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Inhibition of N-acetylglutamate synthase by various monocarboxylic and dicarboxylic short-chain coenzyme A esters and the production of alternative glutamate esters ☆

M. Dercksen [a,b,*], L. IJlst [a], M. Duran [a], L.J. Mienie [b], A. van Cruchten [a], F.H. van der Westhuizen [b], R.J.A. Wanders [a]

[a] Laboratory Genetic Metabolic Diseases, Departments of Pediatrics and Clinical Chemistry, Academic Medical Centre, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

[b] Centre for Human Metabonomics, North-West University (Potchefstroom Campus), Hoffman street 11, Potchefstroom, South Africa, 2520

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ABSTRACT

Hyperammonemia is a frequent finding in various organic acidemias. One possible mechanism involves the inhibition of the enzyme N-acetylglutamate synthase (NAGS), by short-chain acyl-CoAs which accumulate due to defective catabolism of amino acids and/or fatty acids in the cell. The aim of this study was to investigate the effect of various acyl-CoAs on the activity of NAGS in conjunction with the formation of glutamate esters. NAGS activity was measured *in vitro* using a sensitive enzyme assay with ultraperformance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) product analysis. Propionyl-CoA and butyryl-CoA proved to be the most powerful inhibitors of N-acetylglutamate (NAG) formation. Branched-chain amino acid related CoAs (isovaleryl-CoA, 3-methylcrotonyl-CoA, isobutyryl-CoA) showed less pronounced inhibition of NAGS whereas the dicarboxylic short-chain acyl-CoAs (methylmalonyl-CoA, succinyl-CoA, glutaryl-CoA) had the least inhibitory effect. Subsequent work showed that the most powerful inhibitors also proved to be the best substrates in the formation of N-acylglutamates. Furthermore, we identified N-isovalerylglutamate, N-3-methylcrotonylglutamate and N-isobutyrylglutamate (the latter two in trace amounts), in the urines of patients with different organic acidemias. Collectively, these findings explain one of the contributing factors to secondary hyperammonemia, which lead to the reduced *in vivo* flux through the urea cycle in organic acidemias and result in the inadequate elimination of ammonia.

1. Introduction

The degradation of protein and subsequently amino acids is an essential part of human metabolism. The catabolism of amino acids and other nitrogen-containing molecules results in the production of ammonia (NH_3), which is converted into urea in the liver via the urea cycle and excreted as such in the urine. The urea cycle consists of six key enzymes, localized in different compartments within the cell, thus requiring the obligatory participation of different mitochondrial membrane transporters (SLC25A13 and SLC25A15). N-acetylglutamate synthase (NAGS), carbamyl phosphate synthetase 1 (CPS) and ornithine transcarbamylase (OTC) are located in the mitochondrion whereas argininosuccinate synthase (ASS), argininosuccinate lyase (ASL), and arginase (ARG) reside in the cytosol [1]. The urea cycle plays a key role in the control of ammonia levels which need to be kept as low as possible in order to prevent the deleterious consequences of hyperammonemia. Ammonia is especially toxic to the central nervous system (CNS) since slight elevations in ammonia (>100 μM, normal: <45 μM) may cause metabolic encephalopathy and irreversible CNS damage [2].

CPS catalyzes the initial and rate limiting step of the urea cycle and is vital for the regulation of the pathway. Its allosteric activator N-acetylglutamate (NAG), is essential for the function of CPS in the urea cycle [3]. NAGS (E.C. 2.3.1.1), originally identified in *E. coli* by Vogel et al. [4] and later on described in humans by Bachmann et al. [5], is responsible for the synthesis of N-acetylglutamate (NAG) through the conjugation of acetyl-CoA and L-glutamate. The activity of NAGS is stimulated by L-arginine, an allosteric activator [6]. Apart from the short term regulation by NAG, urea cycle activity is also controlled by several other mechanisms, including enzyme induction, the concentration of intermediates and substrates, diet as well as hormonal changes. Although the precise regulation of ureagenesis is

Abbreviations: NAGS, N-acetylglutamate synthase; CoA, coenzyme A; UPLC–MS/MS, Ultraperformance liquid chromatography–tandem mass spectrometry; UCDs, urea cycle defects; CPS, carbamyl phosphate synthetase; CNS, central nervous system; NAG, N-acetylglutamate; GC–MS, gas chromatography mass spectrometry

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* Corresponding author at: Centre for Human Metabonomics, North-West University (Potchefstroom Campus), Hoffman street 11, Potchefstroom, South Africa, 2520. Tel.: +27 18 299 2302.

E-mail address: marli.dercksen@nwu.ac.za (M. Dercksen).

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still poorly understood, it proves to be crucial in the development of primary and secondary hyperammonemia [7,8].

Urea cycle enzyme and transporter defects (UCDs), including NAGS deficiency, are a well defined group of inherited metabolic disorders characterized by episodes of mild to severe hyperammonemia [7,9]. Except for hyperammonemia caused by primary deficiencies in one of the enzymes or transporters involved in the urea cycle, this pathophysiological condition is also observed in numerous other conditions. These include liver disease, anatomical changes such as the portocaval shunt, hyperinsulinism and hyperammonemia due to glutamate dehydrogenase hyperactivity, several organic acidemias, mitochondrial fatty acid oxidation disorders and valproate toxicity [7,10–12]. Fig 1 illustrates the localization of several CoA esters and part of the urea cycle within the mitochondrion.

The elevation of blood ammonia encountered in the classical organic acidemias, including isovaleric-, propionic- and methylmalonic acidemia can be life-threatening [7]. Isolated cases of hyperammonemia have been reported for some disorders e.g. 3-methylcrotonyl-CoA carboxylase- (MCC), multiple acyl-CoA dehydrogenase- (MAD) and succinyl-CoA ligase (associated with mutations in SUCLG1 gene) deficiencies [13–16]. Hyperammonemia in human short-chain acyl-CoA dehydrogenase deficiency (SCADD) is virtually non-existent. Mice with SCADD however have shown elevated blood ammonia levels [17,18]. No cases associated with hyperammonemia have been documented for glutaryl-CoA dehydrogenase- (GA1), short/branched-chain acyl-CoA dehydrogenase- (SBCAD) isobutyryl-CoA dehydrogenase- (IBCAD), 3-ketothiolase- and multiple carboxylase deficiencies as well as succinyl-CoA ligase deficiency due to SUCLA2 gene mutations. [19–24]. These reports and previous findings indicate a clear variation in the occurrence of hyperammonemia associated with short-chain- and short-branched-chain organic acidemias [25]. The inhibition of NAGS is one of the factors which may explain the presence of hyperammonemia, among other. Additional information of these disorders is summarized in Supplementary Table 1.

Since there is accumulation of a range of acyl-CoAs in the different organic acidemias, we decided to study the effect of several acyl-CoAs on the activity of NAGS as well as their behavior as substrates for the enzyme, using an optimized UPLC–MS/MS assay. We argued that these compounds may act as inhibitors and/or substitute substrates for NAGS which would lead to the aberrant *in vivo* regulation of the urea cycle and contribute (in addition to other factors) to episodes of hyperammonemia.

2. Materials and Methods

All materials were supplied by Sigma-Aldrich (St. Louis, MO) unless otherwise specified.

2.1. Preparation and purification of human NAGS protein

The construction of the NAGS wild type plasmid, as well as the expression of human NAGS protein in *E. coli* was performed essentially as described by Schmidt et al. [26] with minor modifications. The coding sequence for mature NAGS, without the mitochondrial targeting

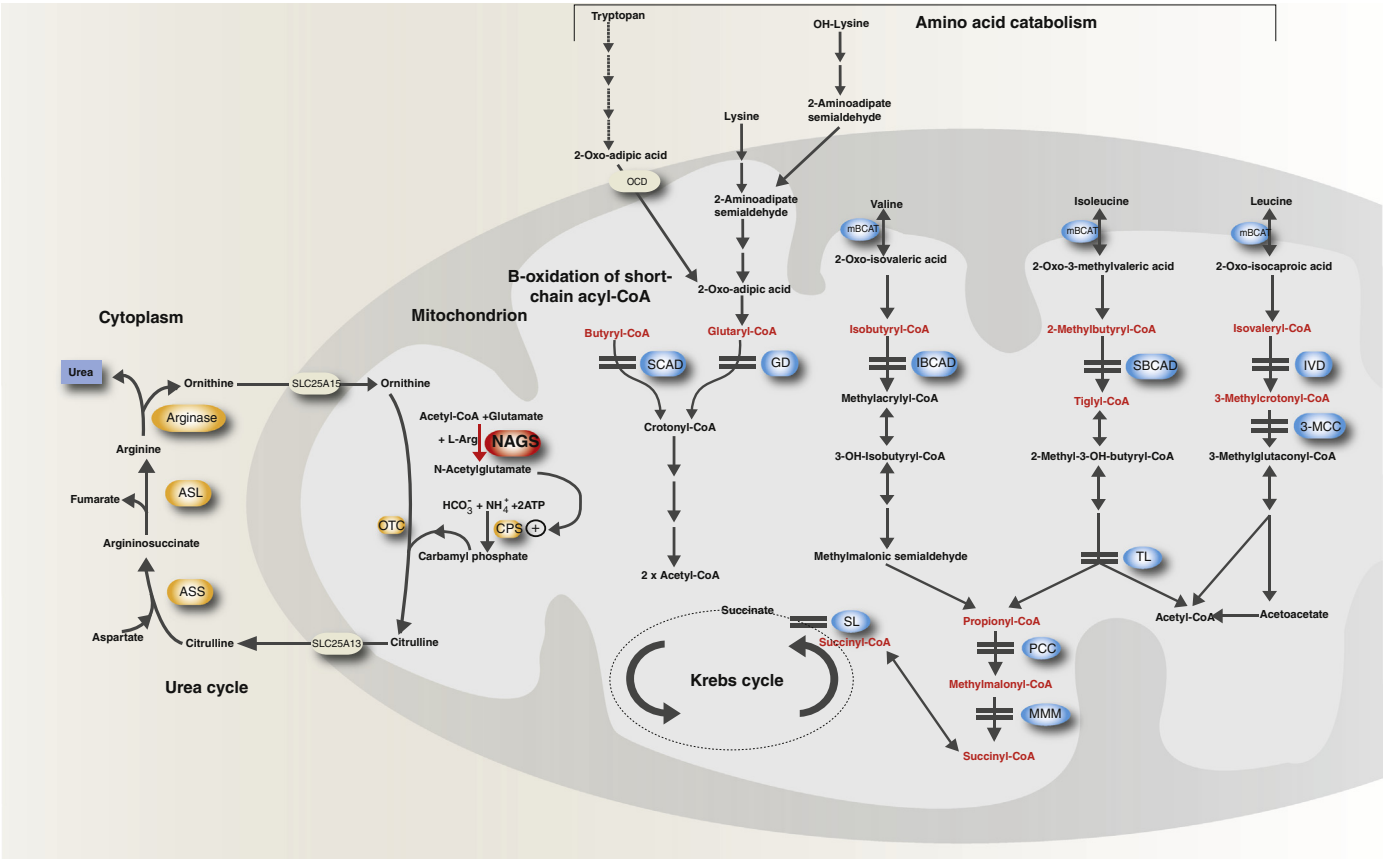

Fig. 1. The localization of branched-short chain and short chain acyl-CoAs and their related metabolic pathways within the mitochondrial matrics. This figure also illustrates urea cycle which is partially in the mitochondria, including NAGS, as well as the enzymatic steps within the cytoplasm. Abbreviations: mBCAT, mitochondrial branched chain aminotransferase; SCAD, short-chain-CoA dehydrogenase; GD, glutaryl-CoA dehydrogenase; IBCAD, isobutyryl-CoA dehydrogenase; SBCAD, short/branched-chain acyl-CoA dehydrogenase; IVD, isovaleryl-CoA dehydrogenase; 3-MCC, 3-methylcrotonyl-CoA carboxylase; TL, 3-ketothiolase; PCC, propionyl-CoA carboxylase; MMM, methylmalonyl-CoA mutase; SL, succinyl-CoA ligase; NAGS, N-acetylglutamate synthase; OTC, ornithine transcarbamoylase; ASS, argininosuccinate synthetase; ASL, argininosuccinate lyase; CPS, carbamyl phosphate synthetase. Membrane transporters include solute carrier transporters, SLC25A13, SL25A15 and the oxodicarboxylate carrier (OCD). Enzyme deficiencies indicated by double black bars, result in the accumulation of short-branched-chain and short-chain CoA esters.

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signal, was PCR amplified from full-length NAGS in pCR2.1 as template (generous gift from Dr. Johannes Häberle) using the forward primer ATATCATATG AGCACCGCCTGGTCGCAGCCC and reverse primer ATATGGATCCTCAGCTGCCTG GGTCAGAAG (*Nde*I and *Bam*HI restriction sites underlined). The PCR product was cloned into pGEM-T (Promega, Madison, WI) resulting in pGEM-T-NAGS and was subsequently sequenced. The *Nde*I/*Bam*HI fragment from pGEM-T-NAGS was subcloned in frame with the N-terminal 10xHis-tag of pET19b.

For protein expression the pET19b-NAGS construct was transformed into *E.coli* BL21 AI strain (Invitrogen, Carlsbad, CA). Cells were grown from an overnight culture in Terrific Broth medium containing glycerol (8 g/L) and ampicilline (100 μg/mL) at 37 °C until an OD600 of 1-1.5 was reached. Thereafter the temperature was adjusted to 22 °C and the mixture was allowed to cool for 1 hour to 22 °C. His-tagged NAGS was expressed overnight at 22 °C by addition of L-arabinose (2 g/L) and IPTG (0.2 mM). Cells were lysed in a buffer containing Tris (25 mM, pH = 7.5) and NaCl (500 mM) by treatment with lysozyme and sonication and the lysate was cleared by centrifugation (20,000 *g*, 15 minutes). To the supernatant imidazole (15 mM) and HisLink Protein Purification Resin (Promega, Madison, WI) was added. Protein binding was allowed batchwise for 30 minutes at 4 °C. The resin was poured into a column and washed with lysis buffer containing imidazole (30 mM). NAGS was eluted from the column by increasing the concentration of imidazole in the lysis buffer to 100 mM (Supplementary Fig. 1). Glycerol (100 g/L) was added to the purified protein and aliquots were snap-frozen in liquid nitrogen and stored at -80 °C.

2.2. NAGS Enzyme Assay

The activity of NAGS, as expressed and purified from *E.coli*, was measured essentially as described by Caldovic et al. with minor modifications [27]. Briefly, the reaction mixture contained 50 mM ammonium acetate buffer (final pH = 8.5), 10 mM glutamate, 2.5 mM acetyl-CoA, and 1 mM arginine in a 75 μl reaction volume. The reaction was started by adding 0.05 μg purified enzyme (prepared in 0.1 mg/mL BSA/PBS solution). The enzyme reactions were stopped with 175 μL acetonitrile, after a 10 minute incubation period at 30 °C. All samples were placed on ice and the internal standard, N-acetyl-L-glutamic-2,3,3,4,4-d5 acid (CDN isotopes: Germany), was added. The precipitated protein was removed by centrifugation (20,000 *g*, 5 minutes, 4 °C) and the supernatant was dried under nitrogen at 40 °C. The product was resuspended in 110 μL of 0.4% heptafluorobutyric acid (HFBA) (pH 4) and 10 μL was injected on the UPLC–MS/MS for monitoring of NAG and other N-acylglutamates. The products were separated by UPLC and analysed by multiple reaction monitoring (MRM) of selected mass pairs as described by Aires et al. [12].

2.3. Kinetic investigation of purified NAGS and inhibitor selection

The purified enzyme was used in a series of kinetic experiments. A complete kinetic investigation was performed with isovaleryl-CoA and butyryl-CoA. The inhibitory effect of isovaleryl-CoA (0–2.5 mM) and butyryl-CoA (0–2.5 mM) on the activity of NAGS was evaluated using the NAGS assay described above. The characterization of the NAGS activity with the two selected inhibitors was done by plotting the measured reaction rates as a function of substrate and inhibitor concentrations. Graph Pad Prism version 5 (GraphPad Software Inc., La Jolla, CA, USA) was used to perform nonlinear regression analysis of the kinetic data and the V_{max}, K_m and K_i as well as the type of inhibition were determined [28].

Additional short-chain acyl-CoAs, i.e. 3-methylcrotonyl-CoA, methylmalonyl-CoA, glutaryl-CoA, succinyl-CoA, isobutyryl-CoA, and propionyl-CoA were tested at a low (0.2 mM) and a high (2.5 mM) concentration. The reaction conditions were the same as described in Section 2.2.

2.4. Chemical synthesis of N-acylglutamate conjugates

Various acylchlorides (propionylchloride, butyrylchloride, isobutyrylchloride, isovaleryl- chloride, succinylchloride, 3-methylcrotonylchloride and glutarylchloride) were used in the synthesis of the glutamate conjugates in this study. Methylmalonylchloride was not commercially available; therefore, we synthesized it by using a modified methodology originally described by Liebich and Först [29]. Two milliliters thionylchloride (0.2 moles) was added to 0.6 moles of methylmalonic acid. The mixture was incubated at 60 °C for 60 minutes. Acylchlorides (1 mL), with an approximate concentration of 0.1 M, were added to 1 mL 2 M L-glutamate prepared in a 10 M NaOH solution. The reaction was carried out on ice for 5 minutes. Thereafter, the solution was kept at 60 °C for 2 hours to enhance further product formation. The solution was subsequently acidified and an ethylacetate extraction was performed. The organic phase was dried under a stream of nitrogen. The dried product, which contained the N-acylglutamates, was stored at -20 °C. Approximately 20 nmoles of the final products were injected on the UPLC–MS/MS in the MRM mode to check for the product and precursor ions.

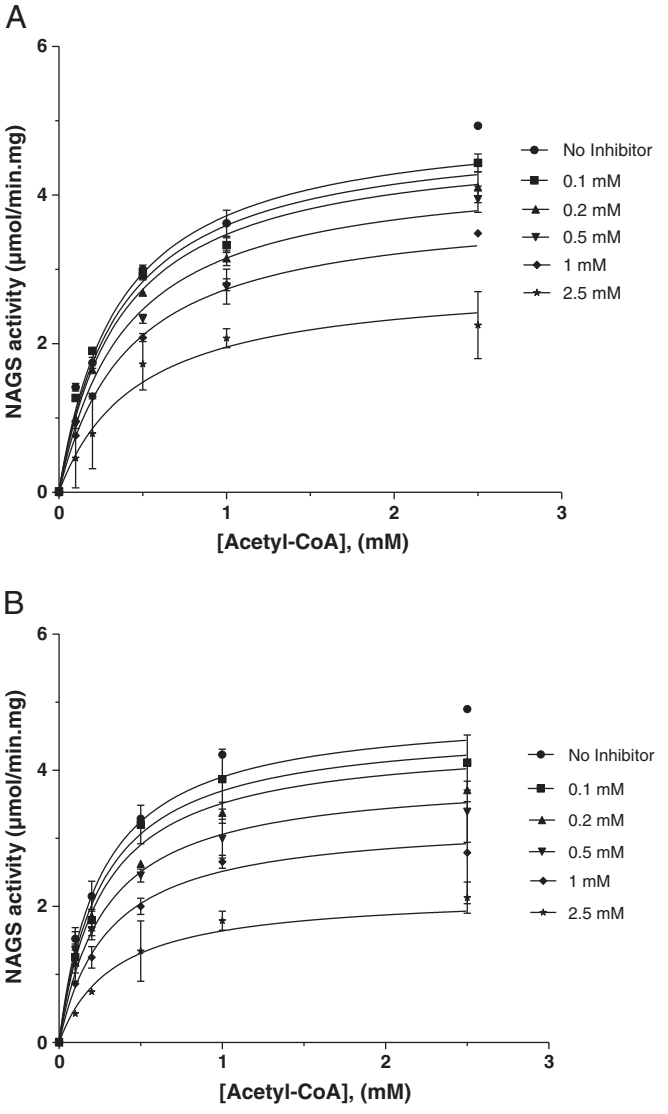

Fig. 2. Inhibition of NAGS by isovaleryl-CoA and butyryl-CoA. The effect of (A) isovaleryl-CoA (0-2.5 mM) and (B) butyryl-CoA (0-2.5 mM) on the activity of NAGS was studied at different substrate concentrations (acetyl-CoA, 0-2.5 mM). Nonlinear regression identified a mixed model inhibition as the best fit mechanism.

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2.5. Identification of N-acylglutamates in urine of patients with short-chain organic acidemias

The presence of N-acylglutamates in urine of patients with different organic acidemias was verified by GC–MS [30]. The synthesized N-acylglutamate and urine samples were analysed under the same GC–MS condition to establish the presence of metabolites. The mass spectra of the synthetic glutamate conjugates were compared to organic acid profiles of patients and control subject (with no metabolic disease) by using the Automated Mass Spectral Deconvolution and Identification System software (AMDIS, version 2.66 from the National Institute for Standards and Technology).

3. Results

In order to study the effect of different acyl-CoAs on the activity of the enzyme N-acetylglutamate synthase, we first expressed human His-tagged NAGS in *E.coli* and subsequently purified the expressed protein using nickel chelate chromatography. To determine the enzyme activity of the eluted protein, we developed a new enzyme assay (see materials and methods) which involves the use of UPLC–MS/MS for acquisition and stable isotope quantification of the reaction product. Using this method, the isolated protein showed abundant NAGS activity.

3.1. Inhibition of NAGS activity by isovaleryl-CoA and butyryl-CoA

Enzyme analyses and kinetics were performed to study the inhibitory properties of isovaleryl-CoA and butyryl-CoA on NAGS. The results are shown in Fig. 2. We determined the kinetic parameters of purified NAGS which resulted in V_{max} and K_m values of 5.0 ± 0.11 μmol/min.mg protein and 0.3 ± 0.07 mM for acetyl-CoA, respectively (values represent the mean and standard deviation of two separate experiments). Isovaleryl-CoA has already been described as an inhibitor of NAGS in rat liver mitochondria, but its inhibitory effect has not been examined in detail with the purified NAGS enzyme [11]. The inhibition constants (K_i) were 1.9 mM for isovaleryl-CoA and 1.3 mM for butyryl-CoA, respectively. The alpha-value, which indicates the probable mechanism of the mixed model inhibition (alpha = 1: non-competitive; alpha < 1: uncompetitive; alpha > 1: competitive inhibition), for isovaleryl-CoA and butyryl-CoA were 1.8 and 1.1, respectively [31]. A comparison of the inhibition constants indicates that butyryl-CoA is a stronger inhibitor of NAGS than isovaleryl-CoA.

3.2. The inhibitory effect of other short-chain acyl-CoAs on NAGS activity

The activity of NAGS was measured using standard reaction mixtures (as described in Section 2.2) with 2.5 mM acetyl-CoA in the presence of different acyl-CoAs (0.2 mM and 2.5 mM) as indicated. The results in Fig. 3 show that all acyl-CoAs including 3-methylcrotonyl-CoA, methylmalonyl-CoA, glutaryl-CoA, succinyl-CoA, isobutyryl-CoA, propionyl-CoA, butyryl-CoA, and isovaleryl-CoA have an inhibitory effect on the activity of NAGS, although to different extent. Propionyl-CoA appeared to be the strongest inhibitor followed by butyryl-CoA. The branched-chain amino acid-related CoA esters had less of an inhibitory effect and the dicarboxylic acyl-CoAs had the least effect on the activity of NAGS.

3.3. Alternative N-acylglutamate conjugates formed by NAGS

Because of the inhibitory effect of various acyl-CoAs (0.2 mM) on NAGS, we also examined the *in vitro* enzymatic formation of N-acylglutamate esters by NAGS in the absence and presence of 0.2 mM and 2.5 mM acetyl-CoA as described in Section 2.4. Fig. 4 shows that several acyl-CoAs were substrates for NAGS and were converted into the corresponding N-acylglutamates. N-butyrylglutamate and N-propionylglutamate were formed at approximately equal rates by NAGS, whereas the remaining branched-chain acyl-CoAs only gave rise to limited amounts of glutamate esters. Trace N-methylmalonylglutamate and no N-glutarylglutamate was detected. Surprisingly, N-succinylglutamate was formed non-enzymatically from succinyl-CoA and L-glutamate.

3.4. The presence of alternative N-acylglutamate conjugates in urine of patients with short-chain related organic acidemias

The results described above prompted us to look for the presence of alternative N-acylglutamate conjugates in urine of patients with

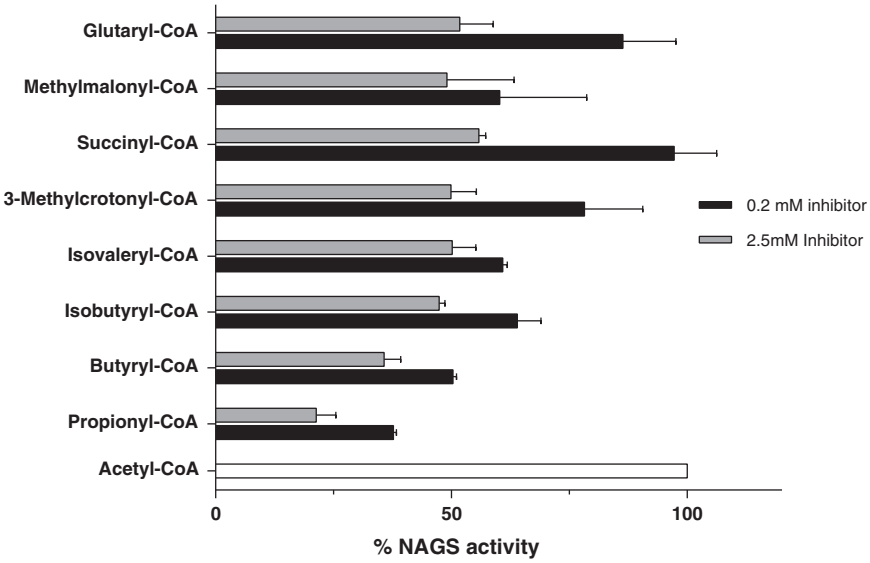

Fig. 3. The inhibitory effect of several acyl-CoAs on NAGS activity. The effect of different acyl-CoAs (0.2 mM and 2.5 mM) on the NAGS activity was studied at an acetyl-CoA concentration of 2.5 mM. The activity in the absence of inhibitors was set at 100%.

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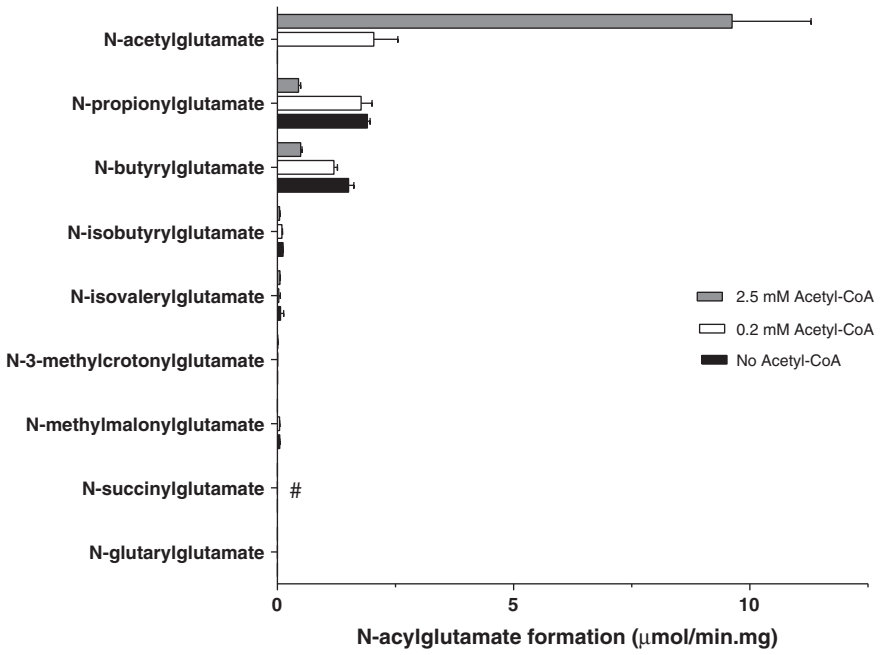

Fig. 4. The enzymatic production of N-acylglutamates from the corresponding acyl-CoA esters by NAGS. Purified NAGS was incubated with various acyl-CoA esters (0.2 mM) in the absence or presence of acetyl-CoA (0.2 mM, 2.5 mM). The N-acylglutamate formation were analysed on the UPLC–MS/MS and quantified with the use of N-acetyl-L-glutamic-2,3,3,4,4-d5 acid as internal standard. #: Formation of N-succinylglutamate by NAGS could not be determined, but nonenzymatic production of N-succinylglutamate was observed.

associated deficiencies. As expected we did observe increased amounts of N-isovalerylglutamate (112 ± 87 mmol/mol creatinine) in the urine of 10 untreated IVA patients. The same was true for N-3-methylcrotonylglutamate (5.6 ± 2.0 mmol/mol creatinine) which was elevated in 3 patients with 3-methylcrotonyl-CoA carboxylase deficiency consistent with previous reports [32–34]. We did not detect N-3-methylcrotonylglutamate in patients with multiple carboxylase deficiency. We also detected trace amounts of urinary N-isovalerylglutamate (5.8 mmol/mol creatinine) and N-isobutyrylglutamate (1.5 mmol/mol creatinine) in a patient with the severe form of MADD. N-isobutyrylglutamate (0.5 mmol/mol creatinine) was also identified in the urine of a patient with presumed isobutyryl-CoA dehydrogenase deficiency (IBCDD). Urine of control subjects did not reflect the presence of any of these glutamate esters. N-isobutyrylglutamate has not reported before in literature. Importantly, the identity was confirmed by GC–MS using a chemically synthesized standard (Fig. 5).

Urinary N-propionylglutamate in patients with propionic acidemia could not be detected in our series of patients. Surprisingly N-butyrylglutamate was absent in urine of patients with SCADD, ethylmalonic encephalopathy, or MADD. In addition, N-methylmalonylglutamate and N-succinylglutamate were not detected in urine of patients with methylmalonic acidemia and/or succinyl-CoA ligase (SUCLA2 gene mutation) deficiency, respectively.

4. Discussion

In this paper we have shown that the activity of purified human N-acetylglutamate synthase is inhibited by a number of monocarboxylic and dicarboxylic short-chain acyl-CoAs. Propionyl-CoA was found to be a strong inhibitor among the tested CoA-esters, fully in line with the life-threatening hyperammonemia which can be observed in the neonatal form of propionic acidemia and methylmalonic acidemia [25]. The ability of NAGS to convert propionyl-CoA to its glutamate ester was quite considerable and is higher than reported before by others [11,35,36]. However, N-propionylglutamate could not be detected in the urines of 20 propionic acidemia patients.

Butyryl-CoA inhibited NAGS to a slightly lesser degree, but was equally effective as propionyl-CoA in its *in vitro* formation of glutamate esters. However, we failed to identify N-butyrylglutamate in the urines of 18 SCADD patients with a variety of homozygous and compound heterozygous mutations. It is generally accepted that patients who accumulate butyryl-CoA, i.e. those with SCAD-deficiency, do not suffer from hyperammonemia, a finding which is seemingly in contrast with our observations [18]. The moderate elevation of the butyryl-CoA in SCADD, as exemplified by the mild elevations of its secondary metabolites ethylmalonic acid and butyrylcarnitine, most probably contributes to the near-absence of hyperammonemia in this condition. Isobutyryl-CoA, the structural isomer of butyryl-CoA derived from the valine catabolic pathway, showed slightly less inhibition of NAGS when compared with its straight-chain analogue. An accumulation of isobutyryl-CoA has been shown to occur in patients with MADD and (presumed) IBCDD (unpublished observations based on the analysis of acylcarnitines and acylglycines). In line with these data we identified N-isobutyrylglutamate in the urine of MADD as well as IBCDD, which has not been reported in literature so far.

Isovaleryl-CoA inhibited the activity of NAGS in a similar way, as compared to butyryl-CoA. Surprisingly, the *in vitro* formation of N-isovalerylglutamate was rather low, in sharp contrast to the observations of a considerable accumulation of N-isovalerylglutamate in the urines of IVA patients during episodes of metabolic decompensation. It cannot be ruled out that the *in vivo* formed N-isovalerylglutamate is quite resistant to degradation by aminoacylase(s) and consequently remains elevated for some time. Earlier observations by Lindner et al. [37] support this theory. They have shown that aminoacylase-1 favors straight-chain acyl-moieties, whereas branched-chain N-acylamino acids are poor substrates for the enzyme. These results explain our findings of only branched-chain glutamate ester in urine of patient with corresponding deficiencies whereas propionyl-glutamate and butyryl-glutamate were not detected.

Dicarboxylic short-chain acyl-CoAs had limited effect on the activity of NAGS, except at an acyl-CoA concentration of 2.5 mM (Fig. 3). Trace N-methylmalonylglutamate and no enzymatic formation of N-succinylglutamate and N-glutarylglutamate were observed. However succinyl-CoA and L-glutamate rapidly formed a glutamate ester

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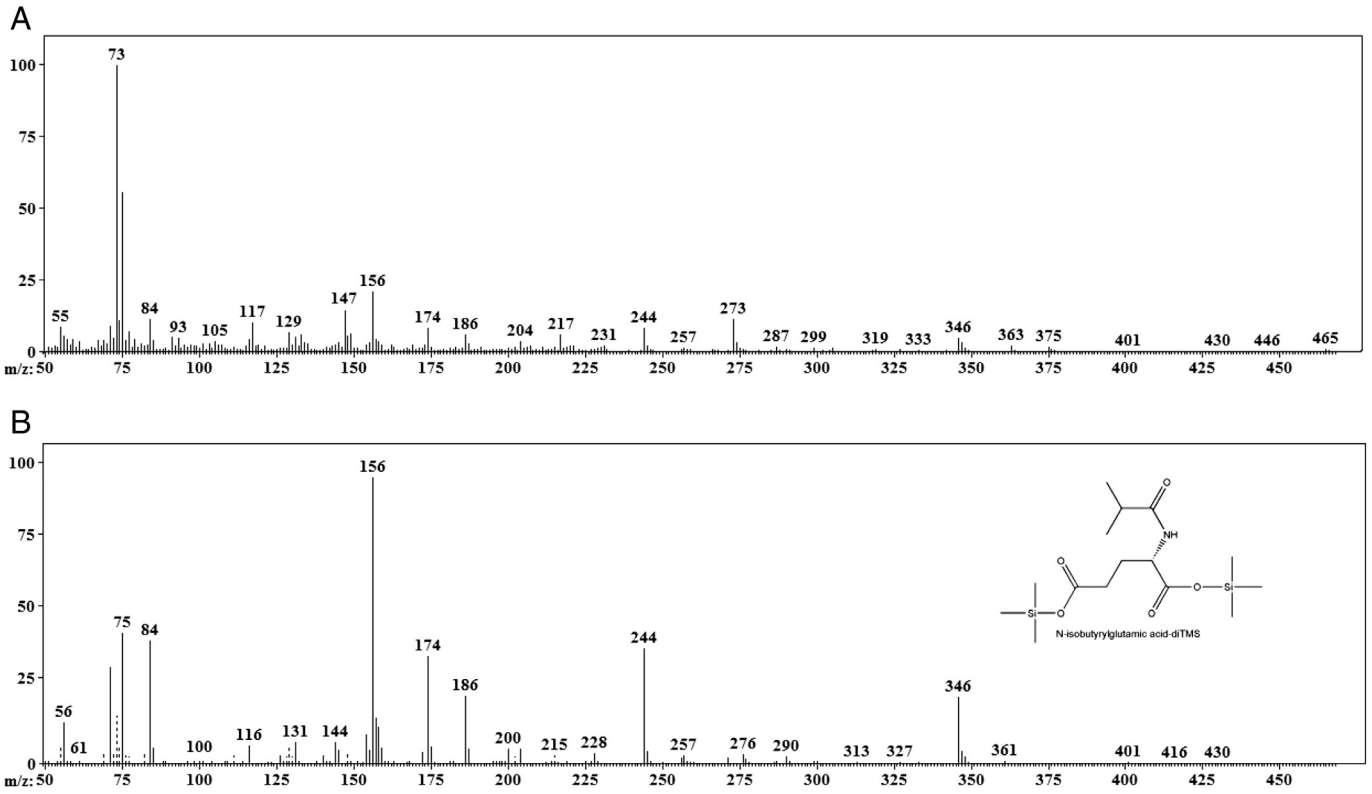

Fig. 5. Mass spectra of the di-TMS derivative of N-isobutyrylglutamic acid (A) The TMS derivative of trace amount of N-isobutyrylglutamate in the urine of a MADD patient, (B) The TMS derivative of chemically synthesized N-isobutyrylglutamate.

in a non-enzymatic reaction. For this reason we tested the hypothesis of inhibition of NAGS by synthetic N-succinylglutamate and proved that this was not the case (data not shown). Hyperammonemia has been reported in a few isolated cases of severe succinyl-CoA ligase deficiency with mutations in SUCLG1. The inhibitory effect of nonenzymatic formed N-succinylglutamate on CPS1 may be the underlying mechanism for the hyperammonemia and further investigation is needed to confirm this phenomenon observed in our *in vitro* study. An alternative explanation may be the tertiary accumulation of propionyl-CoA in this disorder, as the effect of the dicarboxylyl-CoAs is negligible. Although severe hyperammonemia is associated with methylmalonic acidemia, this is most likely due to the secondary accumulation of propionyl-CoA in this disorder and to a lesser degree as a result of elevated methylmalonyl-CoA as previously suggested in the literature [11]. Elevated blood ammonia levels have not been associated with GA1, which concurs with our result of limited inhibition of NAGS activity by glutaryl-CoA. No dicarboxylic N-acylglutamates were found in the urine of patients with relevant organic acidemias.

The difference in the degree of severity of hyperammonemia among the various organic acidemias may be attributed to the secondary *in vivo* metabolic pathways and/or competing enzyme reactions resulting in alleviation of the accumulation of toxic acyl-CoAs as well as formation of alternative conjugates. Indeed, accumulating acyl-CoAs may enter carboxylation, or thiolytic cleavage reactions and subsequently (ω-1)-hydroxylation and ω-oxidation. Butyryl-CoA, isobutyryl-CoA and isovaleryl-CoA possibly serve as substrates for neighboring mitochondrial short-chain dehydrogenases (SBCAD, IBCD or SCAD). The cells also have the ability to conjugate these short-chain acyl-CoAs to glycine, glucuronic acid, and L-carnitine which in all cases result in (partially) detoxified substrates. The affinities of the various short-chain acyl-CoAs for these conjugating enzyme systems differ considerably [38], thus one may expect organic acidemia patients to excrete a variety of different conjugates in their urines. It can be foreseen that metabolomics analysis in this area will unravel the relative importance of each conjugation system in every single patient, thereby opening the way to personalized drug or food additive treatment.

The availability of acetyl-CoA is another factor that should be taken into account when interpreting the hyperammonemia associated with organic acidemias. It is well known that patients with defects of the mitochondrial fatty acid oxidation such as medium-chain acyl-CoA dehydrogenase deficiency (MCADD) or very long-chain acyl-CoA dehydrogenase deficiency (VLCADD) may suffer from hyperammonemia. This can be attributed to the inadequate production of acetyl-CoA hampering the formation of NAG [39]. It can be learned from Fig. 4 that a high level of acetyl-CoA results in a less severe depression of the NAGS activity using the same amount of short-chain acyl-CoA and this is seemingly an advantage of the ketotic state. However, the increased load of ammonia during catabolism to be handled by the urea cycle probably makes the residual NAGS activity insufficient. Immediate treatment with N-carbamylglutamate, a non-physiological activator of NAGS, which is available as the drug Carglumic acid^{R}, as well as reversal of the catabolic state is then indicated.

5. Conclusion

This study contributes to the understanding of hyperammonemia in several organic acidemias. It can be concluded that both inhibition of NAGS as well as formation of alternative N-acylglutamates has a potential effect on the regulatory steps of urea production, especially the activation of CPS. This awareness should prompt the clinician to liberally apply the administration of stable glutamate esters such as N-carbamylglutamate in all neonates with unexplained hyperammonemia until a firm diagnosis has been established. The

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successful application of N-carbamylglutamate in isovaleric academia has recently been demonstrated [40]. Without doubt these studies will eventually turn out to be useful in the design of personalized treatment of patients with secondary hyperammonemia due to organic acidemias, which are also strongly supported by J. Häberle et al. [41].

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