSION

Changes in metabolic rates in perfused livers from fed and fasted rats following infusion of glucagon or adrenaline are shown in table 1. In the fed state, oxygen consumption and glucose production was increased with the two hormones, whereas glycolysis was increased with adrenaline but decreased with glucagon. It has been shown recently [4] that the stimulatory and inhibitory effects on respiration and glycolysis, respectively, by glucagon were kinetically similar and stoichiometrically related. This close relationship was not observed with adrenaline. In the fasted state, glucagon stimulated respiration and gluconeogenesis.

The differences in metabolic rates are reflected by subcellular ATP/ADP systems and pH gradients. the results are summarized in table 2. With glucagon, the ATP + ADP content in mitochondria and the proton gradient across the mitochondrial membrane were increased in the fed and

Table 1

Changes in metabolic rates following infusion of glucagon or adrenaline in perfused livers from fed or fasted rats

	Control		Glucagon		Adrenaline
	Fed	Fasted	Fed	Fasted	Fed
Oxygen consumption	2.32	3.63	+0.55	+0.38	+0.71
Glycogenolysis	2.37	–	+3.66	–	+2.68
Glycolysis	1.89	–	−1.01	–	+0.32
Gluconeogenesis	–	1.31	–	+0.54	–

Livers from fed rats were perfused with substrate-free Krebs-Henseleit bicarbonate buffer, whereas livers from fasted rats were perfused with Krebs-Henseleit bicarbonate buffer containing 3.0 mM lactate and 0.3 mM pyruvate. The rates are mean values of 4–13 experiments calculated from measured arterio-venous concentration differences after 50 min perfusion either in the absence of hormones or following a 10 min infusion of glucagon (10^{-8} M) or adrenaline (10^{-7} M). Values expressed as μmol/g liver per min

Table 2

Effects of glucagon and adrenaline on the subcellular distribution of adenine nucleotides and pH gradients in perfused rat livers

	Total		Mitochondrial		Cytosolic		pH gradient mit.-cyt.
	ATP+ADP (μmol/g dry)	ATP/ADP	ATP+ADP (mmol/l)	ATP/ADP	ATP+ADP (mmol/l)	ATP/ADP	
Fed (13)	13.6	4.2	9.0	0.22	7.2	9.8	0.33
	± 0.6	±0.2	±0.3	±0.04	±0.4	±0.1	±0.02
Fed (4)	11.5	3.0	9.7	0.26	5.4	5.9	0.49
glucagon	± 0.6	±0.2	±0.6	±0.03	±0.3	±1.1	±0.04
Fed (6)	12.2	4.0	16.0	1.10	5.6	8.7	0.79
adrenaline	± 0.4	±0.2	± 2.5	±0.10	±0.4	±1.0	±0.10
Fasted (4)	8.8	2.9	12.8	0.91	3.6	7.0	0.25
	± 0.3	±0.1	± 1.0	±0.10	±0.2	±0.7	±0.07
Fasted (4)	9.2	3.0	14.5	0.90	3.9	8.5	0.49
glucagon	± 0.6	±0.2	± 1.7	±0.14	±0.4	±1.9	±0.07

Experimental conditions as described in table 1. Freeze fixation was performed after 50 min perfusion either in the absence of hormones or following a 10 min infusion of glucagon or adrenaline. Mean values ± SE with number of experiments in brackets

fasted state. Similar changes were also observed in mitochondria isolated from glucagon-treated rats [14,15] and in isolated hepatocytes [15–17]. In the cytosol the ATP/ADP ratio was increased in the fasted state despite enhanced ATP consumption due to gluconeogenesis. Thus, respiration matches the increased ATP demand. The even higher ATP/ADP ratio could suggest a more efficient energy utilisation, e.g. via a diminished futile cycling at the pyruvate(/phospho*enol*pyruvate step due to inhibition of pyruvate kinase [18]. In the fed state, however, the ATP/ADP ratio was decreased (table 2,3). This is consistent with the lowered rate of cytosolic ATP generation due to the observed suppression of glycolysis (table 1).

With adrenaline, the cytosolic ATP/ADP ratio was not significantly changed whereas the mitochondrial ATP+ADP content and ATP/ADP ratio were considerably higher than in control livers. Interestingly, the pH gradient across the mitochondrial membrane was also increased (table 2). Since it is known that α-adrenergic agonists enhance cytosolic and, subsequently, mitochondrial calcium concentrations [19,20], calcium influx into the mitochondria could induce proton extrusion by the respiratory chain. Moreover, since a rise in cytosolic calcium concentration is reported to increase the mitochondrial pyrophosphate content [21] which, on the other hand, is known to exchange for cytosolic ATP via the adenine nucleotide translocase [22], the increased ATP content and ATP/ADP ratio in mitochondria following adrenaline infusion might be explained via a calcium-dependent mechanism. Backflow of ATP, therefore, could counteract partially the increased mitochondrial energy production stimulating both respiration and glycolysis.

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