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]

D. Mlynarčík, I. Lacko and F. Devínsky

Department of Biochemistry and Microbiology, and Department of Inorganic and Organic Chemistry, Faculty of Pharmacy, Comenius University, Kalinčiakova 8, CS-880 34 Bratislava (Czechoslovakia), 13 November 1978

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-

stituted analogue. Synthesis of the compounds studied is described[5,6]. For evaluating the efficiency of these compounds against microorganisms, the method of inhibiting the growth directly in the cultivation media was used[7]. Results are presented as minimum inhibitory concentrations (MIC) in μg/ml (table). Basic requirement valid for the effciency of bis-quaternary ammonium salts was confirmed, i.e. presence of long alkyl substituent (C_9-C_{13}) on the quaternary nitrogen atom, with optimum length C_{11}-C_{12}. Further prolongation of alkyl chain reduces the antimicrobial effect of the compounds studied because of their diminished solubility in water (derivatives Nos 1-5). In comparison with bis-quaternary ammonium salts derived from 1,6-hexanediamine[3], no substantial difference in their efficiency was observed.

Substitution in position 2 of the connecting chain by OH-group moderately decreases the inhibiting effect against microorganisms inspite of the fact that the solubility of these compounds in water is greater than that of preceeding compounds.

Bis-quaternary ammonium salts are antimicrobially more effective than mono-quaternary salts[7,8], particularly on gram-negative bacteria *E. coli*. It is a big advantage in view of the possibility of their being used in pharmaceutical and cosmetic preparations.

Antimicrobial efficiency of bis-quaternary ammonium salts derived from 1,3-propanediamine (MIC in μg/ml)

$R_1(CH_3)_2\overset{+}{N}CH_2CH(R_3)CH_2\overset{+}{N}(CH_3)_2R_2\,2Br^-$

No.	$R_1=R_2$	R_3	*S. aureus*	*E. coli*	*C. albicans*
1.	octyl	H	50	400	100
2.	decyl	H	3	60	10
3.	dodecyl	H	3	20	20
4.	tetradecyl	H	40	200	60
5.	hexadecyl	H	> 1000	> 1000	> 1000
6.	octyl	OH	60	900	300
7.	nonyl	OH	20	200	40
8.	decyl	OH	6	50	8
9.	undecyl	OH	5	60	6
10.	dodecyl	OH	5	90	50
11.	tridecyl	OH	8	50	30
12.	tetradecyl	OH	100	600	90
13.	pentadecyl	OH	90	500	300
14.	hexadecyl	OH	90	> 1000	300

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Effects of calcium-EGTA buffers on active calcium transport in inside-out red cell membrane vesicles

B. Sarkadi, A. Schubert and G. Gárdos[1]

National Institute of Haematology and Blood Transfusion, Budapest, and Department of Physics, University of Agricultural Sciences, Gödöllö (Hungary), 5 October 1978

Summary. In inside-out red cell membrane vesicles, the free calcium concentration half-maximally stimulating active calcium uptake is about 2 orders of magnitude smaller in a calcium-EGTA buffer than in media containing unbuffered calcium. In calcium-EGTA buffer, the maximum rate of calcium uptake is determined by the total calcium concentration present. A possible model for explaining these findings is presented.

Sealed inside-out membrane vesicles (IOV) proved useful in studying the characteristics of active calcium transport of human red cells, since these vesicles show a rapid, ATP + Mg^{2+}-dependent calcium accumulation, and the 'active centre' of the calcium transport system is at the external surface of the IOVs[2-6]. The rate of active calcium uptake by inside-out vesicles is increased by the dialysed supernatant of the red cell hemolysate[2,3,5,6], and the K_{Ca} of about 40 μM observed in the control vesicles is decreased to about 15 μM by the supernatant 'activator protein'[6].

In calcium-loaded resealed ghosts it was shown by Schatzmann[7] that the free calcium concentration required for half-maximum activation of Ca^{2+} + Mg^{2+}-ATPase is about 2 orders of magnitude smaller in a calcium-EGTA buffer than in an unbuffered medium. In the present paper we report comparative studies on the kinetics of active calcium uptake by inside-out vesicles in the presence of unbuffered calcium and of Ca^{2+}-EGTA buffers in the incubation media.

Materials and methods. All the chemicals used were of analytical grade. Deionized water and solutions without added calcium contained less than 3 μM of calcium. 100 mM EGTA - ethylene glycol-bis-(2-aminoethylether)-N,N'-tetra-acetic acid - solutions were titrated with 100 mM $CaCl_2$ solutions before the experiments. A23187 calcium ionophore was a gift of R.L. Hamill (Eli Lilly and Co., Indianapolis).

Stability constants of EGTA for calcium and magnesium, and proton dissociation constants were used as by Schatzmann[7]. The about 20-30% differences in the stability constants in the literature were found to cause the same per cent error in the calculated free, ionized calcium concentrations. In media containing unbuffered calcium, free ionized calcium concentrations were not corrected for binding to ATP as the presence of magnesium makes such a calcium binding insignificant[8].

Inside-out vesicles from human red cells were prepared in chelator-free Tris-HCl buffers as described previously[6]. In the calcium uptake experiments, IOVs with a protein concentration of 20-30 μg/ml medium were preincubated at 37 °C in media containing 120 mM KCl, 20 mM Tris-HCl (pH 7.0), 5 mM $MgCl_2$ and the indicated concentra-