Differential alterations in branched-chain amino acid decarboxylation in liver of hypophysectomized rats[1]

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Summary. Valine decarboxylation was significantly increased and leucine decarboxylation was significantly decreased in rat liver slices following hypophysectomy. In both normal and hypophysectomized rats decarboxylation of leucine exceeded that of valine in slices whereas the reverse was observed with the respective keto acids and mitochondria.

Valine and leucine are reversibly transaminated in mammalian cells to alpha-ketoisovaleric acid and alpha-ketoisocaproic acid, respectively. The carbon skeletons of these essential amino acids are irreversibly altered by mitochondrial oxidative decarboxylation of the keto acids. Since this reaction irreversibly commits the carbon skeletons derived from valine and leucine to degradation it appears reasonable that it would be subject to control mechanisms[2–5].

Valine and leucine decarboxylation by intact mammalian cells depends on the combined effects of many factors including transport across plasma and mitochondrial membranes, cytoplasmic and mitochondrial transamination, compartmentalization, pool size, and mitochondrial keto acid oxidative decarboxylation. We have recently shown that hypophysectomy causes transient and long term differential modulation of branched-chain alpha-keto acid oxidation in purified mitochondria from rat liver[3,4]. Despite the complexities of branched-chain amino acid degradation, alterations in mitochondrial keto acid oxidation caused by hypophysectomy might be reflected in overall amino acid decarboxylation. In this study we have investigated the effects of hypophysectomy on valine and leucine decarboxylation by liver slices and compared the results with the alterations of mitochondrial oxidation of the corresponding keto acids.

Materials and methods. Male Sprague-Dawley rats were obtained from, and operations were performed by, Charles River Breeding Laboratories, Inc., North Wilmington, Mass. Hypophysectomy was carried out on 150 g rats. All rats were fed normal rat chow and water ad libitum. Hypophysectomized rats did not gain or lose weight up to 6 weeks after hypophysectomy, whereas normal rats increased in weight from 150 to 400 g over the same period. We have previously shown that the sham-hypophysectomized rats and normal rats show no changes in levels of mitochondrial alpha-ketoisovaleric acid or alpha-ketoisocaproic acid oxidation throughout the weight range of 150–400 g[3].

For all experiments, rats were killed by cervical fracture and the liver was removed. Liver was sliced 0.5 mm thick with a Stadie-Riggs tissue slicer (Arthur H. Thomas Co., Philadelphia, Pa.). Liver slices were sectioned, weighed, and immediately transferred to reaction vials containing 0.1 ml Krebs-Ringer-Phosphate (KRP) buffer composed of 130 mM NaCl, 5.2 mM KCl, 2.8 mM $CaCl_2$, 1.3 mM $MgSO_4$, and 8.6 mM Na_2HPO_4, pH 7.4. Tissue pieces weighed from 2.0–4.0 mg. Decarboxylase activity increased linearly with tissue weight over the range of 1.0–5.0 mg.

Rat liver hepatocytes were isolated using the collagenase technique of Becker[6]. Assay vials were prepared containing 10^5–10^6 cells in 0.1 ml KRP buffer.

Rat liver mitochondria were prepared by the method of Hogeboom[7]. The final washed mitochondrial pellet was suspended in a small volume of 0.25 M sucrose to give a concentration of 14–36 mg of mitochondrial protein per ml. Mitochondrial suspensions were frozen in aliquots at −70 °C and thawed at room temperature before use.

Assays for amino acid decarboxylation were carried out with rat liver slices and rat liver hepatocytes by the method of Dancis et al.[8]. Tissues were incubated for 60 min at 35 °C in reaction vials containing either 2 mM 1-^{14}C-L-valine or 2 mM 1-^{14}C-L-leucine (0.8 cpm/pmole) in 0.1 ml of KRP buffer. Decarboxylase activity was expressed as pmoles CO_2 released/h/mg fresh wt or pmoles CO_2 released/h/mg protein. With liver slices the specific activities of amino acid decarboxylation measured as pmoles CO_2 released per h per mg protein were about 7 times higher than those measured per mg fresh wt. However, valine/leucine ratios, and differences in absolute levels of activity between normal and hypophysectomized rats, were similar with the 2 methods.

Table 1. Amino acid decarboxylation by rat liver slices*

	Valine	Leucine
Normal	37.6 ± 11.5 (36)	319 ± 129 (36)
Hypophysectomized**	58.9 ± 17.8 (15)	213 ± 55 (15)
p	< 0.001	< 0.001

* Amino acid decarboxylase activity with 1-^{14}C-L-valine or 1-^{14}C-L-leucine is expressed as pmoles CO_2 released per h per mg fresh weight after subtracting non-enzymatic decarboxylation (less than 5%). Activity is shown as the mean ± SD with the number of animals tested in parentheses. The significance of differences between normal and hypophysectomized rats was determined using a non-paired t-test. ** Hypophysectomized rats were killed later than 20 days post-hypophysectomy.

Table 2. Keto acid oxidation by rat liver mitochondria*

	KIV	KIC
Normal	26.2 ± 8.3 (8)	5.0 ± 1.5 (8)
Hypophysectomized**	36.2 ± 9.8 (11)	1.1 (11)***
p	< 0.05	

* Mitochondrial oxidation of alpha-ketoisovaleric acid (KIV) or alpha-ketoisocaproic acid (KIC) is expressed as nmoles $Fe(CN)_6^{-3}$ reduced per min per mg mitochondrial protein after subtracting non-specific mitochondrial ferricyanide reduction (less than 3.0). Activity is shown as the mean ± SD with the number of animals tested in parentheses. The significance of differences between normal and hypophysectomized rats was determined using a non-paired t-test. ** Hypophysectomized rats were killed later than 20 days post-hypophysectomy when a steady state of mitochondrial branched-chain alpha-keto acid oxidation had been reached[4]. *** Alpha-ketoisocaproic acid oxidation in the hypophysectomized rat falls to a level too low for accurate measurements or determination of SD. Individual observations ranged between 0.0 and 2.0.

Table 3. Relative substrate utilization by rat liver preparations

	Ratio of decarboxylase activities	
	Liver slices Valine/leucine	Liver mitochondria KIV/KIC
Normal	0.12	5.3
Hypophysectomized	0.28	33.0

Mitochondrial keto acid oxidation was assayed as previously described[3] by measuring keto acid-specific ferricyanide reduction by mitochondrial suspensions with saturating concentrations of keto acid (5 mM alpha-ketoisovaleric acid or alpha-ketoisocaproic acid).
The protein content of liver slices, hepatocytes and mitochondria was determined by the method of Lowry et al.[9] after digestion with 1 N NaOH.
Results. Valine decarboxylation is increased and leucine decarboxylation is decreased following hypophysectomy, whether measured with liver slices (table 1) or with mitochondria and the respective keto acids (table 2). The ratio of the valine to leucine activities in slices doubled following hypophysectomy and increased six-fold in mitochondria (table 3). In both normal and hypophysectomized rats, decarboxylation of leucine exceeds that of valine in slices whereas alpha-ketoisovaleric acid is oxidized more effectively than alpha-ketoisocaproic acid by mitochondria (table 3).
The results with hepatocyte preparations from 3 rats were similar to those obtained with liver slices from 6 rats providing confidence that the results represent cellular function. Valine decarboxylation ranged from 397–503 pmoles/h/mg protein with hepatocytes and from 96–310 pmoles/h/mg protein for slices. The comparative ranges for leucine were 1372–1883 pmoles/h/mg protein with hepatocytes and 1334–3172 pmoles/h/mg protein with liver slices.
Discussion. Hypophysectomy increases the degradation of valine and decreases the degradation of leucine whether measured by cellular amino acid decarboxylation or by mitochondrial keto acid oxidation. The effects of hypophysectomy on mitochondrial branched-chain keto acid oxidation are reflected in the physiology of the intact cell despite the complex and multiple interactions required of cells. Consistent with these findings is the report that growth hormone increases the decarboxylation of leucine by adipose tissue in hypophysectomized rats[10].
The efficiency of substrate utilization by the intact cell, using either liver slices or isolated hepatocytes differs from that of mitochondria. In the former, leucine is decarboxylated more rapidly than valine whereas the reverse is true with oxidation of the respective keto acids by mitochondria. Degradation of the branched-chain amino acid requires transport into the cell and transamination before mitochondrial oxidation can occur. Transport is unlikely to explain the observed differences because uptake of the branched-chain amino acid by the rat hepatocyte is completed within 1 min without significant intracellular accumulation[11]. The kinetics of transamination offer a more likely explanation. 4 hepatic transaminases for the branched-chain amino acids have been identified, 2 of which are cytoplasmic and 2 are mitochondrial[12]. The 3 branched-chain amino acids are substrates for 1 cytoplasmic and 1 mitochondrial enzyme whereas the other 2 transaminases are effective against leucine and methionine. The rate of decarboxylation of the branched-chain amino acids in rat liver is limited by the low levels of transaminase activity compared with relatively high keto acid decarboxylase activity[13].
Other areas of cellular physiology where knowledge is needed to understand the relative substrate utilization of branched-chain amino acids include amino acid and keto acid transport across the mitochondrial membrane, compartmentalization and pool sizes, and the detailed biochemistry of the mitochondrial branched-chain alpha-keto acid decarboxylase. The differential effects of hypophysectomy on the decarboxylation of valine and leucine by rat liver provide evidence for functional distinctions among the branched-chain amino acids with implications concerning the regulation of their catabolism.

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Antimicrobial effect of bis-quaternary ammonium salts derived from 1,3-propanediamine[1]

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Summary. Antimicrobial activity of bis-quaternary ammonium salts derived from 1,3-propanediamine and 1,3-diamino-2-propanol is described. Effect of the length of alkyl chain and the substitution in the connecting chain on this activity was studied.

Use of quaternary ammonium salts as substances having antimicrobial effects is presently very wide spread. In practice, mainly compounds of relatively simpler structure find application. A review of the properties of quaternary ammonium salts and their uses as antimicrobial substances is presented by Petrocci[2]. Regularity in relationship between the structure and the antimicrobial effects for monoquaternary ammonium salts made us assume that bis-quaternary ammonium salts also might have good antimicrobial activity. This was confirmed in case of some derivatives of 1,6-hexanediamine and 1,2-ethanediamine[3,4].
The scope of this work is to study antimicrobial effects with respect to changes in the length of alkyl chains in the derivatives of 1,3-propanediamine and its 2-hydroxy sub-