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PROPIONATE AND SUCCINATE EFFECTS ON ACETYL GLUTAMATE BIOSYNTHESIS BY RAT LIVER MITOCHONDRIA

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SUMMARY

Acetylglutamate biosynthesis in isolated rat liver mitochondria is dependent on glutamate and stimulated by arginine. Propionate and succinate inhibit this glutamate-dependent synthesis and their effects seem to be cumulative.

Hyperammonemia is frequently associated with deficiencies in human propionyl-CoA carboxylase (1, 2), methylmalonyl-CoA mutase, or racemase (3, 4). In propionyl-CoA carboxylase deficiency the hyperammonemia is related to blood propionate concentrations (5). In the rat, propionate inhibits ureogenesis in liver slices (6) and citrullinogenesis in liver mitochondria (7). Also, propionate appears to have no direct effect on the kinetics of carbamylphosphate synthetase I or ornithine carbamylphosphate transferase. Rather, propionate appears to lower the concentration of substrates such as carbamylphosphate (7). Regarding the propionate-induced decrease in carbamylphosphate, two clues are provided : one by studying the intramitochondrial concentration of ATP (ATP being a substrate of carbamylphosphate synthetase I) and one by studying the biosynthesis of acetylglutamate. Since carbamylphosphate biosynthesis is strictly controlled by acetylglutamate, it was of interest to confirm whether propionate inhibits short term acetylglutamate biosynthesis (8), and if this inhibitory effect of propionate is modified by activators of acetylglutamate synthesis such as arginine (9, 10, 11, 12) or by inhibitors such as succinate (12).

MATERIALS AND METHODS

Male Wistar rats (200-300 g) were fed a standard diet provided by Usine d'Alimentation Rationnelle, 91360 Epinay sur Orge, France, ($A0_2$ diet, 19 % protein). Liver mitochondria were isolated according to the method of Hogeboom (13) and suspended in 250 mM mannitol, 2 mM Tris-HCl, pH 7.4. To study acetylglutamate biosynthesis, mitochondria

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(about 5 mg protein) were incubated for 15 min at 25° C in the following medium : 75 mM Tris-HCl, pH 7.4 ; 15 mM KCl ; 1 mM EGTA ; 5 mM KH_2PO_4 ; 16.6 mM $KHCO_3$; 10 mM ornithine ; 10 mM NH_4Cl ; 3 mM ATP ; 10 mM mannitol. Glutamate, propionate and succinate were added as potassium salts. Rotenone was always added with succinate. At the end of incubation, acetylglutamate was assayed by its activating effect on carbamyl phosphate synthetase I, as described by Meijer et al (8). Acetylglutamate (0 to 15 nanomoles) was added as internal standard. [^{14}C] $NaHCO_3$ (specific activity 53 mCi/mmole) was purchased from CEA, Gif sur Yvette, France. Protein determinations were made by the biuret method (14).

RESULTS

When mitochondria were incubated without glutamate, the acetylglutamate concentration was not significantly different from that obtained with non incubated mitochondria, the concentrations being respectively 0.83 $\pm$ 0.16 and 0.99 $\pm$ 0.08 nmoles/15 min/mg protein (30 and 3 experiments). These values were not modified by arginine, an activator of acetylglutamate synthesis nor by succinate plus rotenone, the values being respectively 0.81 $\pm$ 0.06 and 0.81 $\pm$ 0.13 nmoles/15 min/mg protein (4 and 11 experiments).

There was a strong increase (Table I) in acetylglutamate biosynthesis when glutamate was added (3 times the basal levels in 29 experiments, $p < 0.001$) and this increased biosynthesis could still be stimulated by arginine (maximal stimulation from 1.5 mM arginine on).

Table I : Effect of propionate and succinate on acetylglutamate biosynthesis in rat liver mitochondria

compounds added	without propionate	with propionate	Student's test
None	2.40 $\pm$ 0.49 (29)	1.07 $\pm$ 0.23 (13)	$p < 0.001$
Arginine	4.17 $\pm$ 0.69 (8)	1.37 $\pm$ 0.34 (5)	$p < 0.001$
Succinate	1.44 $\pm$ 0.36 (14)	0.84 $\pm$ 0.19 (7)	$p < 0.001$
Succinate + Arginine	2.30 $\pm$ 0.63 (7)	0.84 $\pm$ 0.16 (6)	$p < 0.001$

Mitochondria (about 5 mg protein) were incubated for 15 min at 25°C in the standard medium (described in Materials and Methods) with 16 mM glutamate. Several compounds were added to the medium : at the final concentration of 2 mM arginine, 10 mM succinate plus 16.6 µM rotenone, 8 mM propionate. Number of experiments are given in brackets. Results are expressed in nmoles of acetylglutamate/15 min/mg protein (mean $\pm$ SD).

Table II : Propionate (8 mM) and succinate (10 mM) effects on K_M and V_M when mitochondria were incubated with various glutamate concentrations.

Compounds added	K_M (mM)	V_M
None	8.30 ± 2.75	2.38 ± 0.27
Propionate	12.20 ± 2.27	1.28 ± 0.11
Succinate	14.90 ± 3.89	1.77 ± 0.23

These results were obtained from linear regression plots calculated from 4 experiments without inhibitor and from 2 experiments with each inhibitor (4 glutamate concentrations for each). Mitochondria were incubated at 25° C for 15 min. Results (means ± SD) are expressed for V_M in nmoles acetylglutamate formed/15 min/mg protein.

Therefore, the effects of propionate and succinate on acetylglutamate biosynthesis were tested with glutamate (Table I). Propionate decreased this synthesis while arginine remained a weak activator ($p < 0.05$ in 5 experiments). Succinate was another inhibitor of acetylglutamate biosynthesis (60% of the synthesis obtained with glutamate alone, $p < 0.001$ in 14 experiments). Rotenone, always added with succinate, had no effect on acetylglutamate synthesis. In presence of succinate, arginine remained an activator ($p < 0.001$ in 8 experiments) while propionate completely inhibited acetylglutamate synthesis and arginine was no longer an activator.

The effects of propionate and succinate in presence of varying glutamate concentrations are shown in Table II. Propionate and succinate increased the K_M for glutamate ($p < 0.001$) and decreased V_M by about 40% for succinate ($p < 0.001$) and 50% for propionate ($p < 0.001$).

DISCUSSION

As Meijer et al (8), we observed noticeable acetylglutamate biosynthesis in presence of glutamate, the substrate of acetylglutamate synthetase together with acetyl-CoA. Our results (0.086 ± 0.019 nmole/min/mg protein for 10 mM glutamate) were similar to those of the same authors who reported a production of 0.1 nmole/min/mg protein at 25° C. This biosynthesis is increased by arginine, an activator of the acetylglutamate synthesis in isolated mitochondria (9, 10) and on partially purified acetylglutamate synthetase (10, 11).

In presence of glutamate, propionate considerably decreases acetylglutamate synthesis. This may be explained by two mechanisms : a decrease of the available acetyl-CoA, and an inhibition of acetylglutamate synthetase by propionyl-CoA and by the synthetized propionylglutamate. Actually, propionate is activated in propionyl-CoA via the acetyl-CoA synthetase pathway (15), using CoA and ATP. This decreases the intramitochondrial pool of acetyl-CoA. It has been shown that, when mitochondria are incubated with propionate and carnitine (16), propionylcarnitine is formed in lieu of acetylcarnitine (acylcarnitine and acyl-CoA derivatives are supposed to be in a steady-state). A decrease of acetyl-CoA would explain our finding of a decreased V_M in presence of propionate. This had already been observed with purified acetylglutamate synthetase (12). A decrease of acetyl-CoA would explain the weak stimulation of acetylglutamate biosynthesis observed with arginine. Furthermore, propionyl-CoA may either inhibit acetylglutamate synthetase (unpublished results) or become its substrate, leading to the formation of propionylglutamate, an inhibitor of acetylglutamate synthetase (12) : this might explain the varying Kms observed with propionate.

The inhibitory effect of succinate may also be explained by a decrease of acetyl-CoA levels. Succinate, through the tricarboxylic acid cycle, forms oxaloacetate, which condenses with acetyl-CoA to yield citrate. The same mechanism is proposed by Meijer et al (8) to explain the inhibitory effect of malate on acetylglutamate biosynthesis. The varying K_M observed with succinate may be explained by a direct inhibition of acetylglutamate synthetase (12).

These inhibitions may be compared to the one induced by 4-pentenoic acid (17). This acid, which decreases acetyl-CoA levels (18), inhibits citrullinogenesis in rat liver slices by decreasing ATP and probably also acetylglutamate. Therefore, propionate reacts at least at one level of ammonia metabolism in mitochondria by decreasing acetylglutamate biosynthesis in presence of arginine and glutamate.

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