Hormonal stimulation of mitochondrial glutaminase

Effects of vasopressin, angiotensin II, adrenaline and glucagon

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Adrenaline (through α_1 -adrenoceptors), vasopressin and angiotensin II stimulate mitochondrial glutaminase activity. This stimulation probably contributes to the ureogenic effect of these hormones. The activity of the enzyme is sensitive to Ca^{2+} depletion. A role of Ca^{2+} in hormonal modulation of glutaminase activity is suggested.

Stimulation of ureogenesis from glutamine by vasopressin, angiotensin II and adrenaline (through α_1 -adrenoceptors) has been reported (Corvera & García-Sáinz, 1981, 1982). This stimulation is accompanied by an enhanced rate of citrulline synthesis by mitochondria isolated from hormone-treated hepatocytes (Corvera & García-Sáinz, 1982; Titheradge & Haynes, 1980). Interestingly, the magnitude of the stimulation of urea synthesis by the hormones was different (glucagon > adrenaline > angiotensin II $\geq$ vasopressin), whereas their effects on citrulline synthesis by isolated mitochondria were nearly identical when NH_4Cl was used as the nitrogen source. However, when glutamine was used instead of NH_4Cl , mitochondria isolated from cells treated with adrenaline or glucagon displayed an enhancement in citrulline-synthesizing capacity, whereas no clear effect was observed with mitochondria isolated from cells treated with vasopressin or angiotensin II (Corvera & García-Sáinz, 1982). These data raised the possibility that the activity of mitochondrial glutaminase could be affected to different extents by the hormones. Stimulation of the activity of this enzyme by glucagon has previously been demonstrated (Lacey *et al.*, 1981). In the present paper, the effects of vasopressin, angiotensin II and adrenaline on the activity of mitochondrial glutaminase are reported. Unlike glucagon, these hormones seem to act through cyclic AMP-independent, Ca^{2+} -dependent, mechanisms. Thus the role of extracellular Ca^{2+} in modulating the basal and hormonally stimulated activities of this enzyme are also shown.

Materials and methods

L-Adrenaline, [arginine]vasopressin, angiotensin II, rotenone and L-propranolol were obtained from Sigma Chemical Co. Collagenase was obtained from Worthington, and bovine serum albumin (fraction V) from Reheis. Glucagon was generously given by Eli Lilly. Other reagents used were of the best quality available.

Female Wistar rats, weighing approx. 200 g, fed *ad libitum* were employed. Hepatocytes were isolated by the method of Berry & Friend (1969) as modified by Tolbert *et al.* (1980). Hepatocytes (60 mg wet wt./ml) were incubated for 10 min with maximally effective doses of the hormones (10 munits of vasopressin/ml, 1 μM -angiotensin II, 10 μM -adrenaline + 10 μM -propranolol, and 0.1 μM -glucagon) in Krebs-Ringer bicarbonate buffer, supplemented with 10 mM-glucose, 10 mM-glutamine, 2 mM-ornithine and 1% bovine serum albumin, saturated with O_2/CO_2 (19:1), pH 7.4 at 37°C. Ca^{2+} and EGTA were added as noted in the Results section. Incubations were ended by centrifuging the cells for 20 s at top speed in a clinical centrifuge; the medium was discarded and the cells were washed once with ice-cold mitochondria isolation medium, which consisted of 250 mM-mannitol, 20 mM-Tris/HCl and 1 mM-EGTA, pH 7.4. A crude mitochondrial preparation was obtained from the cell pellets as described by Hensgens *et al.* (1980). Glutaminase activity was measured as the production of glutamate from glutamine during 10 min, in a medium containing 20 mM-glutamine, 75 mM-Tris/HCl, 15 mM-KCl, 5 mM- K_2HPO_4 , 16 mM- KHCO_3 , 10 mM-succinate, 3 mM- MgCl_2 , 5 μg of rotenone/ml, 25 mM-mannitol and 0.1 mM-EGTA (derived from the

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Fig. 1. Hormonal stimulation of glutaminase of mitochondria isolated from cells incubated with different concentrations of Ca^{2+}

Hepatocytes were incubated with maximally effective doses of the indicated hormones ($\square$, control; $\blacksquare$, vasopressin; ▤ , angiotensin II; ▨ , adrenaline + propranolol; ▩ , glucagon) and various concentrations of Ca^{2+} : (a) 1.25 mM- Ca^{2+} ; (b) no Ca^{2+} , plus 1 mM-EGTA; (c), no Ca^{2+} , no EGTA. Mitochondria were then isolated and glutaminase activity was measured as described in the text. Results are expressed as percentages of control values, which were: (a) 61.6 ± 6 , (b) 17.3 ± 2 and (c) 33.2 ± 5 nmol of glutamate/10 min per mg of protein. Values plotted are the means, and vertical lines represent S.E.M. of duplicate incubations from three to five cell preparations. Statistical significance versus control is indicated: * $P < 0.01$, ** $P < 0.005$, *** $P < 0.001$.

mitochondrial suspension) at 25°C, pH 7.4. The concentration of rotenone used completely inhibits the metabolism of glutamate (Joseph & McGivan, 1978). Glutamate was assayed enzymically (Bernt & Bergmeyer, 1974) in neutralized HClO_4 extracts of the mitochondrial suspension. Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Results

Stimulation of mitochondrial glutaminase activity in mitochondria isolated from cells treated with vasopressin, angiotensin II, adrenaline plus propranolol, or glucagon is presented in Fig. 1(a). Although all the hormones produced a significant stimulation, glucagon and adrenaline were considerably more effective than the vasopressor peptides. The stimulation produced by adrenaline was completely blocked by low concentrations (1 μM) of the α_1 -adrenergic antagonist prazosin (results not shown). The dependency of the hormonal effects on extracellular Ca^{2+} was studied by using mitochondria isolated from cells washed in buffer prepared without Ca^{2+} and incubated in this buffer supplemented with 1 mM-EGTA. The results obtained are presented in Fig. 1(b). Under these conditions, both glucagon and adrenaline stimulated glutaminase activity, whereas neither vasopressin nor angio-

Table 1. Basal glutaminase activity of mitochondria isolated from cells incubated with different concentrations of Ca^{2+}

Hepatocytes were incubated as described in the Materials and methods section with the additions indicated below. Mitochondria were then isolated and glutaminase activity was measured as described in the text. Results are the means $\pm$ S.E.M. for duplicate incubations from three cell preparations.

Additions (mM)		Glutaminase activity (nmol of glutamate/10 min per mg of protein)
EGTA	CaCl_2	
—	1.25	61.6 ± 6
—	—	33.2 ± 5
1	—	17.3 ± 2
1	1.25	49.6 ± 5
1	2.50	50.2 ± 2
1	5	52.9 ± 8
1	10	35.8 ± 6

tensin II produced significant effects. However, the basal activity of the enzyme was much lower than when Ca^{2+} was present during the cell incubation. The variations in the basal activity of mitochondrial glutaminase at different Ca^{2+} concentrations in the cell incubation buffer were studied, and the results are presented in Table 1. The highest basal activity was observed when cells were incubated in the presence of 1.25 mM- Ca^{2+} . When Ca^{2+} was omitted from the cell incubation buffer (which resulted in a

Ca^{2+} concentration of 10–100 μM owing to contamination of the reagents used to prepare the buffer, and to cell lysis) the activity decreased to approx. 53% of this value. When 1 mM-EGTA was added to this Ca^{2+} -free medium, the basal glutaminase activity fell to only approx. 28% of the value obtained in the presence of 1.25 mM- Ca^{2+} . The addition of CaCl_2 to these cells re-established the activity of the enzyme in subsequently isolated mitochondria. These results indicate that cell Ca^{2+} depletion modifies the activity of mitochondrial glutaminase. To study the requirements for extracellular Ca^{2+} for hormonal stimulation of glutaminase activity, hepatocytes that were incubated in the absence of Ca^{2+} but without EGTA were used.

The results are shown in Fig. 1(c); in these experiments glucagon and adrenaline significantly stimulated glutaminase activity, and, although vasopressin and angiotensin II produced in some experiments a slight increase in the activity of the enzyme, the effect was not reproducible.

Discussion

Hormonal stimulation of ureogenesis seems to involve modulation at several steps of this metabolic pathway; stimulation of mitochondrial glutaminase by glucagon has been reported (Lacey *et al.*, 1981). In the present paper, the stimulation of this enzyme by adrenaline (through α_1 -adrenoceptors), vasopressin and angiotensin II is presented. The metabolic effects of vasopressin, angiotensin II and α_1 -adrenergic agonists seem to be related to Ca^{2+} fluxes through the plasma membrane and release of this ion from internal stores, including mitochondria (Blackmore *et al.*, 1982). After this release, mitochondria, isolated from hepatocytes treated with α_1 -adrenergic amines, display enhanced rates of Ca^{2+} uptake and prolonged retention of this ion (Taylor *et al.*, 1980). Our data suggest that the activity of mitochondrial glutaminase is sensitive to the mitochondrial Ca^{2+} concentration. Thus the possibility exists that the effects of the hormones on the activity of the enzyme might be mediated by the changes in mitochondrial ionic fluxes. This interpretation is supported by the marked decrease in the activity of the enzyme when cells were incubated with no Ca^{2+} and 1 mM-EGTA, which decreases intracellular Ca^{2+} stores. When Ca^{2+} was added back to the incubation buffer, the activity of the enzyme returned to near-normal values. This, however, does not rule out the possibility that Ca^{2+} depletion may result in changes in the concentration of other ions (Lardy & Merryfield, 1981) or metabolites in the environment of the enzyme which might themselves modulate its activity.

Joseph *et al.* (1981) found no effect of EGTA on the activity of the mitochondrial glutaminase when

the chelator is added directly to the mitochondria. Our experiments are not comparable, since we studied the effects of EGTA in whole cells, whereas they studied the effect on isolated mitochondria. The possible existence of cytosolic factors that modulate Ca^{2+} efflux from liver mitochondria (Fleschner *et al.*, 1982) further stresses this difference. The stimulation of mitochondrial glutaminase may be partially responsible for the effects of the hormones on urea biosynthesis from glutamine. Both glutaminase activity and urea synthesis are strongly stimulated by adrenaline and glucagon, and to a less extent by vasopressin and angiotensin II. Also, the extracellular Ca^{2+} requirements for the stimulation of mitochondrial glutaminase by the hormones are similar to those observed for the stimulation of urea synthesis from glutamine (Corvera & García-Sáinz, 1982). However, factors besides glutaminase activation also participate in hormonal stimulation of ureogenesis by vasopressin, angiotensin II, adrenaline and glucagon; this is evidenced by the enhanced citrulline synthesis from NH_4Cl by mitochondria isolated from hormone-treated hepatocytes. In summary, glucagon, adrenaline, vasopressin and angiotensin II stimulated the activity of liver mitochondrial glutaminase; this stimulation seems to contribute to the ureogenic action of these hormones. A role of Ca^{2+} in the hormonal stimulation of this enzyme is suggested.

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