Effects of glucagon *in vivo* on the *N*-acetylglutamate, glutamate and glutamine contents of rat liver

James M. STADDON, Norah M. BRADFORD and John D. MCGIVAN

Department of Biochemistry, University of Bristol Medical School, University Walk, Bristol BS8 1TD, U.K.

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Glucagon injected into rats caused an increase in liver *N*-acetylglutamate content, and coincidentally the glutamate content decreased. The liver glutamine content decreased only after 10 min, consistent with an activation of glutaminase after a lag. These observations indicate that the increased *N*-acetylglutamate content was not due to an increase in glutamate content caused by an activation of glutaminase (EC 3.5.1.2).

Stimulation of ureogenesis by glucagon in liver preparations has been observed several times. The mechanism by which this hormonal stimulation is achieved remains to be unequivocally established. Carbamoyl-phosphate synthetase I (EC 6.3.4.16) is a potentially rate-controlling enzyme of ureogenesis. Control of the activity of this enzyme can be mediated by changes in the concentration of its allosteric activator, *N*-acetylglutamate (for a review see Meijer & Hensgens, 1982).

Glucagon stimulated the rate of citrulline synthesis in mitochondria isolated from glucagon-injected rats, and this was attributed in part to an increased *N*-acetylglutamate concentration (Hensgens *et al.*, 1980). Glucagon also stimulated ureogenesis in isolated hepatocytes incubated with glutamine, with a coincident increase in mitochondrial *N*-acetylglutamate concentration (Hensgens *et al.*, 1980). The mechanism by which glucagon increased the *N*-acetylglutamate concentration remains to be elucidated.

Glucagon has been shown to activate glutaminase in isolated hepatocytes, with a consequent increase in intracellular glutamate concentration (Joseph & McGivan, 1978). Correlation between the concentrations of glutamate and *N*-acetylglutamate in liver has been observed (Stewart & Walser, 1980). It is therefore possible that glucagon could indirectly increase *N*-acetylglutamate concentration by activating glutaminase (Hensgens *et al.*, 1980; Kovacevic & McGivan, 1983). In the present paper this hypothesis was tested *in vivo* by measuring the hepatic concentrations of glutamine, glutamate and *N*-acetylglutamate under the influence of glucagon.

Materials and methods

Animals and experimental procedure

Wistar rats (200–300 g) were given a standard diet and water *ad libitum*. Experiments were always started between 10:00 and 11:00 h. The animals were anaesthetized with 1 ml of Sagatal/kg body wt. Glucagon (0.1 ml) was injected into the exposed saphenous vein as a 0.2 mg/ml solution (Yamazaki & Graetz, 1977). The liver was removed at the required time, freeze-clamped, and a HClO₄ extract made as described by Williamson *et al.* (1967). By using a pH-meter, protein-free extracts were adjusted to pH 7.20 with 3 M-KOH/1 M-Mops (4-morpholinepropanesulphonic acid). After cooling the sample on ice for 30 min, the KClO₄ precipitate was removed by centrifugation for 2 min at 2000 g.

Determination of metabolites

Glutamate (Bernt & Bergmeyer, 1965) and glutamine (Lund, 1974) were assayed enzymically. ADP (0.5 mg/ml) was included in the glutamate assays. Glucose was determined by using hexokinase with NAD⁺ and NAD(P)⁺-specific glucose-6-phosphate dehydrogenase. Control concentrations of glutamate and glutamine reported here are similar to those observed by Brosnan & Williamson (1974).

N-Acetylglutamate was determined by the methods described by McGivan *et al.* (1976) and Meijer & van Woerkom (1978) modified so that 0–1 nmol of *N*-acetylglutamate in 0.2 ml of liver extract could be assayed. A partially purified enzyme preparation was used rather than crude mitochondrial

extracts, in order to decrease endogenous *N*-acetylglutamate and ATPase activity. Carbamoylphosphate synthetase and ornithine transcarbamoylase were partially co-purified by the method of Rajman & Jones (1976), which was followed as far as the first $(\text{NH}_4)_2\text{SO}_4$ fractionation. The enzyme preparation was suspended in a buffer containing 50 mM-Mops (K^+ salt), 2.95 M- $(\text{NH}_4)_2\text{SO}_4$, 20% (v/v) glycerol and 2 mM-dithiothreitol, pH 7.20, and stored at -20°C . Before use, the preparation was centrifuged for 30 min at 4500 *g* and the pellet resuspended to a protein concentration of 2 mg/ml in 50 mM-Mops (K^+ salt), pH 7.20. The preparation contained carbamoylphosphate synthetase (75 nmol/min per mg at 30°C), ornithine transcarbamoylase (313 nmol/min per mg) and some ATPase activity (10 nmol/min per mg).

Details of the assay were as follows. Samples (0.2 ml) of liver extract were added to 0.2 ml of 50 mM-Mops (K^+ salt), pH 7.20, containing 0–1 nmol of *N*-acetylglutamate. To each incubation was added 0.5 ml of a medium containing 50 mM-Mops (K^+ salt), 30 mM- MgCl_2 , 40 mM-ornithine, 20 mM- NH_4Cl , 10 mM-ATP, 10 mM- KHCO_3 and 2 μCi of $\text{NaH}^{14}\text{CO}_3$ at pH 7.20. The reaction, at 37°C , was started by adding 0.1 ml of enzyme preparation and terminated after 10 min by adding 25 μl of 70% (w/v) HClO_4 . Precipitated protein was removed by centrifugation for 2 min at 10000 *g*, and incorporation of $\text{H}^{14}\text{CO}_3^-$ into acid-stable products was determined as described by McGivan *et al.* (1976). For each determination this procedure was repeated with, instead of 0.2 ml of liver extract, 0.1 ml of liver extract plus 0.1 ml of extract blank (i.e. 3.5% HClO_4 neutralized and treated in the same way as for the liver extract). An assay blank was determined by replacing the liver extract with 0.2 ml of extract blank.

For each volume of liver extract assayed, radioactivity (c.p.m.) in acid-stable ^{14}C -labelled material was plotted against added *N*-acetylglutamate. The sensitivity of the assay as described was typically 1000 c.p.m./nmol of *N*-acetylglutamate. The *N*-acetylglutamate content of the sample was calculated as described by Meijer & van Woerkom (1978). As determined by this method, the content of *N*-acetylglutamate in control liver was 22 nmol/g, which is comparable with values of 28.5 nmol/g (Gomez *et al.*, 1983), 22 nmol/g (Saheki *et al.*, 1978), 28 nmol/g (Shigesada & Tatibana, 1971) and 35 nmol/g (Stewart & Walser, 1980).

Materials

Enzymes and coenzymes were from Sigma (London) Chemical Co. and from Boehringer Corp. (London). Glucagon was from Sigma.

$\text{NaH}^{14}\text{CO}_3$ (56 Ci/mol) was obtained from The Radiochemical Centre.

Results and discussion

Glucagon injected into rats caused a rapid increase in hepatic glucose content (Table 1). Results (not shown) of control experiments indicated that the hormone alone was the cause of this increase, as no change in rat liver glucose content was observed 20 min after a sham injection. Concomitantly there was an increased hepatic content of *N*-acetylglutamate (Table 1). The increase was observed 2 min after glucagon administration, although it was not statistically significant until 10 min after injection, when it attained an approximately constant value. The magnitude of the increase in *N*-acetylglutamate observed here (40%) is similar to that in mitochondria isolated from glucagon-injected rats (Hensgens *et al.*, 1980). *N*-Acetylglutamate measured in whole liver is primarily of mitochondrial origin (Shigesada & Tatibana, 1971; Hensgens *et al.*, 1980).

Glucagon had no effect on hepatic glutamine content until 15 min after glucagon administration, when a decrease was observed (Table 1). This is consistent with an activation by glucagon of glutaminase *in vivo*, but only after a lag period. A similar lag in the activation of glutaminase by glucagon was observed *in vitro* with isolated hepatocytes (Joseph & McGivan, 1978). However, this interpretation is not unequivocal, as it assumes that other aspects of hepatic glutamine metabolism were invariant for the duration of the experiment.

Glucagon caused a decrease in hepatic glutamate content within 2 min (Table 1). A decrease has previously been observed *in vivo* 15 min (Cook *et al.*, 1977) and 20 min (Siess & Wieland, 1978) after glucagon administration. The glutamate content attained a minimum value 5 min after hormone injection and then started to return to control values (Table 1). It has been shown that in hepatocytes glucagon caused a decrease in cell glutamate content that reflected a change in glutamate concentration in the mitochondrial matrix (Siess *et al.*, 1977), the subcellular location of *N*-acetylglutamate synthetase (acetyl-CoA: L-glutamate *N*-acetyltransferase; EC 2.3.1.1) (Shigesada & Tatibana, 1971). Control experiments indicated that the observed changes in metabolites were due to glucagon. No effect was observed when hormone vehicle alone was injected.

The postulate that *in vivo* a causal relationship exists between the activation of liver glutaminase by glucagon and an increase in the hepatic content of *N*-acetylglutamate is not supported by the data in Table 1, since the increase in *N*-acetylglutamate was temporally associated with a decrease, not an

Table 1. *Effects of glucagon on metabolite concentrations in rat liver*

Rats were injected with glucagon and their livers freeze-clamped at the times indicated as described in the Materials and methods section. The zero-time values are for rats not injected. Results (per g wet weight of liver) are presented as means $\pm$ S.E.M. for the numbers of observations indicated in parentheses. Statistical significance of results with respect to the appropriate zero-time control was determined by Student's *t* test: *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$.

Time (min)	Glucose ($\mu\text{mol/g}$)	Glutamine ($\mu\text{mol/g}$)	Glutamate ($\mu\text{mol/g}$)	N-Acetylglutamate (nmol/g)
0	6.27 $\pm$ 0.23 (5)	4.97 $\pm$ 0.25 (5)	2.09 $\pm$ 0.15 (6)	21.9 $\pm$ 1.4 (10)
2	8.90 $\pm$ 0.29 (5)***	4.99 $\pm$ 0.42 (5)	1.07 $\pm$ 0.04 (5)**	25.1 $\pm$ 2.5 (6)
5	9.96 $\pm$ 0.28 (5)***	5.05 $\pm$ 0.45 (5)	0.86 $\pm$ 0.08 (5)***	27.1 $\pm$ 2.4 (6)
10	11.62 $\pm$ 0.33 (5)***	5.20 $\pm$ 0.42 (5)	1.27 $\pm$ 0.22 (6)*	30.4 $\pm$ 2.3 (6)**
15	11.88 $\pm$ 0.21 (3)***	3.84 $\pm$ 0.41 (3)*	0.98 $\pm$ 0.07 (3)**	33.1 $\pm$ 6.9 (3)
20	11.25 $\pm$ 0.76 (4)***	3.27 $\pm$ 0.22 (4)**	1.63 $\pm$ 0.34 (4)	31.4 $\pm$ 3.9 (5)*

increase, in liver glutamate. The rapid decrease in glutamate content (Table 1) is possibly indirectly caused by an activation of oxoglutarate dehydrogenase, since glucagon has been shown to cause a decrease in hepatic oxoglutarate content *in vivo* (Cook *et al.*, 1977; Siess & Wieland, 1978).

The mechanism by which glucagon caused an increase in hepatic N-acetylglutamate content *in vivo* and the relationship between this increase and the rate of urea synthesis remains to be established. Possible mechanisms, discussed by Hensgens *et al.* (1980), could involve an increase in either arginine (an activator of N-acetylglutamate synthetase) or acetyl-CoA, or decreased mitochondrial efflux of N-acetylglutamate.

In summary, *in vivo* glucagon caused both an activation of liver glutaminase and an increase in the hepatic content of N-acetylglutamate. The increase in N-acetylglutamate, however, did not appear to be a consequence of glutaminase activation.

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