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Commentary

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

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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

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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.

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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