ON THE NATURE OF THE STIMULATION BY GLUCAGON OF CITRULLINE SYNTHESIS IN RAT-LIVER MITOCHONDRIA

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1. Introduction

The stimulatory effect of glucagon on functions in liver mitochondria has been reviewed in [1]. These functions include state-3 respiration, uncoupler-stimulated respiration, succinate dehydrogenase activity and pyruvate carboxylation. The stimulatory effect is observed in mitochondria isolated after treatment of intact rats, perfused livers or isolated liver cells with the hormone. The magnitude of the stimulation by glucagon was elegantly shown to be dependent on the medium used to isolate the mitochondria in [1]. The effects of glucagon were found to be much smaller in mitochondria isolated in a medium with mannitol as osmotic support than in those isolated in a sucrose-containing medium. This difference was mainly due to the fact that the rates of the functions measured in control mitochondria isolated in a mannitol medium were higher than the rates found when a sucrose-containing isolation medium was used. It was concluded [1] that glucagon treatment stabilises the mitochondrial membrane rather than activates mitochondrial functions.

This conclusion is clearly valid for the functions investigated [1]. It is therefore important to know whether this applies to other mitochondrial functions. We show here, that the stimulatory effect of glucagon on citrulline synthesis is due to an activation of carbamoyl-phosphate synthetase and, unlike the stimulatory effect of the hormone on the functions in [1], cannot be ascribed to effects on the stability of the mitochondrial membrane.

2. Methods

Fed male Wistar rats (200–250 g) were anaesthesized and treated with glucagon as in [2]. Liver mitochondria were prepared essentially as in [3] with 250 mM mannitol as the isolation medium. Mitochondria were stored at 0°C at 30–50 mg protein/ml. Citrulline, *N*-acetylglutamate and mitochondrial protein were determined as in [2].

Citrulline synthesis was studied by incubating the mitochondria (2–4 mg protein/ml) at 25°C in closed 25 ml counting vials in a medium (2 ml) containing the following standard components (A): 75 mM Tris–HCl, 15 mM KCl, 5 mM potassium phosphate, 10 mM ornithine, 16.6 mM $KHCO_3$, 10 mM NH_4Cl, 1 mM EGTA, 25 mM mannitol (derived from the mitochondrial suspension) and 1 mM atractylate (to prevent leakage of mitochondrial adenine nucleotides [4]). The pH of the medium was 7.4 and the gas phase was 95% O_2 + 5% CO_2.

Mitochondrial respiration was studied with a Clark-type electrode. Mitochondria (~2 mg protein) were incubated at 25°C in a medium (1.5 ml) containing the following standard components (B): 50 mM Tris–HCl, 15 mM KCl, 2 mM EDTA, 5 mM $MgCl_2$ and 5 mM potassium phosphate (pH 7.4).

3. Results

Table 1 shows that liver mitochondria from rats treated with glucagon, obtained after isolation in a mannitol-containing medium, have enhanced rates of citrulline production (cf. [2]). Citrulline synthesis with glutamate as the respiratory substrate was higher than with succinate because of synthesis of *N*-acetylglutamate, the activator of carbamoyl-phosphate synthetase, during incubation [5,6]. In 6 out of the 10 preparations in each series, *N*-acetylglutamate was also

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Table 1
Effect of glucagon treatment of rats on citrulline synthesis and on respiration in isolated rat-liver mitochondria

Additions	Citrulline production		Rate of respiration		P
	Control	+ Glucagon	Control	+ Glucagon	
	(nmol/mg protein)		(natom O . mg protein^{-1} . min^{-1})		
Succinate	43 ± 5 (10)	88 ± 8 (10)			<0.0005
Glutamate	122 ± 13 (10)	181 ± 16 (10)			<0.005
Succinate:					
state 4			59 ± 3 (10)	63 ± 3 (10)	n.s.
state 3			230 ± 10 (10)	245 ± 9 (10)	n.s.
Glutamate + malate:					
state 4			38 ± 3 (6)	44 ± 3 (6)	n.s.
state 3			184 ± 11 (6)	184 ± 7 (6)	n.s.

For measurement of citrulline production, mitochondria were incubated for 10 min in standard medium A (section 2) with the addition of 10 mM succinate (+2 μg rotenone/ml) or 10 mM glutamate. For measurement of respiratory rates, mitochondria were incubated in standard medium B (section 2) with the addition of either 10 mM succinate (+2 μg rotenone/ml) or 10 mM glutamate +10 mM malate. The same set of 10 different mitochondrial preparations in each group was used for measurement of citrulline synthesis and of oxygen consumption with succinate as the substrate. In 6 out of these 10 preps the rate of respiration with glutamate + malate as substrate was measured. Values are means ± SE; statistical significance was calculated using Student's t-test; n.s., not significant

measured in freshly isolated mitochondria. The values obtained were 0.69 ± 0.05 and 1.02 ± 0.09 nmol/mg protein (means ± SE; $P < 0.025$) in mitochondria from control and glucagon-treated rats, respectively (not shown in table 1; cf. [2]).

In the same mitochondrial preparations in which citrulline synthesis was studied, respiratory rates were also measured. Respiration was measured under state-4 and state-3 conditions with either succinate or glutamate plus malate as substrate. Glucagon treatment of the rats had no significant effect on the rates of oxygen consumption (table 1), in agreement with [1].

4. Discussion

Stimulation of mitochondrial respiration by glucagon was shown to be due to a stabilising effect on the mitochondria [1], and it was suggested that the same effect might be responsible for the stimulatory effect of the hormone on the citrulline-synthesizing capacity of the mitochondria [1]. Our data demonstrate that glucagon stimulated citrulline synthesis under conditions where mitochondrial respiration was not affected. We conclude that the effects of glucagon on citrulline synthesis and on respiration are not related.

Stimulation of citrulline synthesis by glucagon is, at least in part, due to an increased intramitochondrial *N*-acetylglutamate concentration [2,7]. Not only is the *N*-acetylglutamate content of isolated liver mitochondria increased after glucagon administration to either intact rats or isolated hepatocytes, but also the total intracellular amount of *N*-acetylglutamate is enhanced [2]. The latter value is obtained without mitochondrial isolation. Since >70% of intracellular *N*-acetylglutamate is intramitochondrial [2,8,9], we conclude that the *N*-acetylglutamate content of the mitochondria in the intact hepatocyte is also increased by glucagon. The capacity of liver mitochondria to synthesize citrulline is dependent on the magnitude of the intramitochondrial concentration of *N*-acetylglutamate [2,6,9,10]. Thus, the stimulation of citrulline synthesis by glucagon is due to a direct activation of carbamoyl-phosphate synthetase. This effect is clearly different from the stabilising effect of the hormone on several mitochondrial functions described in [1].

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