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Biochemical and behavioural phenotyping of a mouse model for GAMT deficiency

An Torremans[a], Bart Marescau[a], Ilse Possemiers[a], Debby Van Dam[a], Rudi D'Hooge[a], Dirk Isbrandt[b], Peter Paul De Deyn[a,c,*]

[a]*Laboratory of Neurochemistry and Behaviour/Born Bunge Foundation, University of Antwerp (UA), Department of Biomedical Sciences, Universiteitsplein 1, 2610 Antwerp (Wilrijk), Belgium*

[b]*Institute for Neural Signal Transduction, Centre for Molecular Neurobiology Hamburg, University of Hamburg, Martinistrasse 52, 20246 Hamburg, Germany*

[c]*Department of Neurology/Memory Clinic, Middelheim General Hospital, Lindendreef 1, 2020 Antwerp, Belgium*

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Abstract

Deficiency of guanidinoacetate *N*-methyltransferase (GAMT) is the first described creatine (CT) deficiency syndrome in man, biochemically characterized by accumulation of guanidinoacetic acid (GAA) and depletion of CT. Patients exhibit severe developmental and muscular problems. We created a mouse model for GAMT deficiency, which exerts biochemical changes comparable with those found in human GAMT-deficient subjects. CT and creatinine (CTN) levels are significantly decreased and GAA is increased in knockout (KO) mice. In patients, other guanidino compounds (GCs) appear to be altered as well, which may also contribute to the symptomatology. Extensive evaluation of GCs levels in the GAMT mouse model was therefore considered appropriate. Concentrations of 13 GCs in plasma, 24-h urine, brain and muscle of GAMT mice were measured. We also report on the detailed behavioural characterization of this model for GAMT deficiency.

Besides an increase of GAA and a decrease of CT and CTN in plasma, 24-h urine, brain and muscle of KO mice, we observed a significant increase of other GCs in brain and muscle that was sometimes reflected in plasma and/or urine. KO mice displayed mild cognitive impairment.

In general, it could be concluded that the GAMT mouse model is very useful for biochemical research of GAMT deficiency, but shows only a mild cognitive deficit.

Keywords: GAMT deficiency; Knockout; Mouse model; Guanidino compounds; Behaviour; CT deficiency

1. Introduction

Deficiency of guanidinoacetate *N*-methyltransferase (GAMT), the first described creatine deficiency syndrome in man, is an autosomal recessively inherited disorder [1–3]. Creatine (CT) plays an important role in energy metabolism of brain and muscle; it is either taken up from food and/or synthesized endogenously, whereby the enzyme GAMT is essential [4]. GAMT deficiency is biochemically characterized by accumulation of guanidinoacetic acid (GAA) and depletion of CT [1–3]. GAA, as a partial agonist of the $GABA_A$-receptor and inhibitor of Na^+–K^+-ATPase activity, can act as a neurotoxin and/or neuromodulator [5,6]. Patients with GAMT deficiency exhibit a developmental delay or regression, mental retardation and severe abnormalities in expressive and cognitive speech. Extrapyramidal movement disorders, muscular hypotonia and weakness, epilepsy and autistic or self-aggressive behaviour are also observed in patients suffering from GAMT deficiency [1–3,7,8]. Although treatment with oral CT supplementation,

* Corresponding author. Department of Neurology/Memory Clinic, Middelheim General Hospital, Lindendreef 1, 2020 Antwerp, Belgium. Tel.: +32 3 820 26 20; fax: +32 3 820 26 18.

E-mail addresses: peter.dedeyn@ua.ac.be, dedeyn@skynet.be (P.P. De Deyn).

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arginine restriction and ornithine supplementation results in a marked increase of plasma, CSF and brain CT concentrations, a decrease of brain GAA levels and clinical improvement with reduction of seizures, it is not sufficient to cure the disease [1,3,9,10].

Schmidt et al. [11] created a mouse model for GAMT deficiency by targeted disruption of the murine GAMT gene. These GAMT knockout mice exert biochemical changes in body fluids, brain and muscle that are comparable with those found in human GAMT-deficient subjects. CT and creatinine (CTN) levels are significantly decreased and GAA is increased in serum, urine and brain of KO mice. MR spectroscopy of brain and muscle from KO mice showed clearly reduced signals of CT and phosphocreatine, in accordance with findings in GAMT-deficient patients [12]. In addition, increased neonatal mortality, reduction of body weight with reduced body fat content, muscular hypotonia and decreased male fertility were observed in knockouts [11].

In patients, other guanidino compounds (GCs), besides CT, CTN and GAA, are altered as well and may also contribute to the symptomatology. Homoarginine is increased in plasma, urine and CSF, in addition to low arginine and high γ-guanidinobutyric acid concentrations [3]. Extensive evaluation of GCs levels in the GAMT mouse model was therefore considered appropriate. In the present study, the concentration of 13 GCs (α-keto-δ-guanidinovaleric acid (α-keto-δ-GVA), guanidinosuccinic acid (GSA), creatine (CT), guanidinoacetic acid (GAA), α-*N*-acetylarginine (α-*N*-AA), argininic acid (ArgA), β-guanidinopropionic acid (β-GPA), creatinine (CTN), γ-guanidinobutyric acid (γ-GBA), arginine (Arg), homoarginine (Harg), guanidine (G) and methylguanidine (MG)) was measured in plasma, 24-h urine and brain of GAMT mice. Moreover, for the first time, the concentration of GCs was measured in muscle. GAMT-deficient mice that survived the perinatal period displayed no neurological symptoms such as ataxia or epilepsy [11]. However, the possible more subtle behavioural changes of the GAMT knockout mouse model were not yet studied. In this paper, we therefore also report on the detailed behavioural assessment of this model.

The presented research aims at further validation of the mouse GAMT deficiency model as a reliable tool to study this CT deficiency syndrome.

2. Materials and methods

2.1. Animals

A knockout mouse model for GAMT deficiency, generated by Schmidt et al. [11] and backcrossed to C57Bl/6J for at least six generations, was subjected to detailed biochemical and behavioural phenotyping. Animals were housed in standard mouse cages under conventional laboratory conditions (food and water available ad libitum, constant room temperature and humidity, 12/12 h light/dark cycle). Separate groups of mice were used for biochemical and behavioural phenotyping. Mixed-gender groups of GAMT-deficient (KO, −/−), heterozygous (HZ, +/−) and wild type (WT, +/+) mice, aged 8–12 months, were used for determination of GCs in plasma, 24-h urine, brain and muscle (hamstring muscles).

For behavioural tests, 13 KO, 16 HZ and 9 WT male GAMT mice were used. All mice were subjected to the behavioural tests at the age of 6 months and were housed in mixed genotype groups.

Experiments were carried out in compliance with the European Communities Council Directive (86/609/ECC) and the Local Animal Ethics Committee approved all protocols.

2.2. Determination of guanidino compounds

2.2.1. Sample collection and preparation

Twenty-four-hour-urine was collected using a metabolic cage. Afterwards, mice were sedated with Nembutal (sodiumpentobarbital, 60 mg/kg) to collect blood via the orbital sinus (in heparin tubes). Animals were subsequently killed by immersion in liquid nitrogen and kept at −70 °C until dissection of brain and muscle. Blood was centrifuged for 10 min (1000×*g*, 4 °C) and plasma was collected. All samples were kept at −70 °C until analysis. Plasma and urine were deproteinized by adding 1/1 20% TCA (trichloroacetic acid). After 10 min centrifugation (20,800×*g*, 4 °C), the supernatant was diluted with sample buffer (trisodium citrate dihydrate, pH 2.5). Brain (left hemisphere) and muscle tissue were homogenised in 1000 μL ultrapure water followed by addition of the same volume 30% TCA and centrifugation during 30 min (20,800×*g*, 4 °C). Supernatant was diluted with sample buffer if necessary. Finally, 200 μL of sample was injected into the column.

2.2.2. GCs and urea determination

Determination of GCs levels was accomplished with a Biotronic LC 5001 (Biotronik, Maintal, Germany) amino acid analyser adapted for determination of GCs. The GCs were separated over a cation exchange column using sodium citrate buffers and are detected with the fluorescence ninhydrin method as previously reported in detail [13]. A Jasco Model FP-920 fluorescence detector (Jasco International, Tokyo, Japan) is used at 395 nm and 500 nm for Ex. and Em. respectively.

For urea determination, the method with diacetylmonoxime as described by Ceriotti was used [14].

2.3. Behavioural tests

Mice were subjected to a battery of behavioural tests, starting at the age of 6 months and lasting in total 4 weeks.

Cage activity was measured by a technique modified from Crawley and Goodwin [15]. Animals were individu-

ally housed in a standard transparent mouse cage (20×25 cm^2), which was placed between three infrared photo beams (two perpendicular to and one parallel with the length of the cage) interfaced with a computer. Ambulatory activity was measured during the dusk phase during 2 h and overnight during 16 h and expressed as total number of infrared beam crossings.

Visual spatial memory was tested in a Morris water maze (MWM) [16,17]. The circular plastic pool with a diameter of 150 cm was filled with white-coloured water kept at 25 °C. Animals were trained to find a circular escape platform (diameter 15 cm) placed at a fixed position in one of the quadrants, 1 cm beneath the water surface. Escape latency, path length and swimming speed were measured using the San Diego Instruments video tracker and analysed with the accessory software. The animals performed four daily trials with an inter-trial interval of 15 min for 8 days. Data from blocks of those four daily swimming trials, each starting in a semi-random order from one of four positions around the maze, were pooled. Animals that failed to find the platform within 2 min were put on it for 15 s. Data from animals that were unable to swim or floated on all trials were excluded from final analysis. After the eight acquisition trial blocks, a probe trial was recorded. During this test of spatial acuity, the platform was removed from the pool and animals were allowed to swim freely during 100 s. Entries in the previous platform area (target area=northeast (NE)) and time spent in each of the four quadrants (NE, southeast (SE), southwest (SW), northwest (NW)) were recorded.

Subsequently, passive avoidance learning was measured using a step-through box. During the dark phase of their 12/12 h cycle, animals were put into the small brightly lit compartment of the box. After 5 s, the connecting door to the second dark compartment was opened. Upon entry into the dark compartment (four-paw criterion), the connecting door was closed and the animal received a slight foot shock (0.3 mA, 1 s). Exactly 24 h later, the latency to re-enter the dark compartment was timed up to 300 s.

Equilibrium and motor coordination were tested on an accelerating rotarod apparatus (Ugo Basile, Italy). After one adaptation trial of a maximum of 2 min at constant speed (4 rpm), each mouse was placed on the rotating rod for four test trials with an inter-trial interval of 1 min. The time that the animal stayed on the rod was measured up to a maximum of 5 min, during which the rotation speed increased from 4 to 40 rpm. Open field behaviour was assessed with a computerized video tracking system during the dark phase of the mice's 12/12 h cycle. Animals were put in a brightly lit 50×50 cm^2 open field arena and their trajectory was recorded for 10 min following a 1-min adaptation period. Total distance, as well as distance in the center circle was measured. The number of entries in the four corner squares and the centre circle were recorded.

For the social interaction test, the same experimental procedure as in the open field test was used. However, two female mice were put in a cage in the centre circle.

In the final behavioural experiment, animals are put in the dark side of a dark–light transition box with four exits to the lit part of the box. The number of crossings through three infrared beams (one in the dark compartment, two in the lit compartment) was recorded during 10 min.

2.4. Statistics

Statistical analysis of GCs concentrations in plasma, 24-h urine, brain and muscle of WT, HZ and KO mice was performed with one-way analysis of variance (ANOVA) using post hoc Tukey tests to compare in a pair wise fashion between genotypes. Significant effects compared to the WT genotype group are indicated in tables as follows: *P<0.05, **P<0.01, ***P≤0.001; ° was used when obvious differences were observed but no statistics could be done because some values were below detection limit (DL).

Significance of differences in learning curves (MWM experiment) between genotype groups was assessed by two-way repeated measures ANOVA (RM-ANOVA). Groups of WT, HZ and KO mice were statistically analysed with one-way ANOVA. If a significant difference was observed, Student's t-test was used for pair wise comparison of groups. Significant differences are indicated on figures as follows: *P<0.05, **P<0.01 and ***P≤0.001.

3. Results

3.1. Guanidino compound concentration in GAMT mice

GCs concentration in plasma, 24-h urine, brain and muscle of WT, HZ and KO mice are presented as mean ±S.E.M. in Tables 1–4.

Table 1
Concentrations of guanidino compounds and urea in plasma of GAMT wild type (WT), heterozygous (HZ) and knock out (KO) mice

Plasma (μmol/l)	Mice		
Compound	WT (n=5)	HZ (n=7)	KO (n=11)
α-keto-δ-GVA	0.15±0.04	0.22±0.07	0.19±0.02
GSA	0.14±0.02	0.19±0.05	0.16±0.01
CT	172±16	190±24	11.56±7.1***
GAA	1.94±0.41	1.97±0.37	106±29*
α-N-AA	0.74±0.19	1.00±0.18	1.05±0.11
ArgA	0.13±0.05	0.13±0.04	0.08±0.01
β-GPA	<0.007	<0.007–0.03	<0.007–0.47
CTN	8.89±0.73	9.50±0.94	<0.4–2.15°
γ-GBA	0.38±0.10	0.62±0.25	0.52±0.09
Arg	102±8.3	134±11.5	141±10.9
Harg	<0.015–0.3	0.30±0.04	<0.015–0.9
G	0.27±0.01	0.40±0.07	<0.03–1.9
MG	<0.01	<0.01	<0.01
Urea (mmol/l)	8.47±1.04	10.5±1.67	7.91±0.54

Presented as mean±S.E.M. Asterisks indicate significant differences compared to WT genotype (one-way ANOVA and post hoc Tukey: *P<0.05, ***P≤0.001; ° indicates that difference is observed but no statistical test could be done because of values below detection limit (DL)).

Table 2
Concentrations of guanidino compounds in 24-h urine of GAMT wild type (WT), heterozygous (HZ) and knock out (KO) mice

Urine nmol/24 h	Mice		
Compound	WT (n=11)	HZ (n=9)	KO (n=16)
α-keto-δ-GVA	191±37	174±44	129±38
GSA	65±11	85±15	92±11
CT	8394±2739	5672±1203	47±36***
GAA	2023±575	2065±522	19,015±2887***
α-*N*-AA	35±8.2	46±8.1	47±6.9
ArgA	50±10.2	52±11.2	58±8.5
β-GPA	8.8±1.8	7.7±1.3	10.1±1.4
CTN	6491±1336	6206±926	536±124***
γ-GBA	570±174	613±139	943±135
Arg	132±26	141±37	158±21
Harg	<DL–2.0	<DL	<DL–16
G	142±41	158±34	217±34
MG	26±5.3	26±3.7	13±3.1

Presented as mean±S.E.M.; DL=detection limit. Asterisks indicate significant differences compared to WT genotype (one-way ANOVA and post hoc Tukey: ***P≤0.001).

There were no significant differences in GCs concentrations between WT and HZ GAMT mice. Concentration of GAA was increased in plasma, urine, brain and muscle of GAMT-deficient mice (resp. P=0.007, P<0.001, P<0.001, P<0.001). CT and CTN were significantly decreased in all body fluids and tissues tested of KO mice (one-way ANOVA, CT: all P<0.001). Next to these expected differences, concentrations of other GCs were also significantly altered, especially in brain and muscle. One-way ANOVA revealed increased GSA, ArgA, β-GPA and γ-GBA concentrations in brain of GAMT-deficient mice (GSA: P<0.001, ArgA: P<0.001, β-GPA: P<0.001 and γ-GBA: P<0.001). In muscle, β-GPA and γ-GBA were increased in knockouts (one-way ANOVA γ-GBA: P<0.001). Also Harg in brain and muscle and Arg in muscle of KO seemed to be increased (one-way ANOVA, Arg: P=0.05) (Tables 3 and 4). An obvious increase of β-GPA and Harg in the studied tissues of KO mice was observed, while an increase of these compounds could be suggested in the studied biological fluids. Indeed, only 3 out of 11 values of β-GPA in plasma of KO were <0.007 compared to 5 out of 7 in HZ and all values <0.007 in WT mice (Table 1). Harg concentration in plasma of KO was higher (only 1 out of 11 values <0.015) compared to HZ and WT mice (Table 1). Similar increases of β-GPA and Harg could be noticed in urine of KO mice (Table 2).

Table 3
Concentrations of guanidino compounds in brain of GAMT wild type (WT), heterozygous (HZ) and knock out (KO) mice

Brain (nmol/g tiss.)	Mice		
Compound	WT (n=5)	HZ (n=7)	KO (n=11)
α-keto-δ-GVA	0.80±0.08	0.60±0.08	0.73±0.05
GSA	0.17±0.02	0.22±0.03	2.36±0.19***
CT	11,277±422	10,336±465	466±86***
GAA	12.2±1.3	12.8±1.02	1852±61***
α-*N*-AA	0.86±0.14	1.30±0.11	1.14±0.14
ArgA	2.10±0.25	2.33±0.20	4.47±0.19***
β-GPA	0.15±0.03	0.21±0.03	1.05±0.08***
CTN	323±48	246±38	<DL–20.2°
γ-GBA	1.59±0.12	1.73±0.07	11.2±0.84***
Arg	174±6.4	219±51	174±9.31
Harg	<DL–0.69	<DL–1.04	<DL–2.18°
G	2.24±0.12	6.14±2.95	5.03±1.07
MG	1.16±0.34	<DL–1.51	<DL–0.41

Presented as mean±S.E.M.; DL=detection limit. Asterisks indicate significant differences compared to WT genotype (two-way ANOVA and post hoc Tukey: ***P≤0.001; ° indicates that difference is observed but no statistical test could be done because of values below DL).

Table 4
Concentrations of guanidino compounds in muscle of GAMT wild type (WT), heterozygous (HZ) and knock out (KO) mice

Muscle (nmol/g tiss.)	Mice		
Compound	WT (n=4)	HZ (n=6)	KO (n=6)
α-keto-δ-GVA	<DL	<DL	<DL–0.496
GSA	<DL	<DL	<DL
CT	16,508±278	18,788±2157	1501±301***
GAA	1.88±0.43	4.47±1.0	14,450±2088***
α-*N*-AA	<DL	<DL	<DL
ArgA	<DL	<DL–0.95	<DL–0.541
β-GPA	<DL–0.4	<DL–0.6	22±4.3°
CTN	176±20	331±94	<DL°
γ-GBA	0.53±0.11	0.54±0.11	17±3.3***
Arg	121±25	265±115	623±162$_{BS}$
Harg	<DL–0.711	<DL–1.13	4.0±0.8°
G	3.31±0.84	3.42±0.79	3.0±0.89
MG	<DL	<DL	<DL

Presented as mean±S.E.M.; DL=detection limit. Asterisks indicate significant differences compared to WT genotype (two-way ANOVA and post hoc Tukey: ***P≤0.001; BS: borderline significance P=0.05; ° indicates that difference is observed but no statistical test could be done because of values below DL).

3.2. Behaviour of GAMT mice

3.2.1. Cognitive performance assessment

3.2.1.1. Morris water maze. All mice improved their performance during the MWM acquisition phase, as indicated by significant effects of trial block on escape latency and path length in all groups (two-way RM-ANOVA: resp. $F_{7.245}$=59.473, P<0.001/$F_{7.245}$=47.747, P<0.001) (Fig. 1A–B). Furthermore, no effect of the interaction genotype×trial block on escape latency and path length was observed (P=0.087, P=0.152). Two-way RM-ANOVA showed no effect of genotype on escape latency, path length or swim velocity (P=0.595, P=0.231, P=0.684).

Genotype did not affect performance during the acquisition phase of the MWM test. However, during probe trial, two-way ANOVA did reveal a significant effect of the interaction between genotype and the percentage of time spent in each quadrant ($F_{6.140}$=3.742, P=0.002). KO mice exhibited a significantly different search pattern. KO mice spent less time in the target quadrant during the probe trial of the MWM compared to WT and HZ mice (P=0.020 and P=0.017) (Fig. 2A). Although entries in the target quadrant

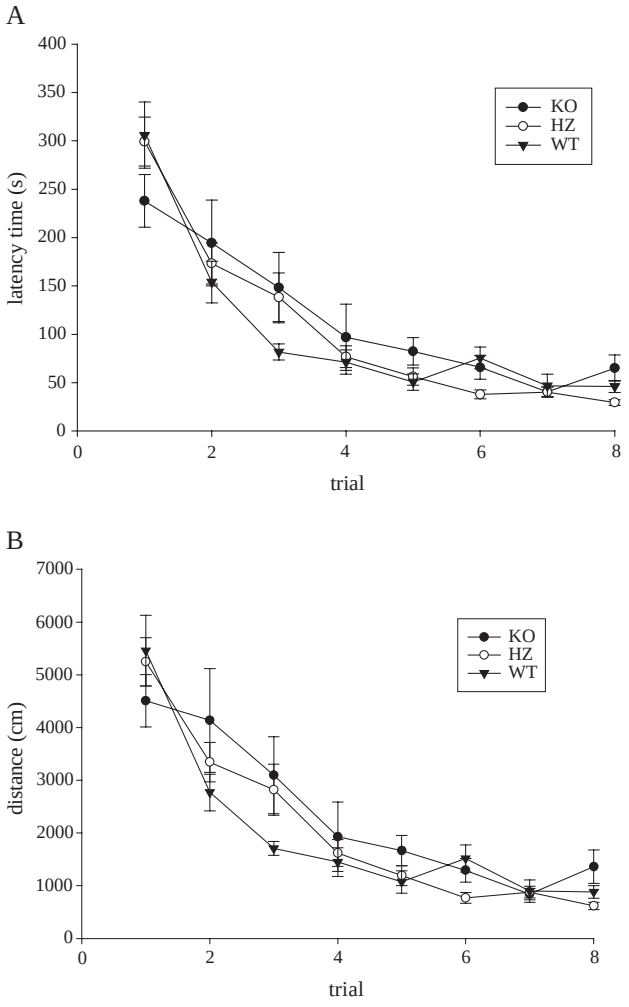

Fig. 1. A. Latency time of GAMT wild type (WT), heterozygous (HZ) and knockout (KO) mice during acquisition trial blocks in the MWM test. B. Path length of GAMT WT, HZ and KO mice during acquisition trial blocks in the MWM test. Each data point represents mean (±S.E.M.) of summed results of four daily trials.

were also lower in the KO group (Fig. 2B), this outcome measure did not reach statistical significance (P=0.216). One-way ANOVA did not reveal a significant effect of genotype on swimming velocity during the probe trial (P=0.963).

3.2.1.2. Passive avoidance. One-way ANOVA comparing all groups showed no significant effect of genotype on latency to re-enter in the dark compartment (P=0.088). Hence, there was no effect of genotype of GAMT mice on passive avoidance learning.

3.2.2. Activity and exploration, and neuromotor assessment

3.2.2.1. Activity. The 2-h and 16-h activity recording did not show any significant difference between groups (one-way ANOVA, 2 h: P=0.745, 16 h: P=0.629). KO, HZ and WT GAMT mice did not display differences in cage activity.

3.2.2.2. Open field exploration. No significant differences in distance, percentage of distance in centre and entries in centre were observed in the open field test. One-way ANOVA, however, did reveal a significant effect of genotype on number of entries in the corners of the arena ($F_{2.35}$=3.309, P=0.048). KO mice entered the corners slightly less than the HZ mice (t=2.177, P=0.04).

3.2.2.3. Social exploration. No significant differences between mice of different genotype were observed in the social exploration test.

3.2.2.4. Dark–light transition box. KO, HZ and WT GAMT mice showed no differences in behaviour in the dark–light transition box.

3.2.2.5. Rotarod. No effect of genotype or interaction of genotype with trial on neuromotor performance was observed. Differences in the above-described tests, there-

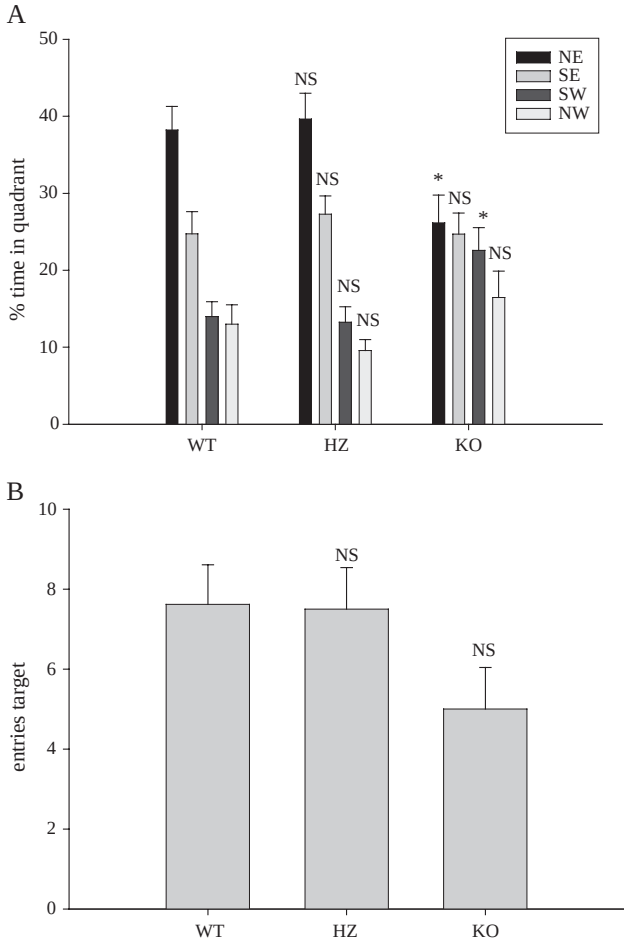

Fig. 2. A. % time spent in each quadrant (northeast (NE), southeast (SE), southwest (SW), northwest (NW)) during probe trial by GAMT wild type (WT), heterozygous (HZ) and knock out (KO) mice. B. Entries of GAMT WT, HZ and KO mice in target quadrant (NE) during probe trial. Presented as mean±S.E.M.; asterisks indicate significance of difference with WT group (Student's t-test: *p<0.05); when no significant difference with WT group was observed, this was indicated as NS (not significant).

fore, could not be attributed to deviant neuromotor performance and equilibrium.

4. Discussion

In this study, we present extensive biochemical and behavioural analyses of GAMT-deficient mice.

We determined the concentration of 13 GCs in plasma, 24-h urine, brain and muscle of WT, HZ and KO GAMT mice. GC levels did not differ between WT and HZ GAMT mice. Therefore, analysis of GCs levels as performed here cannot be used to differentiate between WT and HZ GAMT mice. It can, however, distinguish KO GAMT mice from WT and HZ GAMT mice. GAA concentration in plasma, 24-h urine, brain and muscle of GAMT-deficient mice was increased, while CT and CTN concentrations were decreased. In addition, there was a significant increase of other potentially important GCs in brains of KO mice: GSA, ArgA, β-GPA, γ-GBA and probably also Harg. Besides Arg, β-GPA and γ-GBA were also significantly increased in muscle of GAMT-deficient mice and Harg is suggested to be increased as well. Some of these increases seen in tissues were reflected in plasma and/or 24-h urine.

In plasma and CSF of patients with GAMT deficiency, the following alterations were reported: extremely low CT and CTN, and extremely high GAA levels, increase of Harg, high γ-GBA and low Arg levels [3]. It has been suggested that the depletion of CT and increase of GAA play a significant role in the patients' neurological symptoms [3]. Possibly other GCs are important as well. It was therefore interesting to study putative alterations of these compounds in the GAMT-deficient mouse model. This is also the first study to analyse GCs concentration in muscle of GAMT mice.

The range of decrease of CT and CTN and increase of GAA levels in the GAMT-deficient mice is comparable with human patients [3]. In human plasma, CT is decreased 200-fold, whereas the decrease is 100-fold in plasma of KO GAMT mice. GAA is increased resp. 20-fold and 50-fold in patients and mice. Comparing CSF of patients and brains of KO mice showed a 100-fold decrease of CT and a 200-fold increase of GAA in patients and a 20-fold decrease of CT and a 150-fold increase of GAA in brains of KO.

Increase of brain GSA in GAMT-deficient mice was more pronounced (10- to 14-fold) compared to patients' CSF (about 3-fold) [3]. γ-GBA and Harg are 6–7 and 2–3 times increased in brain of mice, while in CSF of patients γ-GBA and Harg are similarly increased (10- and 4-fold), whereas ArgA and β-GPA are not altered in CSF of patients. Also, low arginine levels seen in patients [3] were not observed in mice. Schmidt et al. [11] reported similar changes in concentrations of some GCs in serum, urine and brain of their GAMT mice, which were backcrossed to C57Bl/6J for our study: increase of γ-GBA (6×), β-GPA (6×) and GSA (13×) in brain of GAMT-deficient mice was reported.

The observed increase of different GCs in the GAMT-deficient mice might help in the adaptation to lowered energy availability or reserve, since there is a lack of phosphocreatine (CT-P), as a consequence of the extremely low CT concentration, in brain and muscle of these mice. Phosphorylation of other GCs as an alternative pathway in order to create energy could be implicated. Phosphagens, as they are called, are repeatedly reported to play a role in resynthesis of ATP and are present at higher concentration in muscle [18–20].

On the behavioural level, the present study showed that GAMT KO mice displayed impaired retrieval of learned information. During the probe trial of the MWM test, GAMT KO mice spent a significant lower percentage of time in the target quadrant. In the passive avoidance learning test, on the other hand, GAMT KO mice did not perform different from WT, indicating that not all forms of learning and memory were affected. The GAMT mouse model showed only a mild cognitive defect, as opposed to patients [1,3]. Except for the open field test, assessment of exploratory behaviour did not reveal differences between genotypes. The observation that KO mice enter corners less than HZ mice in the open field test is difficult to interpret, as exploratory behaviour is not specifically tested in patients.

Schmidt et al. [11] observed no severe neurological symptoms like epilepsy and ataxia in GAMT KO mice, as seen in patients. Although GAA might be involved in epilepsy of GAMT-deficient patients [5] and although this compound was also significantly increased in the brain of the KO mice, epileptic seizures were not observed in our GAMT-deficient mice.

In general, it can be concluded that this GAMT mouse model is very useful for future biochemical research of GAMT deficiency, although it shows only a mild cognitive deficit.

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