Effect of phenylephrine on glutamate and glutamine metabolism in isolated perfused rat liver

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1. Addition of phenylephrine to isolated perfused rat liver is followed by an increased $^{14}\text{CO}_2$ production from $[1\text{-}^{14}\text{C}]\text{glutamate}$, $[1\text{-}^{14}\text{C}]\text{glutamine}$, $[\text{U-}^{14}\text{C}]\text{proline}$ and $[3\text{-}^{14}\text{C}]\text{pyruvate}$, but by a decreased $^{14}\text{CO}_2$ production from $[1\text{-}^{14}\text{C}]\text{pyruvate}$. Simultaneously, there is a considerable decrease in tissue content of 2-oxoglutarate, glutamate and citrate. 2. Stimulation of $^{14}\text{CO}_2$ production from $[1\text{-}^{14}\text{C}]\text{glutamate}$ is also observed in the presence of amino-oxyacetate, suggesting a stimulation of glutamate dehydrogenase and 2-oxoglutarate dehydrogenase fluxes by phenylephrine. Inhibition of pyruvate dehydrogenase flux by phenylephrine is due to an increased 2-oxoglutarate dehydroxygenase flux. 3. Phenylephrine stimulates glutaminase flux and inhibits glutamine synthetase flux to a similar extent, resulting in an increased hepatic glutamine uptake. Whereas the effects of NH_4^+ ions and phenylephrine on glutaminase flux were additive, activation of glutaminase by glucagon was considerably diminished in the presence of phenylephrine. 4. The reported effects are largely overcome by prazosin, indicating the involvement of α -adrenergic receptors in the action of phenylephrine. 5. It is concluded that stimulation of gluconeogenesis from various amino acids by phenylephrine is due to an increased flux through glutamate dehydrogenase and the citric acid cycle.

α -Adrenergic agonists are potent activators of hepatic gluconeogenesis from amino acids. The mechanism is cyclic AMP-independent and has been ascribed to changes in the subcellular concentrations of Ca^{2+} (Exton, 1980; Williamson *et al.*, 1981; Joseph *et al.*, 1981; Taylor *et al.*, 1983). Possible sites of α -adrenergic stimulation of gluconeogenesis include pyruvate kinase (Garrison *et al.*, 1979) pyruvate carboxylase (Garrison & Borland, 1979), phosphoenolpyruvate carboxykinase (Merryfield & Lardy, 1982) and the transfer of reducing equivalents from the cytosol into the mitochondria (Kneer *et al.*, 1979; Yip & Lardy, 1981). Further, α -oxoglutarate dehydrogenase flux has been shown to be stimulated by α -adrenergic agents (Taylor *et al.*, 1983; Sugden & Watts, 1983) and vasopressin (Staddon & McGivan, 1984). During their catabolism, several amino acids, e.g. glutamine or proline, are converted into glutamate. We therefore studied the effect of phenylephrine on glutamate metabolism and its relation to citric acid-cycle and pyruvate dehydrogenase fluxes. The latter has been shown to undergo changes exhibiting a similar time course to that of phenylephrine-

induced Ca^{2+} efflux from perfused rat liver (Sies *et al.*, 1983).

Increased gluconeogenesis from glutamine by α -adrenergic agonists has been attributed to a stimulation of glutaminase (Joseph *et al.*, 1981; Corvera & Garcia-Sainz, 1983) and is accompanied by a fall in intracellular glutamate (Ochs & Lardy, 1983). However, hepatic glutamine metabolism involves fluxes through glutaminase and glutamine synthetase, and the simultaneous activity of both enzymes in the intact liver has been shown (Häussinger & Sies, 1979; Häussinger *et al.*, 1983). In view of the discovery of the intercellular glutamine cycle (Häussinger, 1983), i.e. the simultaneous degradation and resynthesis of glutamine in the periportal and the perivenous areas of the liver acinus, respectively, the effect of phenylephrine on the simultaneous fluxes through glutamine synthetase and glutaminase was studied.

Materials and methods

Haemoglobin-free liver perfusion

Livers of male Wistar rats (100–200 g body wt.), fed on stock diet (Altromin) *ad libitum*, were

perfused as described previously (Sies, 1978), without recirculation of the perfusate, with bicarbonate-buffered Krebs–Henseleit saline plus 2.1 mM-lactate and 0.3 mM-pyruvate (sodium salts), if not indicated otherwise. Perfusion fluid was equilibrated with O₂/CO₂ (19:1). Perfusate flow was approx. 4 ml/min per g and was kept constant throughout the individual perfusion experiment. The temperature was 37°C.

Assays

Perfusate. The concentrations of lactate, pyruvate, acetoacetate, 3-hydroxybutyrate, glutamate, glutamine and ammonia were measured by procedures based on those described in Bergmeyer (1974).

Tissue. Tissue samples (500–1000 mg) of perfused liver, taken by freeze-stop technique, and neutralized HClO₄ extracts were prepared as described by Häussinger *et al.* (1975). Concentrations of glutamate, 2-oxoglutarate citrate and NADP⁺ were determined by enzymic methods based on the procedures described by Bergmeyer (1974).

¹⁴CO₂ production from 1-¹⁴C-labelled compounds was measured as described by Häussinger *et al.* (1975). Perfusate was pooled over 0.5 or 1 min periods and was collected in stoppered conical flasks. CO₂ was trapped in phenylethylamine and radioactivity was determined by liquid-scintillation spectrometry, with Packard Tri-Carb 300C scintillant. Rates of ¹⁴CO₂ release were calculated on the basis of the specific radioactivity of ¹⁴C-labelled compounds in the perfusate. Influent perfusate contained 0.5–1 nCi of [1-¹⁴C]pyruvate or of [3-¹⁴C]pyruvate/ml or 0.5 nCi of [1-¹⁴C]glutamine/ml or 1 nCi of [1-¹⁴C]glutamate or 2 nCi of [U-¹⁴C]proline/ml.

Glutaminase flux was measured as ¹⁴CO₂ production from [1-¹⁴C]glutamine as described previously (Häussinger *et al.*, 1983). Glutamine synthetase flux was calculated as net glutamine release plus glutaminase flux (Häussinger *et al.*, 1983).

Preparation of [1-¹⁴C]glutamine

[1-¹⁴C]Glutamine was prepared from [1-¹⁴C]glutamate as described by Häussinger *et al.* (1983) by the procedures described by Baverel & Lund (1979).

Chemicals

All enzymes, pyruvate, 2-oxoglutarate, NAD⁺ and NADH were from Boehringer (Mannheim, Germany). ¹⁴C-labelled amino acids and pyruvate were from Amersham Buchler (Frankfurt, Germany). L-Lactic acid was from Roth (Karlsruhe, Germany). Phenylephrine, vasopressin and gluta-

minase were from Sigma (St. Louis, MO, U.S.A.). All other chemicals were from Merck (Darmstadt, Germany).

Results

Effect of phenylephrine on glutamate metabolism in isolated perfused rat liver

In isolated perfused rat liver, addition of phenylephrine is followed by a transitory increase in ¹⁴CO₂ production from [1-¹⁴C]glutamate within the first 2 min (Fig. 1). This is not paralleled by an increase in glutamate uptake (Fig. 1). Simultaneously, the tissue contents of 2-oxoglutarate and glutamate are decreased by about 60% (Table 1). This decrease is observed within 1.5 min of phenylephrine addition and is still found 10 min after the onset of phenylephrine. The stimulation of ¹⁴CO₂ production from [1-¹⁴C]glutamate is accompanied by an increased ammonia production by the liver, indicating an increased glutamate dehydrogenase flux (results not shown). Stimulation of ammonia and ¹⁴CO₂ production from [1-¹⁴C]glutamate by phenylephrine is also found in the presence of amino-oxyacetate, an inhibitor of transaminases (Fig. 2). However, with amino-oxyacetate present, the stimulatory effect of phenylephrine on ¹⁴CO₂ production from [1-¹⁴C]glutamate is diminished by about 40%. These data

Fig. 1. Effect of phenylephrine on ¹⁴CO₂ production from [1-¹⁴C]glutamate (●) and on effluent glutamate concentration (○)

For experimental details see the Materials and methods section.

Table 1. Effect of phenylephrine (5 μ M) on the tissue content of oxoglutarate, glutamate, citrate and NADP⁺. Two samples were taken from each liver at 30 and 40 min (see the Materials and methods section). Addition of phenylephrine was started at 28.5 min of perfusion. Data are given as means $\pm$ S.E.M. for *n* perfusion experiments.

Condition	Time of freeze-stop	Content (nmol/g of liver)				<i>n</i>
		Oxoglutarate	Glutamate	Citrate	NADP ⁺	
Control	30 min	441 $\pm$ 66	2286 $\pm$ 93	307 $\pm$ 40	42 $\pm$ 1	5
	40 min	402 $\pm$ 54	2506 $\pm$ 309	289 $\pm$ 46	38 $\pm$ 3	5
Phenylephrine	30 min	102 $\pm$ 18	1242 $\pm$ 114	145 $\pm$ 14	31 $\pm$ 3	7
	40 min	119 $\pm$ 24	1332 $\pm$ 73	154 $\pm$ 25	31 $\pm$ 3	7

Fig. 2. Stimulation of production of ammonia (O) and ¹⁴CO₂ (●) from [1-¹⁴C]glutamate by phenylephrine in the presence of amino-oxoacetate (AOA).

For experimental details see the Materials and methods section.

show that phenylephrine stimulates flux through the sequence of glutamate dehydrogenase, glutamate transamination and 2-oxoglutarate dehydrogenase. The transitory nature of stimulation of ¹⁴CO₂ production from [1-¹⁴C]glutamate, is probably due to a rate-limitation of glutamate oxidation by its transport across the plasma membrane (Ross *et al.*, 1967; Sips *et al.*, 1982; Häussinger & Gerok, 1983) after depletion of intracellular glutamate. This is supported by the observation that, when labelled glutamate is generated intracellularly from added [U-¹⁴C]proline (Fig. 3) or [1-¹⁴C]glutamine (Fig. 4), increased ¹⁴CO₂ production is seen throughout the duration of phenylephrine addition.

Addition of phenylephrine is accompanied by an increase in the ratios of the indicator metabolite couples lactate/pyruvate and 3-hydroxybutyrate/acetoacetate (Fig. 5), as was also shown in livers of starved rats (Taylor *et al.*, 1983), indicating a more reduced state of the cytosolic and mitochondrial NADH systems. In addition, there is a decrease in the tissue content of NADP⁺, also

suggesting an increased NADPH/NADP⁺ ratio (Table 1). This might be due to increased NADPH formation at glutamate dehydrogenase and at NADP-dependent isocitrate dehydrogenase, as shown by the considerable decrease in tissue citrate (Table 1) in the presence of an increased ¹⁴CO₂ production from [3-¹⁴C]pyruvate (Fig. 6). Although the cellular nicotinamide nucleotides are more reduced, citric acid cycle flux is increased by phenylephrine, as evidenced by the stimulation of ¹⁴CO₂ production from [1-¹⁴C]glutamate (Fig. 1) and [3-¹⁴C]pyruvate (Fig. 6).

The observed effects of phenylephrine on ¹⁴CO₂ production from [1-¹⁴C]glutamate are suppressed by prazosin, indicating the involvement of α -adrenergic receptors in the phenylephrine response (Fig. 7).

Effect of phenylephrine on pyruvate dehydrogenase flux

It has been shown that addition of phenylephrine leads to a decreased rate of ¹⁴CO₂ production from [1-¹⁴C]pyruvate (Sies *et al.*, 1983), even though there was no decreased proportion of active (dephospho) form of pyruvate dehydrogenase in the liver tissue. This inhibition of pyruvate dehydrogenase flux by phenylephrine is not explained by a more reduced state of mitochondrial NADH system, because inhibition of pyruvate dehydrogenase flux is transitory (Fig. 8), whereas an increase in the 3-hydroxybutyrate/acetoacetate ratio is observed throughout the addition of phenylephrine (Fig. 5). The similar time course of the effects of phenylephrine on ¹⁴CO₂ production from [1-¹⁴C]glutamate and [1-¹⁴C]pyruvate suggests an inhibition of pyruvate dehydrogenase flux as a consequence of an increased 2-oxoglutarate dehydrogenase flux, which has been demonstrated previously in isolated perfused rat liver (Häussinger *et al.*, 1982). This is further evidenced by the experiment in Fig. 9, showing that the inhibition of ¹⁴CO₂ production from [1-¹⁴C]pyruvate by phenylephrine is no longer observed when 2-oxoglutarate dehydrogenase flux is decreased in favour of glutamate formation from 2-oxoglutarate

Fig. 3. Effect of phenylephrine on $^{14}\text{CO}_2$ production from $[\text{U-}^{14}\text{C}]\text{proline}$ (●) and urea N production (○). For experimental details see the Materials and methods section.

Fig. 4. Effect of phenylephrine on $^{14}\text{CO}_2$ production from $[\text{1-}^{14}\text{C}]\text{glutamine}$ (●) and glutamine concentration in effluent (○). Note the different scales on the right and left ordinates.

Fig. 5. Effect of phenylephrine on the profiles of lactate/pyruvate (○) and 3-hydroxybutyrate/acetoacetate (●) ratios in effluent

Livers were perfused with a medium containing lactate (0.5 mM), pyruvate (0.5 mM), glutamate (0.1 mM), acetoacetate (0.2 mM) and DL-hydroxybutyrate (0.4 mM).

Fig. 6. Effect of phenylephrine on $^{14}\text{CO}_2$ production from [3- ^{14}C]pyruvate

Influent perfusate contained lactate (0.5 mM), pyruvate (0.5 mM) and glutamate (0.1 mM).

Fig. 7. Effect of phenylephrine on $^{14}\text{CO}_2$ production from [1- ^{14}C]glutamate (0.1 mM) in the absence (●) or presence (○) of prazosin (3 ̢M)

Data are from two different perfusion experiments. Influent perfusate contained lactate (0.5 mM), pyruvate (0.5 mM), acetoacetate (0.2 mM) and DL-hydroxybutyrate (0.4 mM).

Fig. 8. Effect of phenylephrine on $^{14}\text{CO}_2$ production from [1- ^{14}C]pyruvate (▲) and [1- ^{14}C]glutamate (●)

Influent perfusate contained lactate (0.5 mM), pyruvate (0.5 mM) and glutamate (0.1 mM).

Fig. 9. Lack of effect of phenylephrine on $^{14}\text{CO}_2$ production from [1- ^{14}C]pyruvate (●) and effluent pyruvate concentration (○) in presence of NH_4Cl

The concentrations of lactate and pyruvate were each 0.5 mM. Pyruvate dehydrogenase flux is stimulated in presence of NH_4^+ ions (Häussinger *et al.*, 1975).

by addition of NH_4^+ ions. Thus the inhibition of pyruvate dehydrogenase flux by phenylephrine is a secondary effect resulting from an increased flux through 2-oxoglutarate dehydrogenase.

Effect of phenylephrine on glutamine metabolism in isolated perfused rat liver

Studies on gluconeogenesis from glutamine suggested an activation of glutaminase by phenylephrine (Joseph *et al.*, 1981; Corvera & Garcia-Sainz, 1983). However, glutaminase and glutamine synthetase are simultaneously active in liver (Häussinger & Sies, 1979; Häussinger *et al.*, 1983), resulting in an intercellular glutamine cycle as a consequence of hepatocyte heterogeneity in glutamine and ammonia metabolism (Häussinger, 1983). As shown in Fig. 4, addition of phenylephrine leads to an increase in glutamine uptake, accompanied by an increased $^{14}\text{CO}_2$ production from [1- ^{14}C]glutamine, reaching a steady state after about 10 min. The initial increase in $^{14}\text{CO}_2$ production from [1- ^{14}C]glutamine within the first 5 min after phenylephrine infusion is probably due to degradation of intracellular [1- ^{14}C]glutamate derived from [1- ^{14}C]glutamine (cf. Fig. 1), whereas $^{14}\text{CO}_2$ production from [1- ^{14}C]glutamine during

the steady state 10 min after phenylephrine addition represents glutaminase flux. The increase in glutamine uptake by phenylephrine is due to a stimulation of glutaminase flux and an inhibition of glutamine synthetase flux (Table 2). As shown in Table 2, the stimulatory effects of NH_4^+ ions (Häussinger *et al.*, 1975) and phenylephrine on glutaminase flux are additive, suggesting different modes of activation. Also, with no added ammonia the effects of phenylephrine on glutaminase and glutamine synthetase fluxes are of similar magnitude but in opposite directions. The inhibition of glutamine synthetase flux by phenylephrine may be explained by the considerable decrease in tissue glutamate (Table 1), in view of the relatively high K_m for glutamate (5 mM) of glutamine synthetase in the presence of 10 mM- Mg^{2+} (Deuel *et al.*, 1978). However, it is at present unclear whether the phenylephrine-induced decrease in cellular glutamate involves all hepatocytes of the liver acinus to a similar extent. Whereas the effects of phenylephrine and glucagon on glutamine synthetase flux are additive, activation of glutaminase by glucagon is strongly inhibited in the presence of phenylephrine (Table 2).

The effects of phenylephrine on glutamine

Table 2. Effect of NH_4^+ , glucagon and phenylephrine on glutaminase and glutamine synthetase fluxes in isolated perfused rat liver in the presence of a physiological portal glutamine concentration (0.6 mM)

Data (nmol per min/g) are given as means $\pm$ S.E.M. for the numbers of perfusion experiments shown in parentheses. Net glutamine release is negative when there is a net uptake of glutamine. The concentrations of NH_4^+ , glucagon and phenylephrine in influent perfusate were 0.8 mM, 0.1 μM and 5 μM respectively. Glutaminase and glutamine synthetase fluxes were determined as described by Häussinger *et al.* (1983).

Treatment	Glutaminase flux	Glutamine synthetase flux	Net glutamine release	Rate of the intercellular glutamine cycle
None (15)	67 $\pm$ 3	148 $\pm$ 10	81 $\pm$ 11	67 $\pm$ 3
Phenylephrine (6)	88 $\pm$ 4	113 $\pm$ 9	25 $\pm$ 8	88 $\pm$ 4
Glucagon (7)	231 $\pm$ 32	84 $\pm$ 20	-147 $\pm$ 30	84 $\pm$ 20
NH_4Cl (4)	157 $\pm$ 8	239 $\pm$ 16	72 $\pm$ 17	157 $\pm$ 8
Glucagon plus phenylephrine (4)	152 $\pm$ 15	64 $\pm$ 13	-88 $\pm$ 22	64 $\pm$ 13
NH_4Cl plus phenylephrine (4)	299 $\pm$ 7	83 $\pm$ 5	-216 $\pm$ 15	83 $\pm$ 5

metabolism are reversed by addition of prazosin (results not shown) and indicate again an involvement of α -adrenergic receptors.

Discussion

The data in this paper show a stimulation of glutamate transamination, glutamate dehydrogenase, 2-oxoglutarate dehydrogenase and citric acid cycle flux by phenylephrine in livers of fed rats. An increased $^{14}\text{CO}_2$ production from added [1- ^{14}C]oxoglutarate in perfused livers from starved rats has also been shown by Taylor *et al.* (1983) and, like $^{14}\text{CO}_2$ production from [1- ^{14}C]glutamate in our experiments, the effect was observed only within the first few minutes after phenylephrine addition. The transitory nature of the increased fluxes when glutamate or 2-oxoglutarate is substrate is due to intramitochondrial substrate depletion as a consequence of the restricted permeability of the liver plasma membrane. However, when the intracellular supply of labelled glutamate is guaranteed, as by addition of [1- ^{14}C]glutamine or [U- ^{14}C]proline, increased $^{14}\text{CO}_2$ production is observed throughout the exposure to phenylephrine. Although these metabolic responses exhibit a similar time course to that of phenylephrine-induced Ca^{2+} efflux from the hepatocytes (Sies *et al.*, 1983; Taylor *et al.*, 1983), and pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase are both activated by Ca^{2+} (Denton & McCormack, 1980; McCormack & Denton, 1981), an inverse correlation of flux through these enzymes occurs in the presence of phenylephrine (see the Results section) and during degradation of proline or glutamine in isolated perfused rat liver (Häussinger *et al.*, 1982).

Stimulation of glutamate dehydrogenase flux by phenylephrine increases the mitochondrial ammonia concentration (Fig. 2), which in turn will activate glutaminase (Charles, 1968; Häussinger *et*

al., 1975; Häussinger & Sies, 1979; Verhoeven *et al.*, 1983). However, stimulation of glutaminase flux by phenylephrine is also observed in presence of a maximally activating ammonia concentration (Table 2) above 0.6 mM (Häussinger & Sies, 1979; Verhoeven *et al.*, 1983) suggesting another mechanism of activation. In this respect, a regulatory role of Ca^{2+} has been suggested (Corvera & Garcia-Sainz, 1983). However, in the presence of a physiological portal glutamine concentration of about 0.6 mM (Yamamoto *et al.*, 1974; DuRuisseau *et al.*, 1975), the present experiments show that increased hepatic glutamine uptake by phenylephrine is also due to an inhibition of glutamine synthetase flux with the consequence of a decreased flux through the intercellular glutamine cycle in presence of NH_4^+ ions. Interestingly, the stimulatory effect of glucagon on glutaminase activity (Joseph & McGivan, 1978; Häussinger *et al.*, 1983) is diminished in the presence of phenylephrine. This is similar to the reported suppression of the gluconeogenic response by glucagon after Ca^{2+} depletion (Joseph *et al.*, 1981). However, at present, there is no satisfactory explanation for such interactions of Ca^{2+} -dependent and cyclic AMP-dependent stimulation of hepatic metabolism.

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