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ORIGINAL PAPER

Paracetamol prevents hyperglycinemia in vervet monkeys treated with valproate

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Abstract Valproate administration increases the level of the inhibitory transmitter, glycine, in the urine and plasma of patients and experimental animals. Nonketotic hyperglycinemia (NKH), an autosomal recessive disorder of glycine metabolism, causes increased glycine concentrations in blood, urine, and cerebrospinal fluid (CSF), most likely due to a defect in the glycine cleavage enzyme or possibly deficits in glycine transport across cell membranes. We investigated the relationship between the hyperglycinemic effect of valproate and induced pyroglutamic aciduria via paracetamol in the vervet monkey. Firstly it was determined if valproate could induce hyperglycinemia in the monkey. The second aim was to increase glutamic acid (oxoproline) urine excretion using paracetamol as a pre-treatment and to assess whether valproate has an influence on the γ-glutamyl cycle. Hyperglycinemia was induced in healthy vervet monkeys when treated with a single oral dose of 50 mg/kg valproate. An acute dose of 50 mg/kg paracetamol increased oxoproline in the urine. Pre-treatment with paracetamol opposed the hyperglycinemic effect of valproate. However, the CSF:serum glycine ratio in a nonketotic monkey increased markedly after paracetamol treatment and remained high following valproate treatment. These results indicate that the γ-glutamyl cycle does indeed play a role in the hyperglycinemic effect of valproate treatment, and that paracetamol may have value in preventing and/or treating valproate-induced NKH.

Keywords Valproate · Hyperglycinemia · Paracetamol · γ-glutamyl cycle · Vervet monkey

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Introduction

Valproic acid (2-propylpentanoic acid, valproate), is a short chain fatty acid compound used to treat epilepsy and bipolar disorders (Keck et al. 1992). It is also useful to treat migraine (Sorensen 1988). The mechanism of action of valproate is unknown, but is primarily related to its ability to increase inhibitory gamma-aminobutyric acid (GABA) transmission in the brain via inhibition of GABA-transaminase or desensitization of GABA autoreceptors (Mclean and Macdonald 1986; Leonard 2003).

Side effects of valproate range from simple gastro-intestinal effects, like nausea and vomiting, to serious central nervous system (CNS) effects. Plasma concentrations of glycine increase (hyperglycinemia) in patients treated with valproate (Mortensen et al. 1980). Hyperglycinemia in patients and rats treated with valproate has been found to be due to the inhibition of the glycine cleavage system (Mortensen et al. 1980). Glycine displays diverse actions in the CNS, acting as an inhibitory neurotransmitter especially in the spinal cord, brainstem, and

retina (Hamosh and Johnston 2001; Leonard, 2003), while it is an obligatory co-agonist with glutamate at N-methyl-D-aspartate (NMDA) receptors in cortical and sub-cortical regions where it facilitates excitatory glutaminergic transmission (Hamosh and Johnston 2001; Harvey and Shahid 2012).

Increased glycine levels in urine, blood and cerebrospinal fluid (CSF) are also found in nonketotic hyperglycinemia (NKH), which is caused by a decrease or absence of the glycine cleavage enzyme system (Hamosh and Johnston 2001; Tada 1987). A CSF:serum glycine ratio larger than 0.08 is the hallmark of diagnosis of NKH (Tada 1987), and reflects a high brain glycine content. Valproate is contraindicated in the treatment of NKH, mainly due to its side effect of increasing plasma and CSF glycine concentrations, which can increase seizure frequency (Hamosh et al. 1992). Since glycine acts as both an inhibitory and excitatory neurotransmitter, the accumulation of glycine to pathological levels will have significant effects on brain function and activity, causing NKH and a resultant increase in seizure activity, possibly due to facilitating glutaminergic activity. The GCS is situated in the mitochondrial inner membrane (Motokawa and Kikuchi 1971), where glycine is catabolized through several metabolic pathways. The major pathway involves the oxidative cleavage of glycine to CO_2, NH_4^+, and a methylene group ($-CH_2$-). The methylene group is accepted by tetrahydrofolate (H_4 folate) in a reversible reaction that is catalyzed by glycine synthase (Kikuchi et al. 2008).

Administration of valproate to rats markedly decreases GCS activity in the liver, most likely due to reduced pyridoxal-phosphate-dependent enzyme activity that is the first step of the GCS (Kochi et al. 1979). It is important to note that valproate-induced inhibition is not restricted to the hepatic GCS, but it also competitively inhibits brain GCS activity (Martin-Gallardo et al. 1985). Valproate therapy can therefore cause elevated CSF glycine levels and CSF:serum glycine ratios. This elevation in glycine is usually observed in CSF and not in plasma and may indicate a different mechanism on the central nervous system when compared to the hepatic cleavage system (Applegarth and Toone 2001). However, glycine homeostasis is not only dependent on the GCS, but also on its transport across cell membranes, especially the blood brain barrier (BBB).

The transport of glycine across the BBB is facilitated by the γ-glutamyl cycle (Fig. 1). The cycle synthesises and degrades reduced glutathione and plays an important role in amino-acid transport across membranes (Orlowski and Meister 1970). The γ-glutamyl cycle in the brain influences amino acid transport indirectly through oxoproline (also known as pyroglutamic acid) (Hawkins et al. 2006) to maintain low concentrations of glutamate, aspartate and glycine in the brain (Lee et al. 1996).

5-Oxoprolinuria is a recognized condition with increased urinary excretion of 5-oxoproline, an intermediate of the γ-glutamyl cycle, and is associated with a deficiency of enzymes in the γ-glutamyl cycle: glutathione synthetase (GS) and 5-oxoprolinase (5-OPase)(Fenves et al. 2006). A number of case reports have suggested that paracetamol can induce pyroglutamic aciduria and increase 5-oxoplroline (Creer et al. 1989; Pitt et al. 1990; Pitt and Hauser 1998). Anion gap acidosis as a result of high levels of 5-oxoproline (pyroglutamic acid) after chronic paracetamol (Pitt and Hauser 1998) and acute paracetamol (Lawrence et al. 2010) exposure has been reported. The paracetamol metabolite, *N*-acetylbenzoquinonimine, reacts irreversibly with glutathione leading to an accumulation of precursors that cannot be converted to glutathione. Glutathione depletion eliminates the feedback inhibition of γ-glutamylcysteine synthetase. As a result, γ-glutamylcysteine production is increased. γ-Glutamylcysteine is then converted via a less favourable pathway by γ-glutamyl cyclotransferase back to 5-oxoproline (Fig. 2; Croal et al. 1998; Pitt and Hauser 1998). This sequence of events explains both the mechanism of pyroglutamic acid accumulation in the urine and paracetamol associated toxicity.

In the current study we investigated whether the γ-glutamyl cycle may also play a role in valproate induced hyperglycinemia. To test this hypothesis, vervet monkeys were used to determine if valproate-induced nonketotic hyperglycinemia could be prevented by simultaneously inhibiting the γ-glutamyl cycle with paracetamol, the latter administered prior to treatment with valproate.

Materials and methods

This study (07D02) was approved by and done in accordance with the guidelines stipulated by the Ethics Committee for the Use of Experimental Animals at the North-West University, Potchefstroom. It was a pilot study that formed part of our investigation of the mechanism of valproate toxicity

Materials

The two experimental drugs, valproate and paracetamol were obtained from Sigma-Aldrich and Fine Chemicals Corporation (Cape Town, RSA) respectively.

Experimental animals

Four vervet monkeys (*Chlorocebus aethiops*, 4 females - 141, 592, 789 and 1 male - 007), weighing between 3.5 and 4.5 kg, were acquired from the Medical Research Council, Tygerberg, South Africa. The monkeys were housed in squeeze-back, stainless steel cages and maintained on a balanced diet purchased from the Medical Research Council and supplemented with fresh vegetables/fruit. Food was given twice daily (9.00–

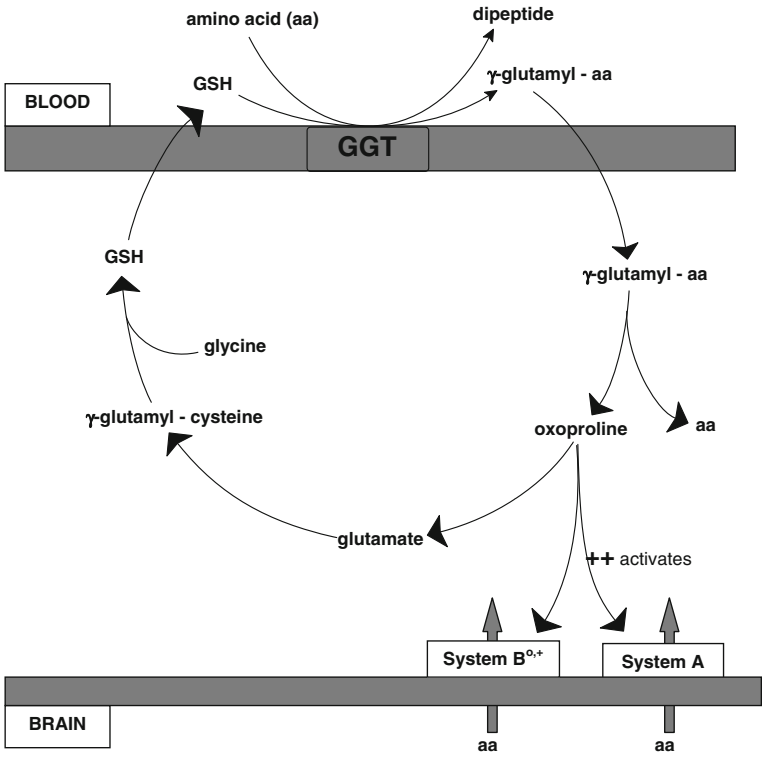

Fig. 1 The γ-glutamyl cycle. Glutamyl aa is formed at the outer surface of the luminal membrane and transferred into the endothelial cell via γ-glutamyl transpeptidase (GGT) where oxoproline is formed. The sodium-dependent transport systems A and B^{o+}, located on the abluminal side, are activated by oxoproline (Lee et al. 1996)

9.30 am and 3.00–3.30 pm), and water was available ad libitum. The monkeys were anaesthetized with approximately 10 mg/kg ketamine hydrochloride to enable handling and blood and CSF collection (Kim et al. 2005). The CSF samples were taken by an anesthetist using the prescribed spinal needles for the procedure and collected in sterile plastic tubes and frozen at −80°C. Blood samples (±3 ml) were drawn from the femoral vein into EDTA tubes. Blood samples were centrifuged at 1000 rpm for 10 min at 4°C. The plasma was removed and divided into three aliquots and stored at −80°C until analysis. Urine samples were collected via catheterization in urine cups and frozen until analysis.

Experimental design

This pilot study, consisting of 3 phases, was done to study the mechanism of valproate toxicity. The experimental setup for each of the phases, are fully described in Table 1. Animals were treated on consecutive days and a rest period of a month or two were allowed between the different phases. It is important to note that the results of the different phases are discussed individually. Valproate and paracetamol were dissolved in purified water and given by gavage. In a study done with epileptic children, treated with 10–41 mg/kg valproate per day (age 8 months to 17 years), elevated levels of glycine serum and hyperammonaemia were found (Iinuma et al. 1988). This

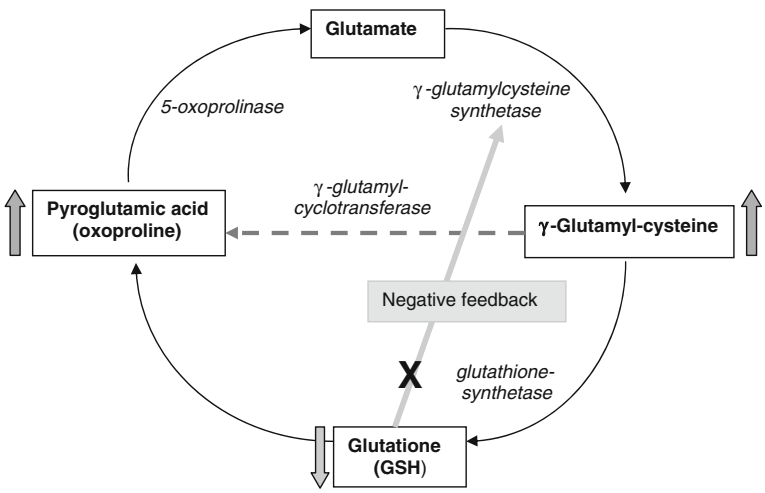

Fig. 2 Glutathione depletion (*grey arrows*) results in the elimination of feedback inhibition of γ-glutamylcysteine synthetase. As a result γ-glutamylcysteine production is increased. Excess γ-glutamylcysteine is converted to pyroglutamic acid (oxoproline) via γ-glutamyl cyclotransferase resulting in an increase in pyroglutamic acid

Table 1 Summary and rationale of the phases in the study

Phase 1	Single oral dose of 50 mg/kg valproate – determine glycine levels in blood, urine and CSF after 1 and 2 h (n=4) (to assess if hyperglycinemia can be induced)(n=3).
Phase 2	Single oral dose of 50 mg/kg paracetamol—determine urine pyroglutamic acid after 1, 2, 3 and 7 h (n=1).
Phase 3	Pre-treatment with a single oral dose of 50 mg/kg paracetamol, followed by a single oral dose of 50 mg/kg valproate – determine glycine in blood, CSF and urine and pyroglutamic acid in urine at ½ hour after paracetamol and ½, 1, 1½, 2½ and 3½ hours after valproate (n=3).

prompted the decision that a single oral dose of 50 mg/kg valproate would be sufficient to induce hyperglycinemia in the vervet monkeys. The oral pediatric dosage for temperature reduction with paracetamol is between 15 and 20 mg/kg (Nabulsi et al. 2005; Scolnik et al. 2002;). It was concluded that an acute single dose of 50 mg/kg paracetamol for vervet monkeys (weighing between 3.5 and 4,5 kg) would inhibit the γ-glutamyl cycle and increase pyroglutamic acid in the urine. Control blood, CSF and urine samples were collected from the test animals. Glycine was determined in blood and CSF and pyroglutamic acid in the urine.

Glycine analysis in blood and CSF

The analysis of amino acids was performed using the EZ: Faast amino acid analysis kit (Phenomenex, Torrance, CA, USA) by GC-MS as described previously (Hušek et al. 2002).

Sample preparation included a solid phase extraction step, followed by a derivatization step and liquid/liquid extraction. Derivatized samples were analyzed by GC-MS (gas chromatography mass spectrometry). Samples (1.5 μL) were injected at 250°C in split mode (1:15) onto a Zebron ZB-AAA column (10 m×0.25 mm i.d., Phenomenex). The oven temperature was initially set at 110°C, followed by an increase to 320°C at a rate of 30°C/min. Helium was used as the carrier gas at a constant flow rate of 1.1 mL/min. Detection was carried out in electron ionization mode in a scan range from m/z 45 to 450.

Pyroglutamic acid analysis in urine

The creatinine levels in the urine samples were determined prior to extraction. The urinary pyroglutamic acid levels were calculated and expressed relative to the creatinine content. Creatinine was determined using a Technicon RA-100 analyser. Organic acids in urine were determined as described by Jooste and co-workers (1994).

After extraction, the organic phase was evaporated under nitrogen at 40°C and the dry samples were frozen at −18°C. Prior to injection into the GC-MS, BSTFA [volume in μl= 2 × mg% creatinine] and TMCS [volume in μl=0.4 × mg% creatinine] were added to the dried samples and incubated at 60°C for 1 h to form TMS derivatives.

One microliter of a derivatised sample was injected into a Hewlett Packard 5880 GC equipped with a Hewlett Packard 5988A mass spectrometer (MS). A Macherey-Nagel (MN 30962–52) column and flame ionization detector were used. The inlet for the GC was splitless while the MS was GC dependent splitless and helium the carrier gas. The settings of the GC were as follows: the carrier gas was hydrogen at 2.5× 100 kPa and a flow rate of 1 ml/min. The makeup gas was nitrogen at a flow rate of 30 ml/min. The final oven temperature was 280°C. The carrier gas in the MS was helium.

Identification and quantification of peaks was done using an Automated Mass Spectral Deconvolution and Identification System (AMDIS) to identify and determine concentrations of organic acids. The concentrations of the organic acids in urine were determined using WsearchPro® software.

Results

Phase 1: Glycine concentrations in CSF and CSF:serum ratio after treatment with 50 mg/kg valproate

Although the CSF glycine levels before treatment varied markedly among the test animals (Table 2), treatment with 50 mg/kg valproate increased these levels in all of the experimental animals, even after two hours following treatment (Fig. 3). Treatment with 50 mg/kg valproate also increased the CSF:serum ratio markedly to levels well above the normal ratio of <0.08. Although the individual results differed markedly, all subjects exhibited an increase in the CSF:serum ratio, which remained high for up to two hours after treatment (Fig. 4).

Table 2 Glycine concentrations and CSF:serum ratios of vervet monkeys following a single oral dose of 50 mg/kg valproate

		T_0	1 h	2 h
Glycine in CSF (μmol/L)	Vervet 141	1.23	58.6	70.65
	Vervet 592	2.19	16.62	13.61
	Vervet 789	6.25	44.59	19.9
CSF:serum ratio	Vervet 141	0.005	0.34	0.41
	Vervet 592	0.009	0.073	0.673
	Vervet 789	0.029	0.182	0.085

Phase 2: Pyroglutamic acid in urine after treatment with 50 mg/kg paracetamol

Pyroglutamic acid levels in the urine increased markedly one hour after treatment with 50 mg/kg paracetamol and returned to normal after two hours and then remained normal for up to seven hours after treatment (Fig. 5). Only one monkey was treated with paracetamol. The results however, are in agreement with the literature, showing an increase in pyroglutamic acid excretion after paracetamol treatment and it was therefore deemed unnecessary to subject more animals to this treatment.

Phase 3: Glycine concentrations in CSF, CSF:serum ratio, and pyroglutamic acid in urine after 50 mg/kg paracetamol pre-treatment followed by 50 mg/kg valproate

Results of the individual animals are given in Table 3. The control samples were collected (time 0), after which paracetamol was given and samples were collected after 30 min (time 30). Valproate was then given and samples were collected ½, 1, 1½, 2½ and 3½ hours after valproate treatment. During this phase monkey 007, diagnosed with hyperglycinemia (ratio>0.08), was included as a positive control.

Paracetamol treatment of the hyperglycinemic monkey (007) increased the glycine levels in the CSF markedly after 30 min. Following treatment with valproate the glycine levels remained relatively constant for up to 3.5 h. The increased glycine levels in the CSF of the normal monkeys (592 and 789) were not as pronounced as in the hyperglycinemic monkey. After treatment with valproate the CSF glycine levels remained almost unchanged in the normal animals for 3.5 h (Table 3; Fig. 6).

The glycine CSF: serum ratio increased markedly in the hyperglycinemic monkey after treatment with paracetamol. Thereafter it remained relatively constant for up to 3.5 h following treatment with valproate. In the normal monkeys paracetamol treatment increased the ratio only slightly. The changes as a result of valproate were relatively small and are possibly of very little clinical relevance (Table 3; Fig. 6).

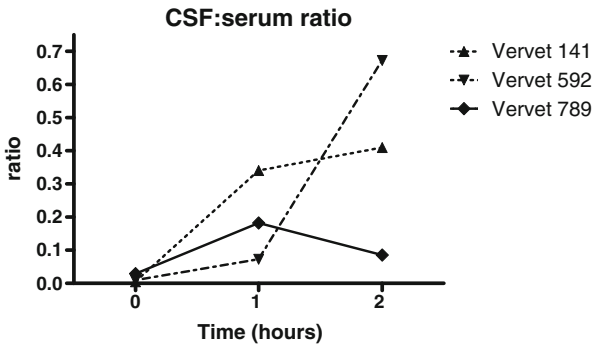

Fig. 4 Glycine CSF:serum ratios of vervet monkeys following a single oral dose of 50 mg/kg valproate

The pyroglutamic acid levels in urine almost doubled after the hyperglycinemic monkey was treated with paracetamol (Table 3; Fig. 5). After valproate treatment it decreased to levels below the initial concentration. Paracetamol treatment of the two normal animals either increased (monkey 789) or decreased (monkey 592) the urinary levels of pyroglutamic acid. Valproate treatment, on the other hand, increased pyroglutamic acid in urine during the first 30 min following treatment. After 30 min the levels either remained relatively constant (monkey 789) or decreased to levels well below initial concentrations (monkey 592) at 3.5 h (Table 3; Fig. 5).

Discussion

The increase in glycine levels (average 39.94 μmol/L; normal 3.22 μmol/L) and the cerebrospinal fluid-to-serum glycine ratio (average 0.198 μmol/L; normal <0.08) in healthy vervet monkeys (141, 592 and 789) one hour after treatment with a single oral dose of 50 mg/kg valproate (Table 2, Figs. 3 and 4) confirms that the animals developed non-ketotic hyperglycinemia. This finding is in agreement with

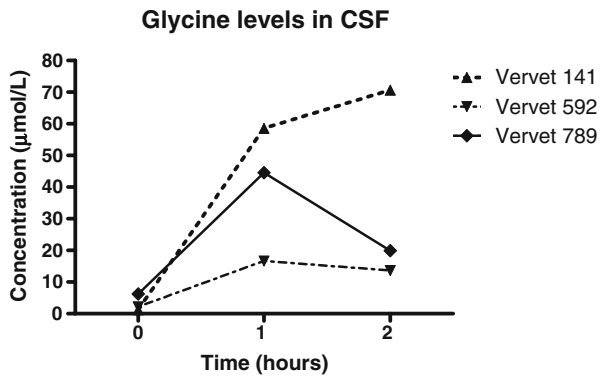

Fig. 3 Glycine levels in CSF of vervet monkeys following a single oral dose of 50 mg/kg valproate

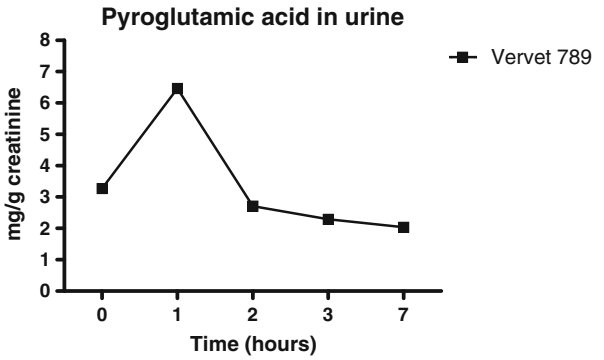

Fig. 5 Pyroglutamic acid levels in urine of vervet monkey 789, following a single oral dose of 50 mg/kg paracetamol

Table 3 Changes in measurements of monkeys treated with 50 mg/kg valproate ½ hour after they received 50 mg/kg paracetamol (monkeys 592 and 789 are normal, where monkey 007 is hyperglycinemic) (T_0=control; T_1=½ hour after paracetamol; T_2=½ hour after valproate; T_3=1 h after valproate; T_4=1½ hours after valproate; T_5=2½ hours after valproate and T_6=3½ hours after valproate)

		Glycine in CSF (μmol/L)	CSF:serum ratio	Pyroglutamic acid (mg/g creatinine)
Vervet 007	T_0	6.06	0.025	4.01
	T_1	38.61	0.169	8.23
	T_2	41.49	0.1958	–[a]
	T_3	36	0.178	3.61
	T_4	34.03	0.214	–[a]
	T_5	44.61	0.2135	–[a]
	T_6	43.84	0.21	2.67
Vervet 592	T_0	4.44	0.0326	4.26
	T_1	5.34	0.0402	3.7
	T_2	4.7	0.0378	5.64
	T_3	4.33	0.0359	4.82
	T_4	5.69	0.0399	3.92
	T5	4.76	0.0379	1.84
	T_6	4.24	0.0378	2.29
Vervet 789	T_0	5.07	0.0295	3.76
	T_1	6.9	0.0357	5.01
	T_2	4.8	0.0274	6.88
	T_3	4.95	0.0307	7.35
	T_4	4.38	0.0264	6.04
	T_5	4.7	0.0314	6.22
	T_6	4.47	0.0338	6.28

[a] not enough urine to determine values

earlier case studies describing the development of nonketotic hyperglycinemia in humans after valproate therapy (Morrison et al., 2006; Korman and Gutman 2002). Our results therefore illustrate that valproate can induce atypical nonketotic hyperglycinemia in vervet monkeys as well.

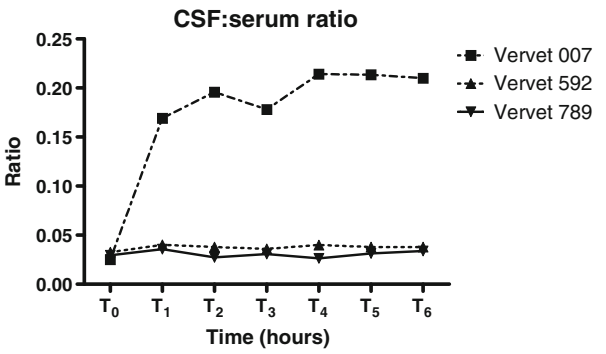

Fig. 6 CSF:serum ratios when the monkeys were treated with 50 mg/kg valproate 30 min after they received 50 mg/kg paracetamol (monkeys 592 and 789 are normal, where monkey 007 is hyperglycinemic) (T_0=control; T_1=½ hour after paracetamol; T_2=½ hour after valproate; T_3=1 h after valproate; T_4=1½ hour after valproate; T_5=2½ hour after valproate and T_6=3½ hour hours after valproate)

A significant increase was seen in the urinary pyroglutamic acid (oxoproline) of vervet 789, 60 min after treatment with paracetamol only (Fig. 5). Pyroglutamic acid excretion can be used as an indirect marker of the extent to which endogenous formation of glycine is able to satisfy the requirement for glycine (Jackson et al. 1987). An important function of glycine is its detoxification of xenobiotics via conjugation. For example, glycine conjugates with sodium benzoate to form hippuric acid, which depletes the glycine pools and leads to an increased urinary excretion of pyroglutamic acid (Jackson et al. 1987, 1996). It is evident from the high pyroglutamic acid excretion, that the vervet monkey has a high capacity to detoxify paracetamol.

Although the rate of pyroglutamic acid (oxoproline) excretion in the urine varied among the test animals receiving paracetamol, these levels showed an overall increase (Table 3). To understand why pyroglutamic acid increases following paracetamol dosing, one needs to revisit the γ-glutamyl cycle (Fig. 1). The γ-glutamyl cycle facilitates amino acid transport into cells. γ-Glutamyl amino acids (γ-Glutamyl-aa) are formed on the outer surface of luminal membranes of the endothelial cells by transfer of a γ-glutamyl moiety of GSH to amino acids. This reaction is catalyzed by γ-glutamyl transpeptidase (GGT). The γ-glutamyl-aa enter the endothelial cell where oxoproline (pyroglutamic acid) is formed via γ-glutamyl cyclotransferase. The Na^+-dependent transport systems A and $B^{o,+}$, located on the abluminal side, are activated by oxoproline. Therefore, if glycine is depleted via glutathione conjugation to N-acetylbenzoquinonimine (paracetamol metabolite), precursors in the γ-glutamyl cycle, including γ-glutamyl-cysteine, glutamate and oxoproline, would accumulate (Fig. 2). This can explain the increase in urinary pyroglutamic acid after 30 min with paracetamol treatment (Table 3).

The CSF:serum ratios and CSF glycine concentrations did not vary significantly in the normal, healthy vervet monkeys (592 and 789) receiving paracetamol pre-treatment followed by valproate treatment (Table 3). It is possible that the γ-glutamyl cycle is always active in normal subjects. When treated with paracetamol, N-acetylbenzoquinonimine reacts irreversibly with glutathione (Pitt et al. 1990), leading to an increase of oxoproline (pyroglutamic acid), which is excreted in the urine (Table 3). Alternatively, glycine is transported from the CSF (brain) to the blood via oxoproline activation. It is possible that paracetamol stimulates the γ-glutamyl cycle, which in turn opposes the hyperglycinemic effect of valproate by transporting the excess glycine in CSF back into the blood via oxoproline activation. This may offer an explanation why the CSF:serum ratio remained normal in animals receiving valproate plus paracetamol treatment (Table 3), as opposed to animals receiving valproate alone (Table 2).

However, the CSF:serum ratio and CSF glycine concentrations in monkey (007) receiving 50 mg/kg paracetamol was distinctly elevated, indicating hyperglycinemia, and this level remained high for up to 3.5 h after 50 mg/kg valproate (Table 3). The biochemical defect causing such an increase in the glycine concentration in blood, urine, and CSF may be attributed to derangements of the glycine cleavage enzyme and/or faulty transport mechanisms of glycine. Indeed, low and high affinity, Na^+-dependent transport systems for glycine are present in both rat and normal human post mortem brain tissue, although a previous study has demonstrated that atypical non-ketotic patients can have a defective glycine transport system in the brain and spinal cord (Mayor et al. 1984). Moreover, although the cause of nonketotic hyperglycinemia is primarily due to defects in GCS, it is plausible that deficits in glycine transport may lead to an inability to transport glycine from the CSF (brain) to the blood, thus leading to increased CSF glycine levels. Since the GCS in the nonketotic hyperglycinemic monkey was probably impaired (Table 3), these results could, at least in part, explain the mechanism for the changes in the glycine CSF:serum ratio (Figs. 6 and 7). Glycine could therefore be synthesized, but not be catabolized. In addition, an acute dose of paracetamol would lead to detoxification via conjugation to glutathione, which in turn can lead to glutathione and glycine depletion. This depletion probably upregulated glycine synthesis and resulted in the initial increase in CSF glycine levels (Table 3, T_1). Therefore challenge of this hyperglycinemic monkey (which has a faulty GCS) with paracetamol lead to increased CSF glycine levels and CSF:serum ratios 30 min after paracetamol treatment (Fig. 6, T_1). In the normal monkeys (with a functioning GCS), however, there was no increase in glycine ratios and concentrations after paracetamol treatment (Table 3, T_1). The additional challenge of this hyperglycinemic monkey with valproate, which also inhibits the glycine cleavage system (Mortensen et al. 1980), further increased CSF glycine levels and CSF:serum ratios for up to 3.5 h after valproate treatment (Table 3; Fig. 6), thus confirming that the addition of valproate leads to an enhanced inhibition of the GCS with increased accumulation of glycine as a consequence (Morrison et al. 2006).

It has been shown in rat models that valproate sodium decreases the hepatic glycine cleavage enzyme system, increasing blood and cerebrospinal fluid glycine concentrations and leading to an increase in seizure frequency (Kochi et al. 1979). Glycine acts as an excitatory neurotransmitter in the cortex at the glutamate NMDA receptor channel complex (Hamosh and Johnston 2001). High levels of glycine in the CSF would lower the seizure threshold and aggravate seizures. In a unique case of atypical nonketotic hyperglycinemia the patient experienced worsening of seizures after introduction of valproate sodium, indicating that the increased glycine levels are responsible for worsening of the underlying metabolic condition and increasing seizure frequency. (Dhamija et al. 2011). Our findings indicate the involvement of the γ-glutamyl cycle in the increased glycine levels after valproate treatment. Furthermore, the opposing effect of paracetamol on valproate-induced hyperglycinemia might provide new therapeutic possibilities in preventing or treating seizures in nonketotic patients treated with valproate.

In conclusion, we have been able to demonstrate that hyperglycinemia can be induced in vervet monkeys treated with valproate. Importantly, pre-treatment of healthy vervet monkeys with paracetamol prevented the hyperglycinemic effect induced by valproate. Treatment of the nonketotic

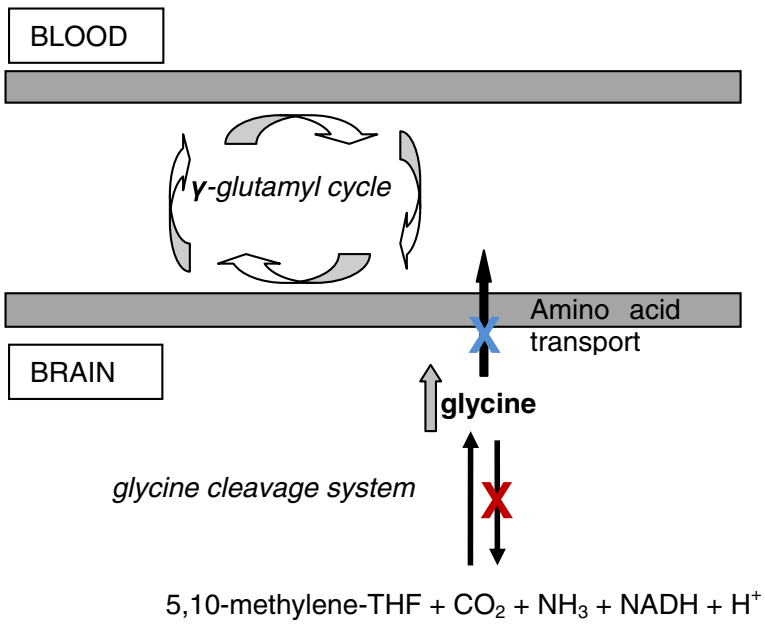

Fig. 7 The increase in glycine CSF concentration in nonketotic hyperglycinemia is the thought to be the consequence of a defect in the glycine cleavage system (indicated in red) and/or a defect in transport mechanisms (indicated in blue) of glycine from the brain back into the blood (Lee et al., 1996)

hyperglycinemic vervet monkey with paracetamol increased CSF glycine levels, most likely because of impaired glycine cleavage and/or deficits in the γ-glutamyl transport system. Since increased glycine levels were reversed with the addition of paracetemol indicate that the γ-glutamyl cycle does indeed play an important yet poorly recognised role in the development of valproate-induced hyperglycinemia. Since glycine displays important physiological effects in the CNS, especially with respect to the excitatory glutaminergic system (Harvey and Shahid, 2012), these findings have importance not only for the understanding of valproate toxicity, but also for its proposed mechanism of action in treating epilepsy, bipolar disorders and other neuropsychiatric illnesses. Further research, using more refined methods such as measuring inhibition of the glycine cleavage system, enzyme studies, detecting free radical levels to determine glutathione depletion and monitoring glutathione conjugates of paracetamol in the urine will be necessary to further define the effects of valproate on the γ-glutamyl cycle.

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