Biochemical Pharmacology 83 (2012) 1331–1339

Contents lists available at SciVerse ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Commentary

Dog bites man or man bites dog? The enigma of the amino acid conjugations☆

Diren Beyoğlu [a], Robert L. Smith [b], Jeffrey R. Idle [a,*]

[a] Hepatology Research Group, Department of Clinical Research, Faculty of Medicine, University of Bern, Murtenstrasse 35, 3010 Bern, Switzerland
[b] Department of Surgery & Cancer, Faculty of Medicine, Imperial College London, South Kensington Campus, London SW7 2AZ, UK

ARTICLE INFO

Article history:
Received 26 October 2011
Accepted 20 December 2011
Available online 29 December 2011

Keywords:
Amino acid conjugation
Neuroregulation
Amino acid scavenging
Hippuric acid
Phenacetylglutamine

ABSTRACT

The proposition posed is that the value of amino acid conjugation to the organism is not, as in the traditional view, to use amino acids for the detoxication of aromatic acids. Rather, the converse is more likely, to use aromatic acids that originate from the diet and gut microbiota to assist in the regulation of body stores of amino acids, such as glycine, glutamate, and, in certain invertebrates, arginine, that are key neurotransmitters in the central nervous system (CNS). As such, the amino acid conjugations are not so much detoxication reactions, rather they are homeostatic and neuroregulatory processes. Experimental data have been culled in support of this hypothesis from a broad range of scientific and clinical literature. Such data include the low detoxication value of amino acid conjugations and the Janus nature of certain amino acids that are both neurotransmitters and apparent conjugating agents. Amino acid scavenging mechanisms in blood deplete brain amino acids. Amino acids glutamate and glycine when trafficked from brain are metabolized to conjugates of aromatic acids in hepatic mitochondria and then irreversibly excreted into urine. This process is used clinically to deplete excess nitrogen in cases of urea cycle enzymopathies through excretion of glycine or glutamine as their aromatic acid conjugates. Untoward effects of high-dose phenylacetic acid surround CNS toxicity. There appears to be a relationship between extent of glycine scavenging by benzoic acid and psychomotor function. Glycine and glutamine scavenging by conjugation with aromatic acids may have important psychosomatic consequences that link diet to health, wellbeing, and disease.

1. Introduction

The amino acid conjugations are often considered to be the poor cousins of drug metabolism and this is clearly reflected by their citation numbers in PubMed relative to, for example, cytochrome P450. The addition of glycine (GLY), glutamine (GLN), and taurine (TAU) to aromatic acids such as benzoic acid (BA) or phenylacetic acid (PAA) by both humans and animals has been from the outset considered as a process of detoxication, a means of rendering BA or PAA more water-soluble, readily excretable and thus less toxic, according to the paradigm first espoused by Williams [1]. Yet, this small patch of biology still comprises many unknowns and therefore retains somewhat of an air of mystery.

What will be argued here is that the amino acid conjugations have not evolved principally to detoxicate aromatic acids. This is merely happenstance. The amino acid conjugations are a means to deplete systemic stores of certain amino acids, those which function in the central nervous system (CNS) as neurotransmitters, thereby serving to regulate their levels in the CNS.

2. Amino acids as agents of conjugation

2.1. A brief history of hippuric acid

According to Williams [1], the conversion of ingested BA into hippuric acid (HA; *N*-benzoylglycine) was the first detoxication mechanism to be reported. Although HA had been isolated from the urine of cows, horses, and a dog in Germany between 1784 and 1831 [1,2], definitive proof that HA arose from BA did not transpire until Keller dosed himself four times within 24 h with 1.9 g (“32 grains”), collected his urine and determined chemically that the

☆ Supported by The Hassan Badawi Foundation Against Liver Cancer (to JRI) and NIH (NIAID) grant U19 AI067773-07 (to JRI).

Abbreviations: BA, benzoic acid; PAA, phenylacetic acid; HA, hippuric acid; GLY, glycine; GLN, glutamine; TAU, taurine; GLU, glutamic acid; EAA, excitatory amino acid; IAA, inhibitory amino acid; EAAT, excitatory amino acid transporter; ECF, extracellular fluid; CSF, cerebrospinal fluid; PAGLN, phenacetylglutamine; PAGLY, phenacetylglycine; PATAU, phenacetyltaurine; 4HBA, 4-hydroxybenzoic acid); 4HHA, 4-hydroxyhippuric acid; 2FA, 2-furoic acid; 2FGLY, 2-furoylglycine; 3IAA, 3-indolylacrylic acid; IAG, 3-indolylacryloylglycine; ECT, electroconvulsive therapy; NMDAR, *N*-methyl-D-aspartate receptor; mGluR, metabotropic glutamate receptor; CNS, central nervous system; BBB, blood-brain barrier; HPLC, high-performance liquid chromatography; LPI, lysinuric protein intolerance; NaPBA, sodium phenylbutyric acid; GPB, glyceryltri(4-phenylbutyrate); 4NB, 4-nitrobenzoic acid; 4ABA, 4-aminobenzoic acid; ARG, arginine.

* Corresponding author. Tel.: +41 31 632 8729; fax: +41 31 632 4997.
E-mail address: jeff.idle@ikp.unibe.ch (J.R. Idle).

0006-2952/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2011.12.031

crystals he isolated were "pure hippuric acid" [2]. The study of HA both in experimental animals and in clinical studies was fashionable for several decades, during which time the view developed that HA was formed from BA in the kidney [3], until reports in 1915 and 1918 strongly suggested that HA was also formed in the liver of dogs [4]. Within a few years, sodium benzoate administration became a popular clinical test for both renal function and liver injury, with claims that healthy subjects could convert doses of up to 40 g into HA with 90% efficiency, provided they had a diet rich in GLY [5]. The American physician Armand J. Quick standardized a test for HA formation involving oral administration of 6 g sodium benzoate with hourly urine collections for 4 h, acidification and concentration of the urine, followed by gravimetric determination of filtered and dried HA crystals. This "Quick's test" yielded approximately 3 g HA in persons with a normal hepatic function and considerably less for those with a range of liver diseases [6].

A debate had ensued regarding the origins and the body reserves of GLY and it is hardly surprising that many investigators sought to manipulate GLY supplies and observe what effects this had on HA formation. HA excretion in rabbits was increased by the co-administration of GLY, but none of six other amino acids, neither glycolic acid, glycol aldehyde, glucose, urea nor sodium acetate [7]. Additional experiments administering BA together with hydrolyzed proteins rich in GLY, specifically, elastin and gelatin, also increased HA urinary excretion in rabbits [8]. Interestingly, hydrolyzed proteins almost entirely lacking in GLY content, specifically, casein, egg albumin, and peanut meal, did not enhance HA excretion, but did so when fortified with free GLY [8]. It was concluded that it was the availability of preformed GLY and not normal protein catabolism that regulated and limited the formation of HA from BA.

2.2. Other common amino acid conjugations

In addition to conjugation with GLY, aromatic acids of various types can undergo conjugation with other amino acids, notably, GLN and TAU. In most respects, the choice of which amino acid is added, GLY, GLN or TAU, depends both upon the chemical class of aromatic acid and the species in question. So, the simplest of acids, BA, is conjugated with amino acids in mammals using only GLY, with GLN and TAU conjugation not being encountered [9]. In contrast, PAA, is conjugated with GLN in humans and certain primate species [10] and with TAU in carnivorous species, such as the dog, cat, and ferret [10,11]. PAA conjugation with GLY is also commonplace, especially among herbivores and rodent species [10]. Occasional bizarre reactions are encountered, such as the absence of GLY utilization for BA and its derivatives in certain bats [12,13] that had been replaced by GLU usage [13,14]. Overall, however, the principal amino acids used for conjugating and apparently detoxicating aromatic acids are GLY, GLN, and TAU.

As far as humans and laboratory mammals are concerned, both BA and PAA are abundant endogenous compounds formed from dietary sources together with several other acidic urinary metabolites by the gut microbiota [15]. In the rat, for example, the urinary excretion profile of these aromatic acids (μmol/24 h after deconjugation) is BA (∼12), PAA (26–31), 3-hydroxyphenylpropionic acid (∼3.5), and 3,4-dihydroxyphenylpropionic acid (∼0.8), with the phenolic acids being excreted unconjugated [15]. BA and HA are found in human urine at concentrations of 106 and 837 μmol/mmol creatinine (900 and 7000 μmol/24 h), respectively [16], while PAA and phenacetylglutamine (PAGLN) are excreted at 3.6 and 1080 μmol/24 h, respectively [17]. Interestingly, PAA is excreted at about 3% creatinine clearance and is therefore mostly reabsorbed by the nephron, whereas PAGLN is actively secreted at 2- to 4-times creatinine clearance [17]. An alternative interpretation of these findings is that PAGLN is formed from PAA in the kidney. HA is formed from BA in adult kidney and liver tissue *in vitro* at similar rates [18].

2.3. Amino acid conjugations – a myth exposed

The central dogma of drug metabolism holds that conjugation reactions render xenobiotics and their primary metabolites more water soluble and, in so doing, assist in their elimination from the body in the urine and the bile. This is ably demonstrated by the formation from BA of benzoyl-β-D-glucuronide. BA has a water solubility of 3.4 g/l, but once conjugated with glucuronic acid, this increases dramatically to 263 g/l [19]. However, formation of HA barely increases water solubility to 3.75 g/l [19]. The case of PAA is more dire, with water solubility for PAA falling from 16.6 g/l to 7.3 and 2.12 g/l when it is conjugated with GLY and GLN, respectively [19]. Conjugation with glucuronic acid is commonplace and there exists a superfamily of at least 117 glucuronidation enzymes, with members expressed in virtually every tissue [20]. It is therefore surprising that amino acid conjugation has not become extinct, for it appears to add relatively little detoxication value to the host, at least based upon physico-chemical arguments, and would appear at first sight to be superfluous. The key question therefore is, what are the evolutionary pressures that are preserving these somewhat vestigial and arcane reactions of aromatic acids, the addition of GLY, GLN or TAU?

3. Amino acids as neurotransmitters

3.1. Overview

A number of amino acids function as either excitatory neurotransmitters or inhibitory neurotransmitters in the vertebrate brain, of which L-glutamic acid (GLU) is the most abundant member of the former category. L-Aspartate, L-cysteine, and L-homocysteine are also excitatory amino acids (EAA). In the inhibitory amino acid (IAA) category are found GLY, TAU, β-alanine, and GABA. Amino acids are primitive neurotransmitters, meaning that they are found as principal neurotransmitters in almost all nervous systems, including worms [21] and spiders [22]. In fact, the most ancient nervous system studied is the motor nerve net neurons of the lion's mane jellyfish, *Cyanea capillata*, which have been reported to use only two β-amino acids, TAU and β-alanine, as neurotransmitters [23].

3.2. Glutamate

GLU is the most ubiquitous free amino acid in the brain [24]. GLU also mediates most excitatory neurotransmission in the mammalian brain and may be regarded as the principal excitatory neurotransmitter in most vertebrate and invertebrate nervous systems. GLU functions not only as a neurotransmitter but also as a fuel reserve for the brain. It can be transaminated to α-ketoglutarate which is a Krebs' cycle intermediate, whose conversion from GLU to oxaloacetate in the Krebs' cycle yields 12 mol of ATP per mole of GLU, similar to glucose as a fuel reserve [24].

GLU acts at two distinct ionotropic receptors, the AMPA/kainate and *N*-methyl-D-aspartate receptors (NMDAR), to mediate excitatory neurotransmission, predominantly in the hypothalamus [25]. In addition, GLU activates metabotropic glutamate receptors (mGluR; group I, mGluR1 and mGluR5; group III, mGluR4, mGluR6, mGluR7, and mGluR8) in magnocellular neurosecretory cells in the hypothalamus [25]. While ionotropic glutamate receptors are ligand-gated ion channels, mGluRs are not. mGluRs are believed both to regulate synaptic efficacy and to maintain homeostasis in the face of acute challenges. They have been described as

"gatekeepers of future synaptic plasticity that may contribute to physiological state transitions and memory in autonomic circuits" [25]. Much work is still required to understand more fully the role of mGluRs.

The action of GLU on ionotropic NMDAR may mediate motor function, cognition, and learning. However, at this type of receptor, GLU alone is insufficient to activate the ion channel. It requires the obligate co-agonist GLY [26] (see below).

3.3. Glycine

In the adult CNS, GLY is a major inhibitory neurotransmitter and when released from glycinergic presynaptic terminals GLY activates postsynaptic strychnine-sensitive ionotropic glycine receptors (GlyR), mediating synaptic inhibition in spinal cord, brainstem, and other CNS areas [27]. GLY has a Janus character, not only acting through GlyR but also co-agonizing NMDAR together with GLU. However, it would appear that GLY acts on NMDAR at concentrations as low as 10 nM [26], while 300–400 μM may be required to activate GlyR [28]. However, the GlyR represents one of the most efficient proteins known, trafficking 10^7 Cl^- ions/s [29]. TAU and β-alanine are also agonists at GlyR [29].

3.4. Movement of amino acid neurotransmitters across the blood–brain barrier

3.4.1. Glutamate

The entire CNS is bounded by the blood–brain barrier (BBB) comprising specialized endothelial cells connected by tight junctions. The BBB therefore has two barrier membranes, one in the lumen of capillary endothelium (luminal) and one on the opposite side of the BBB (abluminal). Molecules entering or leaving the CNS must first pass these two membranes, each of which has specific properties [24]. EAA transporters (EEAT1, 2, and 3) exist only in the abluminal membrane and so these Na^+-dependent glutamate cotransporters traffic GLU from the extracellular fluid (ECF) into the endothelial cell, where it freely diffuses into blood through luminal transmembrane proteins known as facilitative transporters that mediate passive diffusion [30]. These components of the BBB do not permit GLU to enter the brain, but provides for the low concentrations of GLU in ECF that are generally maintained at 0.5–2 μM [24]. This compared with GLU concentrations of 50–100 μM in plasma and 10–12 mM in whole brain, where GLU is compartmentalized within astrocytes and neurons [24]. Clearly, elevated ECF GLU concentrations, that can occur for example with low oxygen tension, when the Na^+ gradient is dissipated, can readily find their way to the peripheral circulation. This appears to be an important defense mechanism because if the ECF GLU concentration rises, GLU becomes neurotoxic. GLN is also removed from the brain by EAAT pumping from ECF into endothelial cells, conversion to GLU and NH_4^+ by glutaminase, and passive diffusion through luminal facilitative transporters into blood [24].

Plasma concentrations of GLU are largely uninfluenced by dietary sources of GLU, for example, large doses of monosodium glutamate, both in clinical and animal studies, because the gut metabolizes dietary GLU and GLN [24].

3.4.2. Glycine

GLY administered at a dose of 2 g/kg to rats caused an elevation of CSF GLY concentration above the ED_{50} value for NMDAR [31]. However, plasma levels of GLY were 100-fold higher than those in CSF and appeared to drive GLY across the BBB by passive diffusion [31]. If brain GLY concentrations rise, as they do in certain inherited metabolic disorders with hyperglycinemia, brain energetics are affected and neurological damage may occur [32]. Experiments with mouse cerebellar slices demonstrated that efflux of GLY is mediated by the EAAT GlyT1 and it was proposed that this transporter mediates GLY efflux under conditions of metabolic impairments such as ischemia [33].

3.4.3. Regarding the hypothesis

It would appear therefore that GLY and GLU freely cross the BBB, with GLU using an outward EAAT. Depletion of GLY by formation and urinary excretion of HA should therefore also occur in brain. Formation and urinary excretion of PAGLN depletes GLN stores and, by knock-on effect, stores of GLU. Falls in GLU plasma levels through this process may draw GLU from the CNS into plasma, since there appears to be no barrier to the outward flow of GLU. Moreover, as the CNS seems to export GLU and GLN to ameliorate neurotoxicity, GLN scavenging by PAA merely provides an irreversible mechanism for the removal of GLN into urine. This principle is illustrated in Fig. 1.

4. Evidence that aromatic acid conjugation may regulate CNS glycine and glutamate

The argument will be made that amino acid conjugation is not so much a process that uses amino acids for the elimination of aromatic acids but rather uses ubiquitous aromatic acids for the irreversible elimination of glycine and glutamine.

4.1. Early studies with Quick's test in psychiatric patients

At the dawn of biological psychiatry, attempts were made to uncover metabolic disturbances in psychiatric diseases through the application of tests such as the glucose tolerance test [34] and the determination of urea production after oral administration of GLY [35], with varied success. However, application of the new Quick's HA test [6,36] to psychiatric problems spawned a body of fascinating literature that would extend over 30 years. Investigating the concept that schizophrenia had a toxic origin, the first study was reported by Quastel and Wales who applied Quick's test to 67 schizophrenic patients, of which were 18 were classified as catatonic and 27 as non-catatonic. Non-catatonic schizophrenics excreted 3.4 ± 0.4 g HA, similar to healthy subjects with no liver disease, while catatonic patients excreted only 2.2 ± 0.5 g HA ($P < 0.0001$ by unpaired Student's *t*-test [authors' calculation]). Impaired renal function was excluded as an explanation [37]. Another group failed to reproduce this finding in 28 catatonic and 34 non-catatonic schizophrenics [38]. Nevertheless, the original findings were reproduced in a second report by Quastel and Wales who also administered sodium benzoate intravenously to catatonics and non-catatonics to obviate absorption differences. They reported the same reduced excretion of HA in catatonics relative to non-catatonics (3.6 ± 0.5 g vs. 5.3 ± 0.8 g, respectively [$P < 0.0001$; authors' calculations]). Moreover, a third group [39] confirmed the original findings of Quastel and Wales. There were unsuccessful attempts to explain the contradictory reports based upon patient body weight [40]. Finally, a Chinese study of over two hundred controls and 120 psychotic patients reported close agreement with Quastel and Wales and suggested that all types of schizophrenic patient might have reduced HA excretion during Quick's test [41].

As had been originally pointed out by Quick [6,36], the formation of HA is critically dependent on the supply of GLY from both dietary protein and *de novo* hepatic synthesis. Therefore, Köersner reported reduced excretion of HA (mg/kg b.w.) as follows: "normal" subjects (20), non-catatonics (21.5), inactive catatonics (16.1), and active catatonics (13.6). But, when he pretreated 11 schizophrenics with 2 g GLY three times a day for 3 days prior to the test, then 4 g GLY 15 min before Quick's test, he reported a >3-fold increase in HA excretion (0.60–1.89 g;

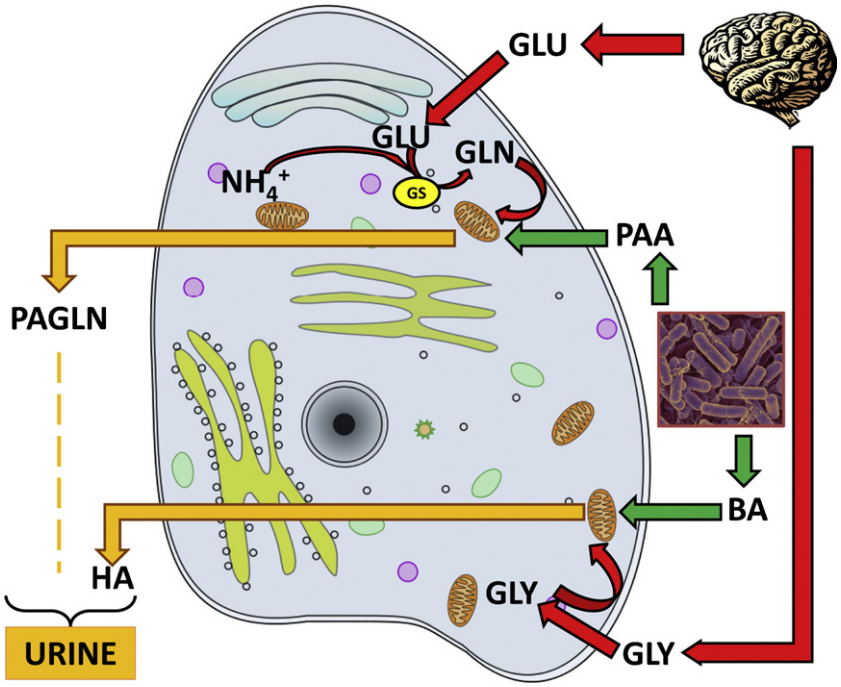

Fig. 1. Schematic representation of the peripheral conversion of glutamate (GLU) and glycine (GLY) from the brain into phenacetylglutamine (PAGLN) and hippuric acid (HA, benzoylglycine) by mitochondrial conjugation with phenylacetic acid (PAA) and benzoic acid (BA), respectively, that have been produced by the gut microbiota (purple box). Glutamine (GLN) is first synthesized from GLU and ammonia by cytosolic glutamine synthase (GS). PAGLN and HA are irreversibly removed by urinary excretion, representing a net loss of GLU and GLY.

$P < 0.0001$ by paired Student's *t*-test [authors' calculation]) for a subset of 6/11 patients. Only two patients did not show an increased HA excretion after GLY loading [42]. In 11 patients with senile dementia and 11 controls, Quick's intravenous test was applied on day 1 and day 3, but on day 2, 3 g GLY in three divided doses was also administered around the time of the 1.77 g sodium benzoate intravenous dose [43]. Without GLY loading, the controls excreted 1.1 ± 0.2 g HA in 0–1 h after dosing and the senile dementia patients 0.6 ± 0.2 g HA ($P < 0.0001$; unpaired Student's *t*-test [authors' calculation]). After GLY loading, both the controls and the senile dementia patients increased their HA excretion to 1.3 ± 0.2 g ($P = 0.020$) and 0.8 ± 0.2 g ($P = 0.017$) [authors' calculations], respectively [43]. Senile dementia was therefore associated with a diminished formation of HA, which could be partially resolved by the administration of GLY.

The increased excretion of HA consequent to GLY loading represents an increased GLY excretion of ~15% of the 4 g administered dose [42] and ~3% of the 3 g administered dose [43]. From the known characteristics of GLY absorption [44], it must be assumed that the administered GLY dose was quantitatively absorbed in these experiments. Therefore, the pool of GLY available for HA formation is relatively small and presumably conjugation of BA efficiently depletes this GLY compartment.

HA excretion was studied in US WW2 veterans with post-combat neuroses that were characterized as displaying "free anxiety" [45]. Such individuals exhibited symptoms such as frequent panic attacks, dilated pupils, facial and manual muscular tremor, tachycardia, and hyperpnoea [45]. This is the other end of the scale from the catatonic patients referred to above. An intravenous dose of 1.77 g sodium benzoate was administered to 17 such patients, together with 14 less affected, 10 depressed, and 11 "normal" subjects. These four groups excreted HA 1.93 ± 0.68, 1.37 ± 0.24, 1.23 ± 0.19, and 1.27 ± 0.19 g/h, respectively. ANOVA revealed a statistically significant difference between groups ($P < 0.01$) that was due to a difference between the free anxiety group and the other three groups combined ($P < 0.001$) [45]. When corrected for excretion of endogenous (dietary) HA, the difference between six free anxiety patients and six control subjects was still significant ($P < 0.01$).

Therefore, free anxiety is associated with an increased synthesis of HA [45], in stark contrast to catatonic schizophrenics who displayed an impaired synthesis in several studies [37,39,41,42]. Most interestingly, when six free anxiety patients were treated with either psychotherapy, electroconvulsive therapy (ECT) or insulin shock therapy, HA excretion fell from 2.4 ± 0.8 to 1.6 ± 0.2 g ($P = 0.035$; paired Student's *t*-test [authors' calculation]) [45]. Another study was reported in which ECT was used on 20 schizophrenic patients and 15 depressed patients, and Quick's test was performed on each before and after ECT. Both schizophrenic and depressed subjects had impaired HA synthesis, which was partially rectified in both groups by ECT [46]. These authors stated, "It appears that a low rate of excretion of hippuric acid parallels states of severe withdrawal accompanied by psychomotor retardation. It does not seem to be of decisive importance whether this state of withdrawal is part of a schizophrenic or depressive process" [46].

4.1.1. Regarding the hypothesis

Hepatic conjugation of BA with GLY, as measured by Quick's oral and intravenous test protocols, has been reported in multiple studies to be impaired in schizophrenia with and without catatonia, depression, and senile dementia. In all cases, these patients displayed various levels of withdrawal consistent with reduced psychomotor activity. This impairment has been reported to be partially or fully alleviated by loading doses of GLY and by successful therapeutic intervention with ECT. Moreover, in a study of WW2 veterans with free anxiety, enhanced conjugation of BA with GLY was reported. Such subjects displayed increased psychomotor activity. It is therefore tempting to speculate that BA has been utilized by the body to modify GLY concentrations in some compartment.

GLY is a major inhibitory neurotransmitter in the mammalian CNS and also acts as an essential co-agonist of GLU at NMDARs [47]. NMDAR function is believed to be impaired in schizophrenia, in part because NMDAR antagonists have psychotomimetic actions [48]. Clinical trials that have administered GLY, and the other GLY receptor agonists D-serine and sarcosine, have produced measurable improvements in the psychopathology of schizophrenia [49]. On the other hand, GLY administration to rats has pronounced anxiogenic effects [50]. Free anxiety may therefore be associated with excessive glycinergic activity.

We propose, therefore, based upon the foregoing evidence, that impaired HA synthesis is schizophrenia, catatonia, depression, and senile dementia result in reduced GLY scavenging by BA in these conditions due to impaired GLY availability. In addition, free anxiety is associated with increased GLY scavenging by BA due to enhanced GLY availability in this psychiatric condition. This is consistent with the hypothesis that BA conjugation with GLY is a neuroregulatory event.

4.2. Studies in autism

In an attempt to define a neurobiological component in autism, overnight urine collections from 19 autistic children were analyzed for hippuric acids, the nicotinamide metabolite N^1-methyl-2-pyridone-5-carboxamide, and uric acid metabolites of purines [51]. Three GLY conjugates, HA, 4-hydroxyhippuric acid and 2-furoylglycine (2-furoic acid is believed to be dietary in origin [52]) were measured. Total GLY conjugate excretion "was often massive, indicating extensive detoxification by glycine conjugation (note also patient no. 11, for whom hippurate excretion was low, but a massive amount of furoylglycine was excreted)" [51]. An NMR-based metabolomic study of 39 autistics, 28 nonautistic siblings, and 34 age-matched healthy volunteers, failed to find a statistically significantly altered HA excretion in autism [53], but this report has no mention of the GLY conjugates of 4-hydroxyhippuric acid and 2-furoic acid. Of intense interest is the report of a large unidentified peak on HPLC which was found specifically in urines from autistic persons [54]. This substance was identified as 3-indolylacryloylglycine (IAG), and subsequently became the subject of many publications discussing its status as a possible marker for autism. However, three GLY conjugates, in addition to HA, have been reported as being significantly elevated in the urine of autistic persons, suggesting a broader upregulation of GLY conjugation in this disease.

A case report of an autistic child with hyperactivity showed a low plasma GLY concentration. Oral GLY supplements reduced his hyperactivity [55]. Unfortunately, no urinary measurements were made. Serum GLU, but not GLY, was elevated in autism (89.2 ± 21.5 μM) relative to healthy controls (61.1 ± 16.5 μM; $P < 0.001$), the authors suggesting that an abnormality in glutamatergic neurotransmission may underlie autism [56]. It was recently proposed that antagonism of the GLY site of the NMDA receptor could be a new strategy for autism treatment [57].

4.2.1. Regarding the hypothesis

There is an accumulation of various lines of evidence that suggest that elevated glutamatergic neurotransmission through NDMA receptors contributes to the pathobiology of autism. GLU and GLY are co-agonists at this receptor. GLY is removed by the liver of autistic subjects by excessive conjugation of a number of different organic acids. GLY and GLU would also appear to play a role in autism through hyperactivity of glutamatergic neurons. Although there is only one report of elevated GLU and no reports of elevated GLY in autism [56], it is possible that the excessive scavenging of GLY by multiple organic acids keeps serum GLY concentrations within normal limits and helps reduce CNS concentrations.

4.3. The use of aromatic acids to promote glycine and glutamine excretion

In 1914, Lewis reported that 6–10 g sodium benzoate administered to man results in a rapid urinary excretion of HA and that the increase in urinary nitrogen excretion due to HA was almost quantitatively compensated by a fall in urea and ammonia excretion [58]. The same principle was later demonstrated for PAA conversion to PAGLN [59]. This led to the idea that the administration of BA or PAA could be an efficient means to promote nitrogen excretion in persons with inborn errors of urea cycle enzymes [60]. Simply put, BA and PAA could be used to scavenge GLY and GLN, respectively.

Several investigations proved this concept to be correct. Urea cycle enzymopathies lead to hyperammonemia. Interestingly, hyperammonemia has been generated experimentally under carefully controlled conditions in subjects with lysinuric protein intolerance (LPI) [61], a condition in which ornithine, arginine, and lysine become depleted due to excessive renal excretion and poor intestinal absorption as a result of mutations in the transporter gene *SLC7A7*. The shortfall of cationic amino acids corrupts the urea cycle leading to hyperammonemia after a protein-rich meal [62]. When LPI patients received an intravenous infusion of L-alanine, hyperammonemia developed. The co-infusion of sodium benzoate and, on a separate day, sodium phenylacetate, did not abolish the hyperammonemia but BA and PAA administration accounted for 11.5% and 22.1% of urea nitrogen as urinary HA and PAGLN, respectively. The conversion of BA to HA and PAA to PAGLN in the 0–6 h urine was 65% and 51% of the dose, respectively [61]. Moreover, there is biochemical evidence in the form of elevated 5-oxoproline urinary excretion that BA administration depletes GLY stores [63].

PAA quickly replaced BA in clinical practice because excretion of GLN as PAGLN involves the excretion of two nitrogen atoms compared to only one for GLY as HA. Different delivery forms that might enhance the bioavailability of PAA have been evaluated in urea cycle enzymopathies and include sodium phenylbutyrate (NaPBA) and glyceryltri(4-phenylbutyrate) (GPB), both of which are metabolized to PAA and then on to PAGLN [64]. NaPBA is approved at a dose up to 20 g/day, which exceeds recommended daily limits for sodium intake. A dose of 17.4 ml GPB delivers the same dose of phenylbutyric acid without any sodium. Ten subjects with urea cycle enzymopathies who completed the trial in a Phase 2 crossover study took NaPBA or GPB and had blood and urine (0–6, 6–12, 12–24 h) analyzed for parent drugs, PAA, PAGLN, PAGLY, phenylbutyrylglycine, and phenylbutyrylglutamine [64]. 0–24 h urinary PAGLN accounted for 54% of the dose of both drugs. All the other aforementioned metabolites each accounted for <1% of dose.

A safety evaluation of NaPBA and GPB has been carried out. Twenty-four healthy adult volunteers were given NaPBA or GPB (both 3 g/m^2; equivalent to PBA) in a Phase I crossover study [65]. Bloods were collected up to 48 h together with 0–4, 4–8, 8–12, and 12–24 h urines. Samples were analyzed for PBA, PAA, PAGLY, PAGLN, phenylbutyrylglycine and phenylbutyrylglutamine, together with intact GPB. These investigators also conducted in the same study an investigation in 24 cirrhotic patients and eight healthy controls using oral doses of GPB of 100–200 mg/kg, once or twice daily [65]. Interestingly, cirrhotic subjects excreted similar amounts of PAGLN, even those with severe cirrhosis (Child–Pugh score C). Metabolic patterns and blood metabolite levels were also similar between healthy and cirrhotic subjects. Accordingly, the GLN synthesis from GLU and mobilization must be unimpaired even in severe hepatic functional impairment.

PAA has also been evaluated in a Phase I trial as an anticancer drug, being administered initially at a dose of 150 mg/kg i.v. over 2 h to 17 patients with various tumors which were refractory to other

treatments [66]. Serum concentrations of PAA peaked at ~460 μg/ml and PAGLN rose to 224 ± 81 μg/ml. During the 10 h of the investigation, serum GLN concentration fell from 109 ± 29 μg/l pre-dose to a sustained low of 60–70 μg/ml before finally recovering at 10 h. These investigators conducted a second Phase I study of PAA in 18 cancer patients in which PAA was administered by a 1-h infusion twice daily for 2 weeks at two dose levels (125 and 150 mg/kg) [67]. PAGLN and unchanged PAA accounted for 76 ± 15% and 3 ± 1% in the 0–24 h urine, respectively, of the administered dose. The authors noted, "Although we could not demonstrate sustained declines in plasma glutamine concentrations after repeated administration of phenylacetate, the urinary excretion of glutamine (as phenylacetylglutamine) from a 70-kg patient receiving 125 mg/kg/dose of phenylacetate twice daily would nevertheless exceed 90 mol [*sic*] per day" [67]. The authors surely meant 90 mmol, equivalent to 13.1 g GLN (not 13.1 kg). Nevertheless, this is a considerable depletion of GLN. The patients exhibited dose-dependent excretion of GLN as PAGLN and also dose-dependent neurological side-effect. The potential relationship between these two variables will be examined later.

4.3.1. Regarding the hypothesis

Numerous investigations in human subjects have demonstrated that BA and PAA (free and as metabolic precursors) are able to scavenge significant amounts of GLY and GLN respectively. In one study were GLN serum concentrations were determined, there was a sustained decline in GLN levels. Since GLN is synthesized from GLU and ammonia, it must be assumed that these pharmacological manipulations also reduce stores of GLU. However, no direct experimental evidence exists in support of this proposition. Nevertheless, humans excrete between 2 and 4 mmol/day HA [68], equivalent to 150–300 mg GLY. In addition, ~1 mmol PAGLN is excreted daily in urine, equivalent to 264 mg of either GLN or GLU [17]. Therefore, these two aromatic acids alone, without the intervention of 4-hydroxybenzoic acid, 2-furoic acid, 3-indolylacrylic acid, and perhaps many more, scavenge over 500 mg GLY plus GLN per 24 h. However, it should be stated that other mechanisms may exist to help regulate systemic concentrations of GLY and GLN/GLU. Since GLY is mobilized rather slowly in response to an oral dose of benzoic acid (see above), these other mechanisms may involve less responsive equilibria than GLY scavenging by conjugation with benzoic acid. This pathway, which ultimately leads to urinary excretion of GLY, is therefore irreversible.

4.4. CNS effects of administered aromatic acids

There have been many human investigations administering BA, PAA, or PAA precursors and in all of these side-effects were recorded. It is important to note that a large proportion of untoward effects of BA and PAA administration originate in the CNS. For example, both BA and PAA i.v. administration were reported to cause dizziness, nausea, and vomiting [61]. Intravenous PAA caused confusion, lethargy and vomiting, which were dose-related [66] and in a second study, i.v. PAA was commonly associated with neurological toxicity, including somnolence, fatigue, headache, lightheadedness, and dysgeusia [67]. The oral administration of NaPBA was linked with six CNS adverse events, specifically, one each of clonus, dizziness, dysgeusia, encephalopathy, nystagmus, and tremor [64]. In seven cirrhotics, all of whom excreted normal amounts of PAGLN, oral GPB administration was associated headaches [65].

From the side-effect profiles, it would appear that BA and PAA administration may result in reduced psychomotor activity.

4.4.1. Regarding the hypothesis

The adverse event profiles of BA, but in particular, PAA and PAA precursor administration have a major CNS component. We propose that these may be due, in part, to depletion of GLU in the CNS as a consequence of excessive GLN scavenging by PAA.

4.5. Blood glutamate scavenging

Accumulated evidence suggests that there exists an efflux of excess GLU from brain ECF into blood. The question remains as to the role of plasma GLU concentrations in this process, in other words, is this efflux simply driven from within the CNS or can changing circumstances in plasma assist the process? During pathological states such as traumatic brain injury or ischemia stroke, GLU is uncontrollably released from astrocytes and neurons and this buildup of GLU in ECF may lead to neuronal death throughout the brain [69]. A number of salient facts should be considered here; (1) the brain is highly vascularized with 100 million capillaries that have a surface area of ~12 m^2, (2) almost all neurons are close to a capillary within a distance of 8–20 μm, (3) these capillaries are dense with EAATs [69]. This massive network permits the efflux of GLU from the brain. In addition, it would appear that this process may be enhanced by a process of GLU scavenging. Experiments have been reported in which rats were either injected intracerebroventricularly or perfused cerebroventricularly with radiolabeled GLU. To decrease blood GLU concentrations in these animals the investigators utilized the GLU scavenging properties of two blood-resident enzymes glutamate–pyruvate transaminase and glutamate–oxaloacetate transaminase, which both metabolize GLU to α-ketoglutarate in the presence of the respective GLU co-substrates, pyruvate and oxaloacetate [70]. The decrease in blood GLU concentrations hastened the brain efflux of radiolabeled GLU [70]. It has been stated that the scavenging of blood GLU by transaminases is a neuroprotective mechanism [69].

Transaminases are not the only route for GLU removal. Conversion to GLN by glutamine synthase in the liver followed by GLN conjugation with PAA and urinary excretion of PAGLN also offers a powerful mechanism for scavenging GLU, which, unlike transaminase activity, is not reversible. The same argument surely applies for GLY, which seems to cross the BBB freely and is depleted by scavenging with BA.

4.5.1. Regarding the hypothesis

Experimental evidence in the rat demonstrates that GLU efflux from the brain can be accelerated by blood scavenging of GLU. This principle illuminates the likelihood that GLU and GLY efflux from brain can be directly linked to hepatic scavenging mechanisms that ultimately result in the urinary excretion of HA and PAGLN and perhaps other GLY and GLN conjugates too. This process is illustrated in Fig. 2.

4.6. Insects and spiders

All the foregoing scientific evidence was drawn from investigations on humans and laboratory mammals. It is instructive to establish if similar arguments apply at the opposite end of the phylogenetic tree. Remarkably, the conjugation of BA and its derivatives 4-nitrobenzoic acid (4NBA) and 4-aminobenzoic acid (4ABA) has been studied in both insects and spiders. BA derivatives are conjugated almost exclusively with GLY in man. In house spiders (*Tegenaria* sp.), 4NBA and 4ABA were reported to be conjugated with GLU and arginine (ARG) [71]. 4ABA produced the ARG conjugate in millipedes, centipedes, and harvest spiders [71]. In contrast, BA, 4NBA, 4ABA, and salicylic acid (2-hydroxybenzoic acid) were detected as their respective GLY conjugates in the excreta of locusts (*Locusta migratoria*) dosed with the parent acids. ARG and GLU conjugates of these acids were not looked for in these studies [72]. How do these excretory patterns of BA derivatives

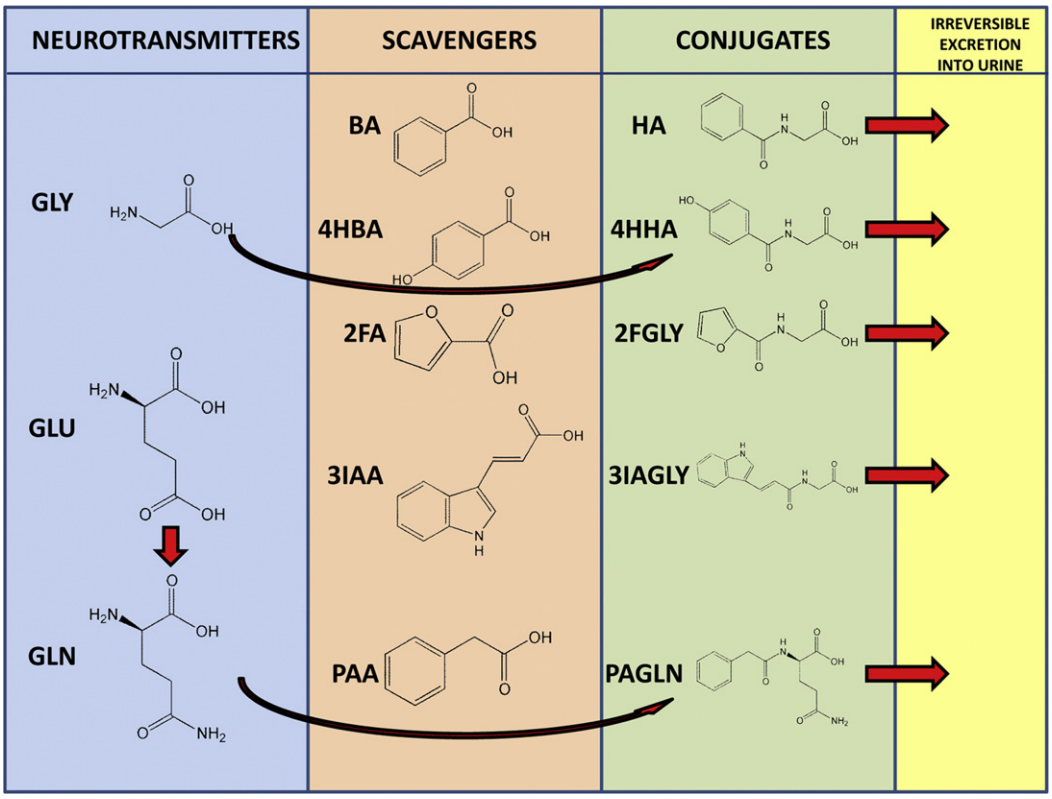

Fig. 2. Chemical structures of the amino acids, GLY, GLU, and GLN, as present in brain (blue box). Chemical structures of the known scavengers BA, 4-hydroxybenzoic acid (4HBA), 2-furoic acid (2FA), 3-indolylacrylic acid (3IAA), and PAA (orange box). Chemical structures of the amino acid conjugates of GLY and GLU formed in the scavenging process and includes HA, 4-hydroxyhippuric acid (4HHA), 2-furoylglycine (2FGLY), 3-indolylacryloylglycine (3IAGLY) and PAGLN (green box). Scavenged amino acids are irreversibly excreted into urine (yellow box). See also legend to Fig. 1 for key.

compare with amino acid occurrence as neurotransmitters in the primitive nervous systems of these species?

The occurrence and function of excitatory and inhibitory amino acid neurotransmitters have been studied in five families/suborders of spiders. In the protocerebellum, GLU and TAU were the predominant amino acid neurotransmitters [22]. The locust *Schistocerca gregaria* has two behavioral phenotypes, gregarious and solitarious. Thirteen different potential neurotransmitters have been assayed in the CNS of locust nymphs as they undergo the transition between long-term solitarious and gregarious adults [73]. Acetyl choline was by far the most abundant neurotransmitter with values of the order of 6–7 μmol/nervous system. The aromatic amines tyramine, dopamine, serotonin, octopamine, and *N*-acetyldopamine were present only in trace amounts (0.1–4 nmol/nervous system), while the amino acid neurotransmitters GLU, GLY, and ARG were dominant and all rose significantly after the change from long-term gregarious to third generation solitarious [73].

To recap, locust species and certain spiders conjugate BA derivatives with GLY, GLU, and ARG. Conjugation of aromatic acids with ARG has never been described to our knowledge in a vertebrate species. The CNS of closely related species contains GLY, GLU, ARG, and TAU as neurotransmitters. It is possible in these lower invertebrates that BA and its derivatives are used to scavenge unwanted amino acid neurotransmitters.

4.7. Accumulation of evidence

The proposition posed in this commentary has been that the value of amino acid conjugation to the organism is not, as in the traditional view, to use amino acids for the detoxication of aromatic acids. Rather, the converse is more likely, to use aromatic acids that originate from the diet and gut microbiota to assist in the regulation of body stores of amino acids, such as GLY and GLU, that are key neurotransmitters in the CNS. As such, the amino acid conjugations are not so much detoxication reactions, rather they are neuroregulatory processes. Experimental data have been culled in support of this hypothesis from a broad range of scientific and clinical literature. Below we summarize the arguments in enumerated points:

1. Compared with glucuronic acid conjugation, for example, amino acid conjugation does not render aromatic acids significantly more water soluble. In many cases, the converse is true. Thus, the detoxication value of amino acid conjugation is low.
2. The principal amino acid conjugations in vertebrates utilize GLY, GLN, and TAU.
3. GLY, GLU, and TAU are neurotransmitters in the CNS.
4. GLU is also an efficient fuel reserve for the brain.
5. GLU and GLN are removed from brain by the outward transport of GLU to blood by EAAT1, 2, and 3.
6. GLY is trafficked from brain to blood by GlyT1.
7. This reversible amino acid efflux acts as a neuroprotective mechanism against elevated, neurotoxic concentrations of amino acids.
8. Trafficked amino acids are scavenged by metabolic processes that lead to irreversible urinary excretion of amino acid conjugates of aromatic acids.
9. Experiments in rats demonstrate that scavenging of GLU by activation of blood transaminases removes GLU from the brain.
10. Both BA and PAA are used clinically to scavenge GLY and GLN for the purposes of excess nitrogen excretion in urea cycle defects.

11. Clinical use of PAA and its precursors NaPBA and GPB, which result in considerable GLN scavenging, are commonly associated with CNS side-effects, a possible reflection of brain GLU depletion.
12. Psychiatric conditions with reduced psychomotor activity display reduced GLY scavenging by BA, which can be reversed by GLY loading.
13. Psychiatric conditions with enhanced psychomotor activity display enhanced GLY scavenging by BA.
14. GLY scavenging by various aromatic acids is enhanced in autism.
15. GLY, GLU, and ARG are scavenged by BA and its derivatives in certain species of insects and spiders that use these same amino acids as neurotransmitters in their CNS.

4.8. Postscript

Assuming the verity of this proposition advanced here, there would be a clear link between dietary constituents that are processed by the gut microbiota into aromatic acids and the disposition of GLY and GLU in the body. The consequences of this could be that diet has a major impact on psychomotor function via this amino acid scavenging pathway. The otherwise banal conjugation of aromatic acids with GLY and GLN might have greater biological and clinical consequences than hitherto thought. The poor cousin may now have inherited a fortune.

Conflict of interest

The authors have no conflicts of interest to declare.

References

[1] Williams RT. Detoxication mechanisms. New York: John Wiley & Sons Inc.; 1959.
[2] Keller W. On the conversion of benzoic acid into hippuric acid. Prov Med J Retrosp Med Sci 1842;4:256–7.
[3] Bunge G, Schmiedeberg O. Ueber die Bildung der Hippursäure On the formation of hippuric acid Arch Exp Pathol Pharmakol 1877;vi:233–55.
[4] Lackner E, Levinson A, Morse W. The role of the liver in hippuric acid synthesis. Biochem J 1918;12:184–9.
[5] Bryan AW. Clinical and experimental studies on sodium benzoate: the value of the sodium benzoate test of renal function, and the effect of injury of the liver on hippuric acid synthesis. J Clin Invest 1925;2:1–33.
[6] Quick AJ. Clinical value of the test for hippuric acid in cases of disease of the liver. Arch Int Med 1936;57:544–56.
[7] Griffith HW, Lewis HB. Studies in the synthesis of hippuric acid in the animal organism. V. The influence of amino-acids and related substances on the synthesis and rate of elimination of hippuric acid after administration of benzoate. J Biol Chem 1923;57:1–24.
[8] Griffith WH, Lewis HB. Studies in the synthesis of hippuric acid in the animal organism. VI. The influence of the protein of the diet on the synthesis and rate of elimination of hippuric acid after administration of benzoates. J Biol Chem 1923;57:697–707.
[9] Bridges JW, French MR, Smith RL, Williams RT. The fate of benzoic acid in various species. Biochem J 1970;118:47–51.
[10] James MO, Smith RL, Williams RT, Reidenberg M. The conjugation of phenylacetic acid in man, sub-human primates and some non-primate species. Proc R Soc Lond B Biol Sci 1972;182:25–35.
[11] Idle JR, Millburn P, Williams RT. Taurine conjugates as metabolites of arylacetic acids in the ferret. Xenobiotica 1978;8:253–64.
[12] Bababunmi EA, Smith RL, Williams RT. The absence of hippuric acid synthesis in the Indian fruit bat. Life Sci II 1973;12:317–26.
[13] Emerole GO, French MR, Williams RT. Effect of p-nitro-substitution on the metabolism of benzoic acid in the Indian fruit bat. Biochem Exp Biol 1977;13:385–90.
[14] Idle JR, Millburn P, Williams RT. Benzoylglutamic acid, a metabolite of benzoic acid in Indian fruit bats. FEBS Lett 1975;59:234–6.
[15] Goodwin BL, Ruthven CR, Sandler M. Gut flora and the origin of some urinary aromatic phenolic compounds. Biochem Pharmacol 1994;47:2294–7.
[16] http://www.hmdb.ca/ [accessed September 27, 2011].
[17] Yang D, Beylot M, Agarwal KC, Soloviev MV, Brunengraber H. Assay of the human liver citric acid cycle probe phenylacetylglutamine and of phenylacetate in plasma by gas chromatography-mass spectrometry. Anal Biochem 1993;212:277–82.
[18] Pacifici GM, Mogavero S, Giuliani L, Rane A. Conjugation of benzoic acid with glycine in the human fetal and adult liver and kidney. Dev Pharmacol Ther 1991;17:52–62.
[19] www.chemspider.com [accessed September 27, 2011].
[20] Mackenzie PI, Bock KW, Burchell B, Guillemette C, Ikushiro S, Iyanagi T, et al. Nomenclature update for the mammalian UDP glycosyltransferase (UGT) gene superfamily. Pharmacogenet Genomics 2005;15:677–85.
[21] Mano I, Straud S, Driscoll M. *Caenorhabditis elegans* glutamate transporters influence synaptic function and behavior at sites distant from the synapse. J Biol Chem 2007;282:34412–9.
[22] Meyer W, Poehling HM. Regional distribution of putative amino acid neurotransmitters in the CNS of spiders (Arachnida: Araneida). Neurochem Int 1987;11:241–6.
[23] Anderson PA, Trapido-Rosenthal HG. Physiological and chemical analysis of neurotransmitter candidates at a fast excitatory synapse in the jellyfish *Cyanea capillata* (Cnidaria, Scyphozoa). Invert Neurosci 2009;9:167–73.
[24] Hawkins RA. The blood–brain barrier and glutamate. Am J Clin Nutr 2009;90:867S–74S.
[25] Kuzmiski JB, Bains JS. Metabotropic glutamate receptors: gatekeepers of homeostasis. J Neuroendocrinol 2010;22:785–92.
[26] Johnson JW, Ascher P. Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 1987;325:529–31.
[27] Betz H, Laube B. Glycine receptors: recent insights into their structural organization and functional diversity. J Neurochem 2006;97:1600–10.
[28] Schmieden V, Grenningloh G, Schofield PR, Betz H. Functional expression in Xenopus oocytes of the strychnine binding 48 kD subunit of the glycine receptor. EMBO J 1989;8:695–700.
[29] Langosch D, Becker CM, Betz H. The inhibitory glycine receptor: a ligand-gated chloride channel of the central nervous system. Eur J Biochem 1990;194:1–8.
[30] Takahashi M, Billups B, Rossi D, Sarantis M, Hamann M, Attwell D. The role of glutamate transporters in glutamate homeostasis in the brain. J Exp Biol 1997;200:401–9.
[31] Kawai N, Bannai M, Seki S, Koizumi T, Shinkai K, Nagao K, et al. Pharmacokinetics and cerebral distribution of glycine administered to rats. Amino Acids 2011. doi: 10.1007/s00726-011-0950-y.
[32] Busanello EN, Moura AP, Viegas CM, Zanatta A, da Costa Ferreira G, Schuck PF, et al. Neurochemical evidence that glycine induces bioenergetical dysfunction. Neurochem Int 2010;56:948–54.
[33] Huang H, Barakat L, Wang D, Bordey A. Bergmann glial GlyT1 mediates glycine uptake and release in mouse cerebellar slices. J Physiol 2004;560:721–36.
[34] Quastel JH. Biochemistry and mental disorder. Lancet 1932;220:1417–9.
[35] Lockwood MR, Davies DR. Blood-urea changes in abnormal mental states after the administration of amino-acid. Biochem J 1932;26:745–52.
[36] Quick AJ. The conjugation of benzoic acid in man. J Biol Chem 1931;92:65–85.
[37] Quastel JH, Wales WT. Faulty detoxication in schizophrenia. Lancet 1938;232:301–5.
[38] Strom-Olsen R, Greville GD, Lennon RW. Hippuric acid synthesis in schizophrenia. Lancet 1938;232:995–6.
[39] Davies DR, Hughes TPE. Faulty detoxication in mental disorder. Lancet 1940;235:403–5.
[40] Greville GD. The hippuric acid excretion test in schizophrenia. Proc R Soc Med 1945;38:671–4.
[41] Wong YT. The hippuric acid liver function test in schizophrenia. J Nerv Ment Dis 1945;102:183–4.
[42] Koersner PE. Some observations on the hippuric acid test in schizophrenia. Acta Psychiatr Scand 1947;22(Suppl. 47):145–55.
[43] Stern K, Tyhurst JS, Askonas BA. Note on hippuric acid synthesis in senility. Am J Med Sci 1946;212:302–5.
[44] Cook GC. Impairment of glycine absorption by glucose and galactose in man. J Physiol 1971;217:61–70.
[45] Persky H, Grinker RR, Mirsky IA. The excretion of hippuric acid in subjects with free anxiety. J Clin Invest 1950;29:110–4.
[46] Fabisch W, Fellner C. Excretion of hippuric acid in schizophrenic and depressive patients after E.C.T.. Psychosom Med 1957;19:320–5.
[47] Betz H, Gomeza J, Armsen W, Scholze P, Eulenburg V. Glycine transporters: essential regulators of synaptic transmission. Biochem Soc Trans 2006;34:55–8.
[48] Field JR, Walker AG, Conn PJ. Targeting glutamate synapses in schizophrenia. Trends Mol Med 2011;17:689–98.
[49] Lin CH, Lane HY, Tsai GE. Glutamate signaling in the pathophysiology and therapy of schizophrenia. Pharmacol Biochem Behav 2011. doi: 10.1016/j.pbb.2011.03.023.
[50] Dolu N. Dose-related anxiogenic effect of glycine in the elevated plus maze and in electrodermal activity. J Basic Clin Physiol Pharmacol 2007;18:141–7.
[51] Lis AW, McLaughlin I, Mpclaughlin RK, Lis EW, Stubbs EG. Profiles of ultraviolet-absorbing components of urine from autistic children, as obtained by high-resolution ion-exchange chromatography. Clin Chem 1976;22:1528–32.
[52] Arribas-Lorenzo G, Morales FJ. Estimation of dietary intake of 5-hydroxymethylfurfural and related substances from coffee to Spanish population. Food Chem Toxicol 2010;48:644–9.
[53] Yap IK, Angley M, Veselkov KA, Holmes E, Lindon JC, Nicholson JK. Urinary metabolic phenotyping differentiates children with autism from their unaffected siblings and age-matched controls. J Proteome Res 2010;9:2996–3004.
[54] Anderson RJ, Bendell DJ, Garnett I, Groundwater PW, Lough WJ, Mills MJ, et al. Identification of indolyl-3-acryloylglycine in the urine of people with autism. J Pharm Pharmacol 2002;54:295–8.

[55] Khan SG, Levy HL, Legerski R, Quackenbush E, Reardon JT, Emmert S, et al. Xeroderma pigmentosum group C splice mutation associated with autism and hypoglycinemia. J Invest Dermatol 1998;111:791–6.
[56] Shinohe A, Hashimoto K, Nakamura K, Tsujii M, Iwata Y, Tsuchiya KJ, et al. Increased serum levels of glutamate in adult patients with autism. Prog Neuropsychopharmacol Biol Psychiatry 2006;30:1472–7.
[57] Ghanizadeh A. Targeting of glycine site on NMDA receptor as a possible new strategy for autism treatment. Neurochem Res 2011;36:922–3.
[58] Lewis HB. Studies in the synthesis of hippuric acid in the animal organism. II. The synthesis and rate of elimination of hippuric acid after benzoate ingestion in man. J Biol Chem 1914;18:225–31.
[59] Shiple GT, Sherwin CP. Synthesis of amino acids in animal organisms. I. Synthesis of glycocoll and glutamine in the human organism. J Am Chem Soc 1922;44:618–24.
[60] Brusilow SW, Valle DL, Batshaw M. New pathways of nitrogen excretion in inborn errors of urea synthesis. Lancet 1979;2:452–4.
[61] Simell O, Sipila I, Rajantie J, Valle DL, Brusilow SW. Waste nitrogen excretion via amino acid acylation: benzoate and phenylacetate in lysinuric protein intolerance. Pediatr Res 1986;20:1117–21.
[62] Sebastio G, Sperandeo MP, Andria G. Lysinuric protein intolerance: reviewing concepts on a multisystem disease. Am J Med Genet C Semin Med Genet 2011;157:54–62.
[63] Jackson AA, Badaloo AV, Forrester T, Hibbert JM, Persaud C. Urinary excretion of 5-oxoproline (pyroglutamic aciduria) as an index of glycine insufficiency in normal man. Br J Nutr 1987;58:207–14.
[64] Lee B, Rhead W, Diaz GA, Scharschmidt BF, Mian A, Shchelochkov O, et al. Phase 2 comparison of a novel ammonia scavenging agent with sodium phenylbutyrate in patients with urea cycle disorders: safety, pharmacokinetics and ammonia control. Mol Genet Metab 2010;100:221–8.
[65] McGuire BM, Zupanets IA, Lowe ME, Xiao X, Syplyviy VA, Monteleone J, et al. Pharmacology and safety of glycerol phenylbutyrate in healthy adults and adults with cirrhosis. Hepatology 2010;51:2077–85.
[66] Thibault A, Cooper MR, Figg WD, Venzon DJ, Sartor AO, Tompkins AC, et al. A phase I and pharmacokinetic study of intravenous phenylacetate in patients with cancer. Cancer Res 1994;54:1690–4.
[67] Thibault A, Samid D, Cooper MR, Figg WD, Tompkins AC, Patronas N, et al. Phase I study of phenylacetate administered twice daily to patients with cancer. Cancer 1995;75:2932–8.
[68] Mulder TP, Rietveld AG, van Amelsvoort JM. Consumption of both black tea and green tea results in an increase in the excretion of hippuric acid into urine. Am J Clin Nutr 2005;81:256S–60S.
[69] Teichberg VI, Cohen-Kashi-Malina K, Cooper I, Zlotnik A. Homeostasis of glutamate in brain fluids: an accelerated brain-to-blood efflux of excess glutamate is produced by blood glutamate scavenging and offers protection from neuropathologies. Neuroscience 2009;158:301–8.
[70] Gottlieb M, Wang Y, Teichberg VI. Blood-mediated scavenging of cerebrospinal fluid glutamate. J Neurochem 2003;87:119–26.
[71] Smith JN. Detoxications of aromatic acids with glutamic acid and arginine in spiders. Nature 1962;195:399–400.
[72] Friedler L, Smith JN. Comparative detoxication. 3. Hippuric acid formation in adult locusts. Biochem J 1954;57:396–400.
[73] Rogers SM, Matheson T, Sasaki K, Kendrick K, Simpson SJ, Burrows M. Substantial changes in central nervous system neurotransmitters and neuromodulators accompany phase change in the locust. J Exp Biol 2004;207:3603–17.