Propionate enters GABAergic neurons, inhibits GABA transaminase, causes GABA accumulation and lethargy in a model of propionic acidemia.

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Running head: Propionic acid inhibits GABA transaminase

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Abbreviations used: GABA: γ-amino butyric acid; GSH: glutathione; i.v.: intravenous; NMRS: nuclear magnetic resonance spectroscopy; TCA cycle: tricarboxylic acid cycle.

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Abstract

Propionic acidemia is the accumulation of propionate in blood due to dysfunction of propionyl-CoA carboxylase. The condition causes lethargy and striatal degeneration with motor impairment in humans. How propionate exerts its toxic effect is unclear. Here we show that intravenous administration of propionate causes dose-dependent propionate accumulation in the brain and transient lethargy in mice. Propionate, an inhibitor of histone deacetylase, entered GABAergic neurons, as could be seen from increased neuronal histone H4 acetylation in striatum and neocortex. Propionate caused an increase in GABA levels in the brain, suggesting inhibition of GABA breakdown. *In vitro* propionate inhibited GABA transaminase with a K*i* of ~1 mmol/L. In isolated nerve endings propionate caused increased release of GABA to the extracellular fluid. *In vivo*, propionate reduced cerebral glucose metabolism in both striatum and neocortex. We conclude that propionate-induced inhibition of GABA transaminase causes accumulation of GABA in the brain, leading to increased extracellular GABA concentration, which inhibits neuronal activity and causes lethargy. Propionate-mediated inhibition of neuronal GABA transaminase, an enzyme of the inner mitochondrial membrane, indicates entry of propionate into neuronal mitochondria. However, previous work has showed that neurons are unable to metabolize propionate oxidatively, leading us to conclude that propionyl-CoA synthetase is probably absent from neuronal mitochondria. Propionate-induced inhibition of energy metabolism in GABAergic neurons may render the striatum, in which >90% of the neurons are GABAergic, particularly vulnerable to degeneration in propionic acidemia.

Key words: caudate; GABA transaminase; autism; propionic aciduria; propionyl-CoA synthetase.

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Introduction

Propionic acidemia is a devastating genetic disorder that causes lethargy, mental retardation, and degeneration of the striatum, leading to severe motor impairment. Often the disease is fatal [1,2]. The underlying cause is reduced activity of propionyl-CoA carboxylase. This deficiency leads to impaired metabolism of propionate (a metabolic product of gut bacteria) and of propionyl-CoA (an intermediate in the metabolism of several amino acids) in the liver and other organs, causing accumulation of propionate in blood [1-3]. Serum values as high as 5 mmol propionate/L have been reported [4]. Irrespective of its role in propionic acidemia, a contribution of propionate to the development of autism has been proposed [5].

It is not clear how propionate exerts its toxic effect. Several biochemical alterations have been noted in brains of experimental animals after administration of propionate, including changes in protein carbonylation, lipid peroxidation, and antioxidant capacity [6,7]. A decrease in the levels of several neurotransmitters [8], and an increase in histone acetylation [9] and microRNA levels [10] have also been reported; the latter two alterations could directly affect gene expression, an effect that may be of special relevance for autism development. Two metabolites that are formed during mitochondrial metabolism of propionate, propionyl-CoA and 2-methylcitrate, have inhibitory effects on several enzymes of the tricarboxylic acid (TCA) cycle [11-14] or glutamate dehydrogenase [15]. These inhibitory effects are probably important for the liver failure and ensuing hyperammonemia that accompany propionic acidemia, but they do not readily explain the neurodegeneration. Since propionate is not oxidized by neurons [9], formation of propionyl-CoA and 2-methylcitrate from propionate would not be expected to occur in neuronal mitochondria. Therefore, accumulation of propionyl-CoA and 2-methylcitrate are unlikely causes of the specific vulnerability of the striatum in propionic acidemia.

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More than 90% of striatal neurons are GABAergic [16]. Given the vulnerability of striatum in propionic acidemia [1,2], we asked if propionate itself somehow targets GABAergic neurons. Here we report that propionate enters striatal neurons from the circulation and inhibits GABA transaminase. This effect may help explain both the lethargy in propionic acidemia, because brain GABA levels increase, and the vulnerability of the striatum in this disorder, because TCA cycle activity and energy production in GABAergic neurons to a large degree involves formation and breakdown of GABA through the so-called GABA shunt (Fig. 1) [17].

Materials and methods

Animals

The animals were female NMRI mice (Bomholt, Ry, DK), 30 g bodyweight. They had free access to food and tap water. Mice that received ^{14}C-labeled glucose were fasted for 12 hours prior to experiments, but had free access to water. Air humidity was 50%, and the light/dark cycle was 12 hours. Animal treatment was approved by the ethics committee at the Norwegian Defense Research Establishment, and the experimental work was in strict accordance with institutional and national ethical guidelines. After intravenous (i.v.) or intraperitoneal administration of sodium propionate or sodium chloride, animal behavior was closely observed until the animals were killed.

Determination of the level of propionate in brain and serum

To determine the level of propionate in brain and serum after i.v. injection, mice received sodium [1-^{13}C]propionate (99% isotopic enrichment; Isotec, Sigma, St. Louis, MO, USA), i.v. Doses were 1.25, 2.5, or 5 μmol sodium [1-^{13}C]propionate/g bodyweight. The injected

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solution was 125, 250, or 500 mmol [1-^{13}C]propionate/L. Control animals received sodium chloride, 500 mmol/L. The 125 and 250 mmol/L solutions of [1-^{13}C]propionate were supplemented with sodium chloride, 375 or 250 mmol/L, respectively, so that all solutions had similar osmolarity. At 5 or 15 minutes, the animals were anesthetized with a lethal dose of pentobarbital i.v. and perfused transcardially with 15 mL ice-cold phosphate-buffered saline (NaCl 140 mmol/L, NaH_2PO_4 10 mmol/L; pH 7.4) over 15 seconds, which caused brains and livers to become pale. Blood was collected from the right atrium. To see if this procedure would flush significant amounts of [1-^{13}C]propionate out of the brains, some animals were not perfused transcardially, but were decapitated, and blood was collected from the severed vessels. The two groups were almost identical with respect to the level of propionate in the brain, and only the values obtained after transcardial perfusion will be reported. Brains were rapidly removed, frozen in liquid nitrogen, weighed, and homogenized in 2 volumes of ice-cold perchloric acid, 7% (vol/vol). Protein was removed by centrifugation, and perchloric acid was precipitated with KOH, 9 mol/L. Prior to nuclear magnetic resonance (NMR) spectroscopy, 400 μL of brain extracts were mixed with 100 μL of D_2O containing dioxane, 1%, as an internal concentration standard. Sera, 150 μL, were mixed with 300 μL D_2O containing dioxane, 0.3%. Inverse-gated ^{13}C NMR spectroscopy was done as described [9]. The amount of [1-^{13}C]propionate in brain and serum was determined from the NMR spectra.

Confocal microscopy of histone acetylation

To see if propionate entered striatal neurons, we made use of the inhibitory effect of propionate on histone deacetylase [18]. Six mice received an i.v. injection of sodium propionate, 5 μmol/g body weight (0.5 mol/L, pH 7), and six mice received sodium chloride, 0.5 mol/L. At 15 minutes, the animals were deeply anesthetized with pentobarbital and

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perfused transcardially with 4% paraformaldehyde. Brains were cryosectioned coronally in 10 μm sections and collected on glass slides, so that each glass slide had sections from two propionate-treated mice and two controls.

Brain sections were incubated overnight at room temperature with mouse antibodies against acetylated histone H4 (2 μg/ml; Upstate, New York, USA) and rabbit antibodies against glial fibrillary acidic protein (GFAP; Sigma, St Louis, MO, USA). Secondary antibodies were species-specific and coupled to different fluorochromes, Alexa 555 and Alexa 488 (Molecular Probes, Eugene, OR, USA). Microscopy was done with a Zeiss LSM 5 Pascal axioplan 2 imaging confocal laser scanning microscope. The pinhole size was set to about 1 Airy unit, and the images were acquired sequentially with a scan speed of 12-13 μs/pixel. Laser wavelengths for the green and red channels were 488 nm and 543 nm, respectively. High resolution micrographs were acquired as composites of confocal optical sections, 0.77 μm thick, through the entire nuclei. The amount of acetylated histone H4 was quantified as fluorescence intensities, and data from each optical section were used. Quantification was done with Zeiss LSM 5 Pascal software. Nuclei not coinciding with GFAP staining were considered neuronal. Nuclei coinciding with GFAP staining were considered astrocytic and were not analyzed; we have shown previously that propionate enters (and is metabolized oxidatively by) astrocytes [9]. Histone H4 acetylation was analyzed for 7 nuclei in the striatum and 7 in the overlying neocortex in each animal.

Determination of amino acids

To determine the effect of propionate on brain amino acid levels, awake mice received sodium propionate 1.25, 2.5, or 5 μmol/g bodyweight as 125, 250, or 500 mmol/L solutions i.v. Control animals received sodium chloride, 500 mmol/L, as described above. Five minutes

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after the injection, the animals were killed by cervical dislocation and decapitation. The heads were dropped into liquid nitrogen within 1 second of decapitation. Brains were removed in the frozen state and homogenized in 4 mL of ice-cold perchloric acid, 3.5% (vol/vol). Proteins were removed by centrifugation, and perchloric acid was precipitated with KOH, 9 mol/L. The volume of the supernatant was measured, and α-aminoadipate, 1 mmol/L, was added 1:1. Amino acids and glutathione (GSH) were quantified fluorimetrically after pre-column derivatization with o-phthaldialdehyde and separation by HPLC, using α-aminoadipate as an internal concentration standard [19].

Enzyme analyses

GABA transaminase activity was determined as the formation of glutamate from GABA and α-ketoglutarate in homogenates of mouse forebrain, 5% (weight/volume), as described [20], with a final GABA concentration of 10 mmol/L. Aspartate aminotransferase activity was analyzed similarly [20] as the formation of glutamate from aspartate and α-ketoglutarate, substituting GABA with aspartate (final concentration 20 mmol/L). Succinate dehydrogenase activity was analyzed as described [21] in mouse brain homogenates as the reduction of tetrazolium in the presence of succinate, 0.3 mmol/L. Incubation time was two hours, and the reaction product, formazan, was extracted and assayed spectrophotometrically. Blank values obtained in the absence of succinate were ~12% of measurements in the presence of succinate. Propionate was present in the enzyme assays at final concentrations of 1-100 mmol/L.

Isolated nerve endings. Exposure to propionate.

Nerve endings (synaptosomes) were isolated from the cerebral cortex of mice by the method of Gray and Whittaker [22], as recently described [23]. The nerve endings were reconstituted

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in artificial CSF with (in mmol/L) NaCl 120, KCl 25, $CaCl_2$ 1, $MgCl_2$ 1, NaH_2PO_4 0.3, glucose 2.5, glutamine 0.1, pH 7.3. Nerve endings were incubated with Na-propionate at 0, 0.3, 1, 3, 10, or 30 mmol/L, by adding Na-propionate, 150 mmol/L (or NaCl, 150 mmol/L), to the nerve endings, final volume 200 μL. Incubation took place at 37°C for 15 minutes. Then nerve endings were pelleted gently by centrifugation at 500 g. The supernatants were kept for analysis of amino acids and glutathione. The pellets were lysed in 200 μL water, frozen and thawed and re-centrifuged. The resulting supernatants were analyzed with respect to amino acid levels.

Cerebral metabolism of [^{14}C]glucose

Mice that were fasted overnight received propionate, 5 μmol/g bodyweight, as a 0.5 mol/L solution, or sodium chloride, 0.5 mol/L, i.v. into a tail vein over 15 seconds. At 5 minutes they received 10 μCi [U-^{14}C]glucose (3 mCi/mmol; ARC, St. Louis, MO, USA) in 200 μL physiological saline i.v.; after another 10 or 15 minutes the animals were killed by cervical dislocation and decapitation. The heads were immediately dropped in liquid N_2, and the striatum and overlying neocortex were dissected out from the frozen brains. Tissue samples were homogenized in 2 mL ice-cold perchloric acid, 3.5% (vol/vol), containing α-aminoadipate, 50 μmol/L, as an internal amino acid concentration standard, and centrifuged. Supernatants were neutralized with KOH, 9 mol/L, and the precipitating $KClO_4$ was removed by centrifugation. Extracts were lyophilized to dryness and redissolved in 60 μL water. Amino acids were quantified fluorimetrically after pre-column derivatization with o-phthaldialdehyde and separation by HPLC [19]. Radiolabeling of amino acids was determined by scintillation counting after collection of separated amino acids from the HPLC eluate [23].

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Results from animals with a 10-minute survival after injection of [U-^{14}C]glucose were essentially the same as those with a 15-minute survival, and only the former results are given.

Data presentation and statistics

Data are given as mean + SD values. Values for succinate dehydrogenase activity and histone H4 acetylation level are given as percent of control values; other enzyme activities, levels of metabolites, and radiolabeling are given in absolute values. Differences between groups were analyzed with the Student's *t*-test, paired or unpaired, or with one-way ANOVA with Dunn's or Dunnet's correction for multiple comparisons, as appropriate.

Results

Levels of propionate in brain and serum after i.v. injection

The level of propionate in the brain increased dose-dependently after i.v. injection, as could be seen 5 minutes after injection of [1-^{13}C]propionate, 1.2-5 μmol/g bodyweight. The mean levels varied between 0.3 and 2.3 nmol propionate/mg tissue (Fig. 2a). Assuming a water content of 80% and a uniform distribution of propionate, these levels would correspond to concentrations of propionate of 0.4-2.8 mmol/L. The corresponding mean serum levels were 0.7-17 mmol propionate/L (Fig. 2b).

Fifteen minutes after injection of [1-^{13}C]propionate, 5 μmol/g, the level of propionate in the brain was 0.8 ± 0.1 nmol/mg tissue, and the level in serum was 2.3 ± 0.9 mmol/L.

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Behavioral effects of intravenous injection of propionate

Within 30 seconds after intravenous injection of sodium propionate, 5 μmol/g bodyweight, the animals appeared lethargic, a state that lasted for approximately 20 minutes. The animals lay down, but kept their heads elevated above the floor of the cage. Some animals lay with their hind feet splayed, but all animals could be stimulated to walk about the cage without displaying ataxia. Animals that received sodium propionate, 2.5 μmol/g, appeared quiet compared to control animals, but the response was less pronounced than in animals that received 5 μmol sodium propionate/g. Animals that received 1.25 μmol sodium propionate/g were not behaviorally different from saline-treated animals; these animals explored their cages and were highly active. Injection of a larger amount of propionate (15 μmol/g bodyweight) intraperitoneally, caused a lethargic state for approximately 45 minutes.

Effect of propionate on histone H4 acetylation in striatum

Propionate, 5 μmol/g bodyweight, caused an increase in histone H4 acetylation in striatal and neocortical neurons, as could be seen at 15 minutes after i.v. injection (Fig. 3), indicating entry of propionate, a known inhibitor of histone deacetylase [18], into neurons. The striatal neurons, as defined by their nuclei, were medium-sized, typical GABAergic striatal projection neurons [24]. The labeling intensity of acetylated histone H4 in neurons was approximately doubled by propionate administration (Fig. 3).

Effect of propionate on brain levels of GABA and aspartate

Injection of propionate, 1.25-5 μmol/g bodyweight, into awake mice caused a dose-dependent increase in the brain level of GABA at 5 minutes (Fig. 2c); the highest dose of propionate

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caused an increase in GABA of approximately 40%. At the same time the level of aspartate decreased dose-dependently (Fig. 2d); the maximal decrease was approximately 20%. The levels of glutamate, glutamine, glycine, taurine, or GSH were not altered by propionate treatment (not shown).

Effect of propionate on enzyme activities

Propionate inhibited GABA transaminase with a K*i* of approximately 1 mmol/L (Fig. 4) in mouse forebrain homogenates. Propionate did not affect aspartate aminotransferase or succinate dehydrogenase activities at any concentration (Fig. 4).

The effect of propionate on amino acid levels in isolated nerve endings

GABA levels increased in isolated nerve endings that were exposed to propionate. The total level of GABA (that in nerve terminals + that found extracellularly in the incubation medium) increased significantly by approximately 40, 50, and 70% with propionate at 3, 10, and 30 mmol/L, respectively ($p<0.001$). The GABA concentration in the extracellular fluid varied between 14 and 17% of the total amount of GABA. Extracellular GABA increased significantly with propionate ≥1 mmol/L (Fig. 5). The levels (total or extracellular) of aspartate, alanine, or taurine did not change significantly in nerve endings exposed to propionate. Extracellular glutamate increased (by 40%; $p = 0.02$) only in the presence of propionate, 30 mmol/L. Extracellular GSH, which was 35±4% of total GSH in controls, decreased significantly with propionate ≥ 3 mmol/L (Fig. 5), whereas intracellular GSH was not significantly different between groups.

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Effect of propionate on brain glucose metabolism

Propionate inhibited glucose metabolism in both striatum and neocortex of awake mice, as could be seen from a decrease in the radiolabeling (specific activity) of GABA, glutamate, and glutamine from [U-^{14}C]glucose (Fig. 6). Propionate treatment reduced the radiolabeling of GABA to approximately 60% of control. The radiolabeling of glutamate and glutamine was reduced to 76% and 50% of control, respectively. The radiolabeling of aspartate was also reduced by propionate treatment, but since the total level of aspartate decreased similarly (by ~20%), the specific activity (radioactivity/nmol amino acid) did not decrease significantly.

Discussion

Propionate inhibits GABA transaminase

This study shows that propionate enters the brain from the circulation in a concentration-dependent manner. Propionate, a known inhibitor of histone deacetylase [18], was shown to enter neurons in striatum and neocortex, as could be seen from the increase in neuronal histone H4 acetylation. The striatal neurons that took up propionate had nuclei consistent with medium-sized, GABAergic projection neurons [24].

Accumulation of propionate in the brain was accompanied by increased levels of GABA. This increase could be explained by an inhibitory effect of propionate on the GABA-degrading enzyme, GABA transaminase, since the level of propionate in the brain after i.v. administration of propionate was similar to concentrations that caused significant inhibition of GABA transaminase *in vitro*. The accompanying reduction in the level of aspartate is typically seen with inhibitors of GABA transaminase [17,25,26]. Aspartate is concentrated in GABAergic neurons [27-29], where it is formed from oxaloacetate downstream of GABA transaminase (Fig. 1). Therefore, the decrease in aspartate seen in this study agrees well with propionate being an inhibitor of GABA transaminase.

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The increased concentration of GABA in the brain of propionate-treated animals probably caused increased release of GABA from GABAergic nerve endings, as suggested by the results with isolated nerve endings in this study. The cytosolic concentration of GABA in GABAergic nerve endings determines the degree of filling of synaptic vesicles [30], and so a higher concentration of GABA in nerve endings leads to a greater release of GABA during neuronal activity, leading to greater activation of GABA receptors. An increased release of GABA probably caused the lethargic state elicited by propionate administration; a similar reaction is seen with γ-vinyl GABA, a specific inhibitor of GABA transaminase [17]. Therefore, it is likely that an elevated level of propionate leads to increased inhibitory (GABAergic) neurotransmission and thereby contributes to the lethargy observed in patients with propionic acidemia [1].

Propionate-treated animals had reduced cerebral metabolism of glucose, as could be seen from the diminished radiolabeling of amino acids from [^{14}C]glucose. This most likely reflected the inhibitory effect of GABA on neuronal activity. The same effect can be seen after treatment with $GABA_A$ receptor agonists [31,32].

Neurons probably lack mitochondrial propionyl-CoA synthetase

The inhibitory effect of propionate on neuronal GABA transaminase (e.g. in isolated nerve terminals) implies that propionate entered neuronal mitochondria, because GABA transaminase is a mitochondrial enzyme associated with the inner mitochondrial membrane [33]. It has previously been shown that neurons, in contrast to astrocytes, are unable to metabolize propionate oxidatively although they do metabolize propionyl-CoA, which is formed during the metabolism of isoleucine [9]. The present finding of propionate-mediated inhibition of GABA transaminase therefore strongly suggests that propionate enters neuronal mitochondria, but that it is not further metabolized [9] due to a lack of mitochondrial

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propionyl-CoA synthetase. A lack of mitochondrial propionyl-CoA synthetase would leave neurons unable to dispose of even mildly elevated levels of propionate during episodes of propionic acidemia, adding to the vulnerability of the brain in this condition.

Propionate-induced vulnerability of GABAergic neurons?

Inhibition of GABA transaminase implies inhibition of TCA cycle activity in GABAergic neurons, because, in these neurons, TCA cycle activity involves formation and breakdown of GABA to a high degree [17,26] (cf. Fig. 1). Inhibition of energy metabolism could render GABAergic neurons vulnerable to other stressors, e.g. lipid peroxidation and protein carbonylation, which have been shown to occur in the brains of experimental animals exposed to propionate [6,7] as well as to the effects of hyperammonemia, which follows from the inhibitory effect of propionate on N-acetyl-glutamate synthase [34]. The reduction in glutathione levels that were observed in isolated nerve endings exposed to propionate could contribute to this vulnerability.

GABAergic neurons make up more than 90% of the neurons in striatum [16]. Therefore, the inhibitory effect of propionate on GABA transaminase and the TCA cycle activity of GABAergic neurons may render striatum especially vulnerable in patients with propionic acidemia. This could explain why the striatum so frequently undergoes degeneration in patients with this disorder [1-3]. We cannot at present explain the reduction in extracellular GSH when nerve terminals were exposed to propionate, but the finding mimics the observation that plasma GSH is reduced in children with propionic acidemia [35] and it corroborates earlier findings of reduced GSH levels in rodent models of propionic acidemia [7].

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Disclosure/Conflict of Interest

The authors have no conflict of interest to declare.

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Author contributions:

All authors contributed to study design and rationale, laboratory work, data analysis, and writing of the manuscript.

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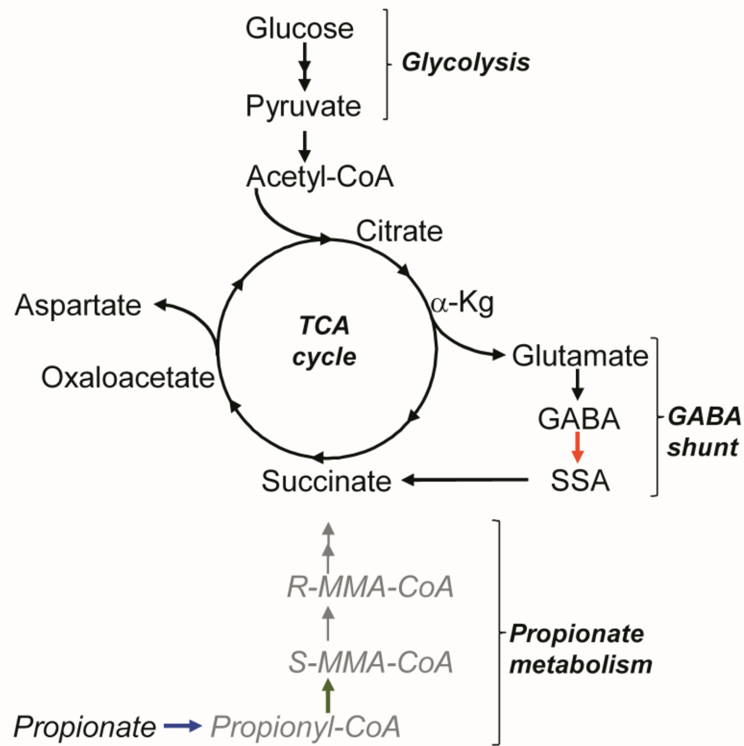

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Figure 1 Simplified scheme of glycolysis, the TCA cycle, and the GABA shunt, and how glutamate, GABA, aspartate, and propionate relate to the TCA cycle. In GABAergic neurons, α-ketoglutarate (α-Kg) may be converted into succinate along two pathways: the conventional TCA cycle pathway (through α-ketoglutarate dehydrogenase and succinyl-CoA ligase) or the GABA shunt. In the GABA shunt, α-ketoglutarate is converted to glutamate, which is decarboxylated to GABA. GABA is transaminated to succinic semialdehyde (SSA) by GABA transaminase (red arrow), which is inhibited by propionate. Succinic semialdehyde is converted into succinate. Succinate is successively converted into fumarate, malate, and oxaloacetate; the latter gives rise to aspartate. In GABAergic neurons, the GABA shunt accounts for approximately 50% of the flux from α-ketoglutarate to succinate and is therefore important for energy production in these neurons [17].

The lower part of the scheme shows conversion of propionate to propionyl-CoA by propionyl-CoA synthetase (blue arrow; probably not expressed in neuronal mitochondria) and subsequent formation of L-methylmalonyl-CoA (MMA-CoA) by propionyl-CoA carboxylase (green arrow), which is dysfunctional in propionic acidemia. L-MMA-CoA is converted to R-MMA-CoA, which is converted to succinyl-CoA and hence to succinate.

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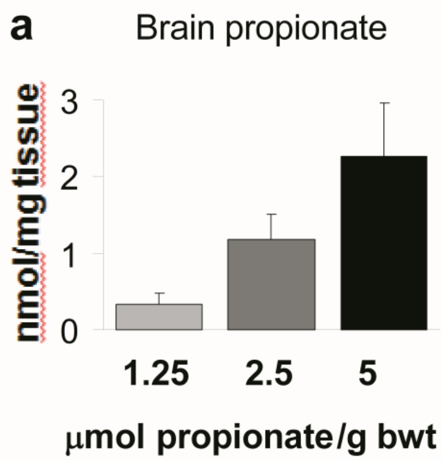

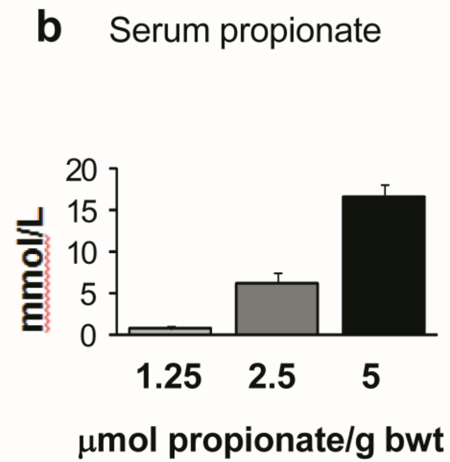

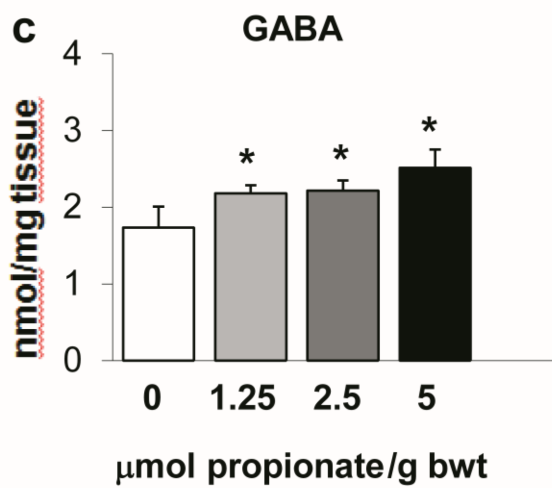

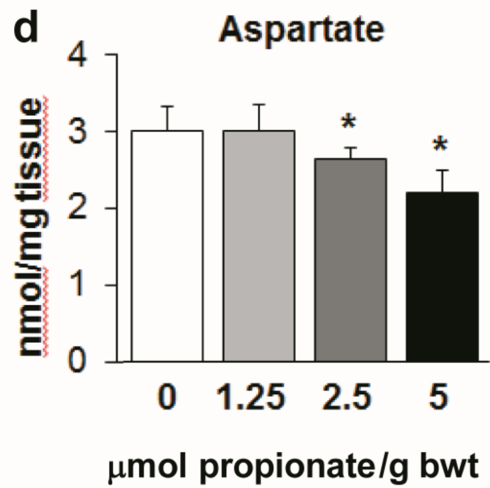

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Figure 2 Concentrations of propionate, GABA, and aspartate in mouse brain or serum 5 minutes after i.v. injection of propionate. Mice received sodium [1-^{13}C]propionate, 1.25, 2.5, or 5 μmol/g bodyweight, i.v. Brains (a) and sera (b) were analyzed by ^{13}C NMR spectroscopy for content of [1-^{13}C]propionate (mice were perfused transcardially with saline to remove blood from the brain). c and d: Awake mice received sodium propionate, 1.25, 2.5, or 5 μmol/g bodyweight, i.v. At 5 minutes the animals were killed by decapitation, and the heads were immediately frozen in liquid nitrogen. Brains were analyzed for GABA and aspartate. Data are (in a, c, and d) nmol/mg tissue, (in b) mmol propionate/L; mean + SD values; N = 4-7 animals in each group. Asterisks: difference from control; *: $p < 0.05$.

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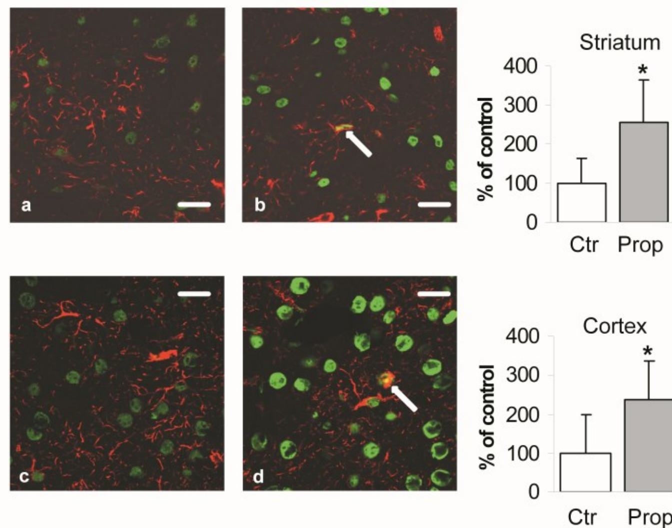

Figure 3 Effect of propionate on histone H4 acetylation in neurons. Wake mice received sodium propionate, 5 μmol/g bodyweight, or saline, and were killed 15 minutes later. Confocal photomicrographs show histone H4 acetylation (green) and glial fibrillary acidic protein (GFAP; red) in striatum of controls (a) and propionate-treated (b) mice (upper panels) and in the overlying neocortex of controls (c) and propionate-treated (d) mice (lower panels). Nuclei not coinciding with GFAP staining were considered neuronal. Acetylated histone signal may also be seen in astrocytes (arrow), in which GFAP and acetylated histones are present in close approximation to GFAP. Scale bars 10 μm. Diagrams to the right show histone acetylation as percent of control in striatum and cortex, respectively, mean + SD; N = 6 animals in each group. *: $p \leq 0.02$.

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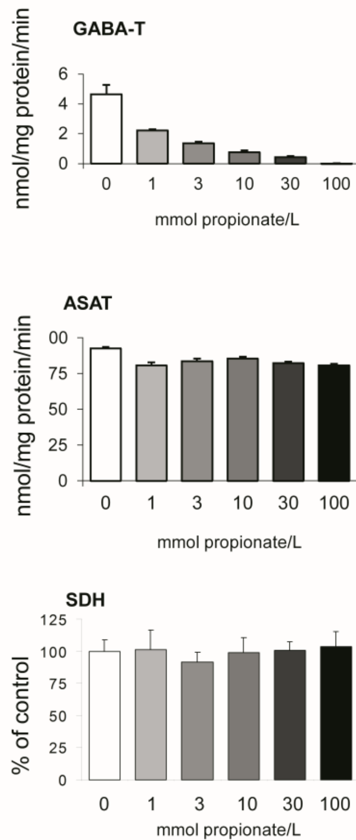

Figure 4 Inhibition of GABA transaminase by propionate. Mouse brain homogenates (N = 5) were assayed for GABA transaminase (GABA-T), aspartate aminotransferase (ASAT), and succinate dehydrogenase (SDH) activities in the presence of propionate, 0-100 mmol/L. Data are nmol/mg tissue x min^{-1} or (in the case of SDH) percent of control; mean + SD values.

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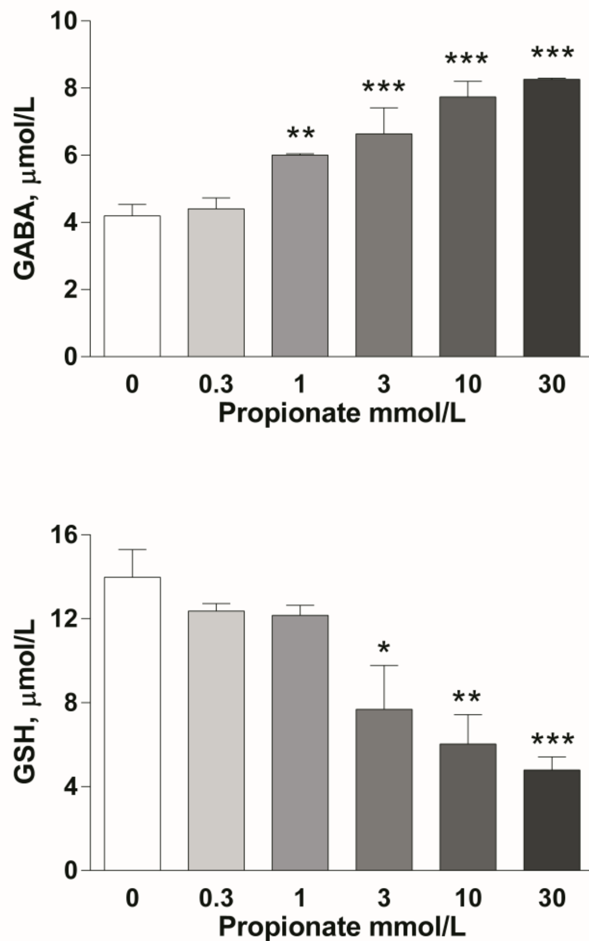

Figure 5 Effect of propionate on the extracellular concentration of GABA and glutathione (GSH) in preparations of isolated nerve endings. Isolated nerve endings (synaptosomes) were incubated at 37°C in incubation medium containing sodium propionate, 0-30 mmol/L, and potassium at depolarizing concentration (25 mmol/L). At 15 minutes nerve endings were gently pelleted by centrifugation, and the extracellular fluid (supernatant) was analyzed with respect to amino acids. Data are mean + SD values; N=3 per value. Asterisks: difference from control; *: $p < 0.05$; **: $p < 0.01$; ***: $p<0.001$, one-way ANOVA with Dunn's correction.

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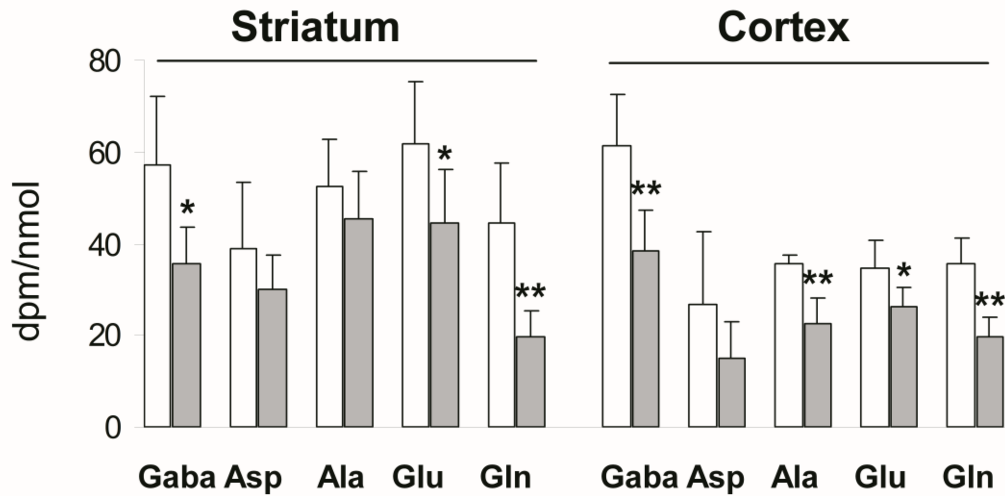

Figure 6 Effect of propionate on metabolism of glucose manifesting as radiolabeling of amino acids from [^{14}C]glucose in striatum and neocortex. Mice received saline or sodium propionate, 5 μmol/g bodyweight, i.v. At 5 minutes, they received 10 μCi [U-^{14}C]glucose i.v., and they were killed 10 minutes later. Data are dpm/nmol; mean + SD values; N = 7 animals in each group. Asterisks: difference from control; *: $p < 0.03$; **: $p < 0.003$; one-way ANOVA with Dunnet's correction for multiple comparisons. Abbreviations: Ala: alanine, Asp: aspartate, Gln: glutamine, Glu: glutamate.

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