Chemistry and Neurochemistry of the Kynurenine Pathway of Tryptophan Metabolism

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1 Introduction

The kynurenine pathway was first described as a major route for the metabolism of tryptophan in 1947.[1] The pathway was of interest as both a source of nicotinamide and as the first metabolic pathway affected by the onset of vitamin B6 deficiency.[2] However, when 5-hydroxytryptamine (5HT) was found to be an important neurotransmitter, the major focus of tryptophan research shifted to the investigation of its role as a precursor of 5HT. The alternative kynurenine pathway was perceived to be less important and work in this area declined. Interestingly, in peripheral tissue only 1% of dietary tryptophan is actually converted to 5HT, while greater than 95% is metabolised to kynurenines. It has only been more recently that there has been a resurgence of interest. This has arisen as a result of some very interesting developments concerning the biological activities of kynurenine metabolites.[3,4] The most important work concerns the neurological activity of quinolinic acid, which is implicated as an etiological factor in a range of neurodegenerative diseases including AIDS related dementia, Huntington's disease, Lyme disease and septicemia.[5] In the design of novel therapies to treat these conditions, the kynurenine pathway has been identified as an important target for drug action. Mechanistic work on the enzymes involved in the pathway has therefore been stimulated, to provide information so that specific and effective inhibitors can be designed. The kynurenine pathway contains a number of very interesting enzymes that catalyse chemical reactions not usually found in metabolism. Currently many of the chemical mechanisms are poorly understood and so even without the recent pharmaceutical interest the kynurenine pathway poses some challenging questions to bioorganic chemists. The aim of this review is to describe the pathway, its biological importance and the chemistry of the enzymes involved.

2 The Kynurenine Pathway of Tryptophan Metabolism

The kynurenine pathway (Scheme 1) begins with oxidative cleavage of the amino acid tryptophan (1) by one of two different enzymes, tryptophan 2,3-dioxygenase (EC 1.13.11.11) or indo-§leamine 2,3-dioxygenase (EC 1.13.11.17), both of which are haem dependent. The product of this cleavage is formylkynurenine (2),

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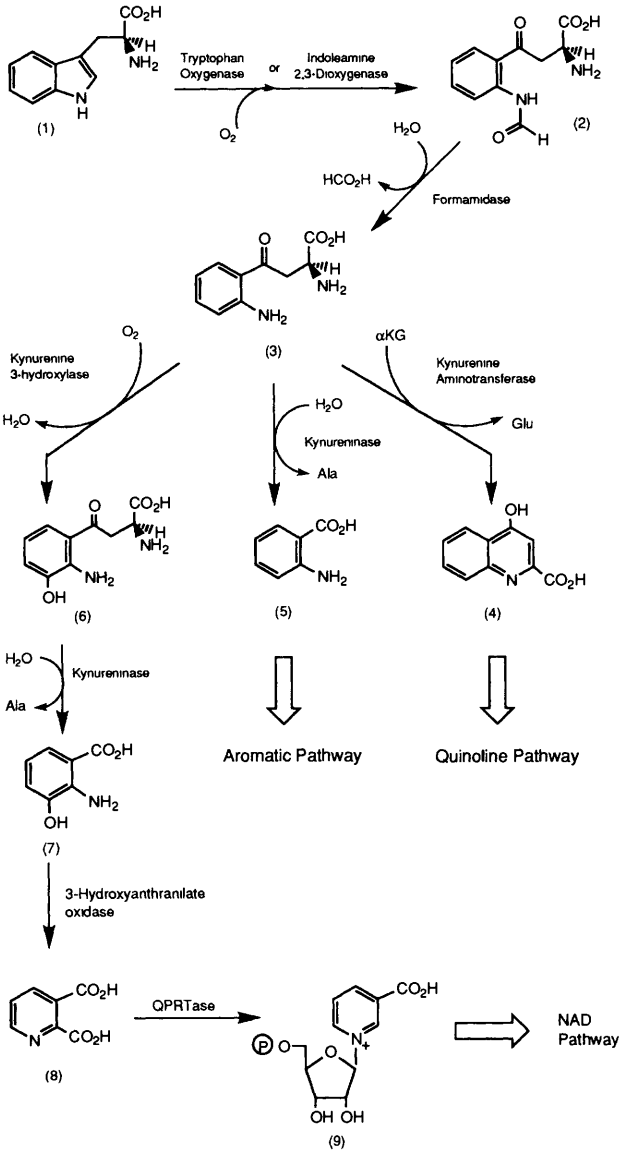


Scheme 1

which is then hydrolysed by a formamidase enzyme (EC 3.5.1.9) to give kynurenine (3), the amino acid which gives the pathway its name. Kynurenine is at a branch point in the pathway and may have a number of different fates. Kynureninase (EC 3.7.1.3) catalyses the cleavage of kynurenine to give anthranilic acid (5) and alanine, and the anthranilic acid may then be utilised in the biosynthesis of aromatic amino acids. Alternatively transamination catalysed by kynurenine aminotransferase (KAT, EC 2.6.1.7) produces kynurenic acid (4), a stable aromatic metabolite sometimes employed in the biosynthesis of quinolines. Both KAT and kynureninase are pyridoxal-5′-phosphate (PLP) dependent. The third possibility is oxidation of the benzene ring catalysed by kynurenine 3-hydroxylase (EC 1.14.13.9). At this point kynureninase can make a second contribution to the pathway and cleave the 3-hydroxy kynurenine (6) to give 3-hydroxyanthranilic acid (7). There are

some reports of a further enzyme present in some tissues that will hydroxylate anthranilic acid at the 3-position providing an alternative route to this metabolite. Rearrangement of 3-hydroxyanthranilic acid produces quinolinic acid (8), the most neurologically important metabolite. This reaction is catalysed by 3-hydroxyanthranilic acid oxidase (EC 1.13.11.6). Finally, quinolinic acid is converted to nicotinic acid mononucleotide (9) by the action of quinolinate phosphoribosyl transferase (QPRTase, EC 2.4.2.19). Further transformations may then follow, leading to the nicotinamide coenzymes. The 5HT pathway diverges from the kynurenine pathway at the very beginning, where its first step is oxidation of tryptophan at the 5-position. Later steps produce kynurenine derivatives which therefore contain a hydroxy group at the 5-position. However, there is no evidence for an enzyme that will 5-hydroxylate kynurenine directly, to allow it to crossover into the 5HT pathway.

2.1 Oxidative Cleavage of Tryptophan

The first step on the kynurenine pathway is the oxidative cleavage of the five-membered heterocyclic ring of tryptophan. Two distinct enzymes can catalyse this reaction and both are haem dependent. The first, tryptophan 2,3-dioxygenase (EC 1.13.11.11), is essentially localised in the liver, while the second, indoleamine 2,3-dioxygenase (EC 1.13.11.17), is widely distributed in mammalian tissue, including the brain, lung and small intestine.

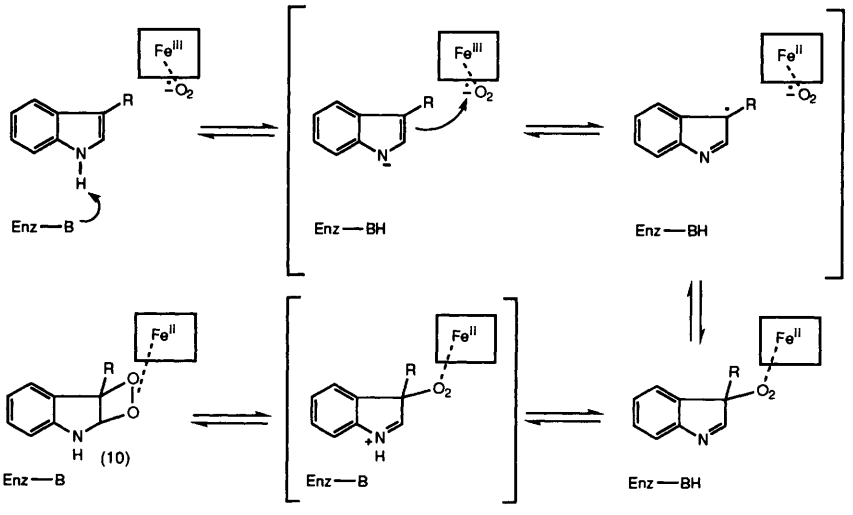
2.1.1 Tryptophan 2,3-Dioxygenase

As tryptophan 2,3-dioxygenase is rate limiting for the entry of tryptophan into the pathway, it is subject to several different regulatory mechanisms. Such regulation is to be expected for a pathway which catalyses the irreversible catabolism of what is, to mammals, an essential amino acid. Tryptophan 2,3-dioxygenase has a short half life of only about two hours and induction of its *de novo* synthesis is regulated by the glucorticoid hormones. Tryptophan and some of its analogues, including α-methyltryptophan, also have an allosteric activating effect on the enzyme.[6] They bind to the enzyme at a distinct activating site, and do not compete with the substrate for the active site. This results in stabilisation of the holo-enzyme (*i.e.* enzyme–haem complex), and produces a reduction in K_M for the haem cofactor. This effect complicates the kinetics for turnover of tryptophan as it binds to both sites, acting as a substrate and an activator. The enzyme is also activated by non-specific reductants, *e.g.* ascorbic acid, borohydride and hydrogen peroxide. The function of these species is to initiate the catalytic reaction by reducing the ferric (inactive) form of the haem iron, which is formed by autoxidation. However, this property is thought to be an *in vitro* artefact and not significant *in vivo*.

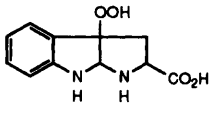
The mammalian liver enzyme is a tetramer, with a subunit molecular weight of 103 000, and containing two haem units per tetramer. The fungal and bacterial enzymes have essentially the same structure. Molecular oxygen is utilised in the catalytic cycle and this binds after tryptophan. Although the enzyme is activated by a range of tryptophan analogues, the only substrate for the enzyme is tryptophan itself. The results of isotope effect studies have allowed a mechanism to be proposed (Scheme 2).[7] A primary solvent deuterium isotope of 4.4 implied that abstraction of the indole proton was at least partially rate limiting, while an inverse secondary isotope effect of 0.96 was observed for L-[2-^{3}H]tryptophan, indicative of a change in hybridisation from sp^2 to sp^3 at this position. This is consistent with carbon–oxygen bond formation at carbon-2. Changes in the isotope effects with pH implied that proton abstraction must occur before C–O bond formation, and the variation with oxygen concentration indicated ordered binding of oxygen before substrate. Stepwise rather than concerted formation of the oxetane (10) was proposed as the initial deprotonation of the indole would make the concerted pathway energetically inaccessible. The final product then arises *via* decomposition of the oxetane, consistent with labelling studies that show both of the new oxygen atoms in the product derive from molecular oxygen. Studies on the dye-sensitised photooxidation of tryptophan also revealed formation of 3-hydroperoxytryptophan as the first step.[8] However there was evidence from quenching model reactions that a cyclic intermediate (11) was involved. The 3-hydroxy analogue was isolated and its X-ray crystal structure determined. On warming (11) is converted through to the product presumabley via intramolecular attack of the peroxy group on C-2 to give an oxetane, as in Scheme 2 (10). There is no evidence for the formation of such a cyclic intermediate in the enzyme catalysed reaction, possibly owing to binding of the substrate in a specific conformation at the active site preventing cyclisation.

2.1.2 Indoleamine 2,3-Dioxygenase

Indoleamine 2,3-dioxygenase shows some important mechanistic differences from tryptophan 2,3-dioxygenase. It has a much broader substrate specificity and will accept a range of related indoleamines. These include both D- and L-tryptophan, tryptamine (12, R = CH_2NH_2), 5-hydroxytryptophan, 5-hydroxytryptamine and melatonin. The enzyme utilises superoxide rather than molecular oxygen, and its activity is inhibited by superoxide dismutase. Superoxide will not act as a oxygen donor for tryptophan 2,3-dioxygenase. Finally the enzyme is not induced by the same mechanisms as tryptophan 2,3-dioxygenase and contains no activating site for tryptophan analogues. However, the enzyme is induced by γ-interferon, which is released in response to pathological stress.[9] Induction of the enzyme results in depletion of available tryptophan, which is the least abundant of the essential amino acids required for cellular integrity. Hence, its availability affects protein



Scheme 2



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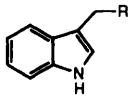

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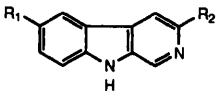


(13)

synthesis, genome replication, and organismal growth Increased indoleamine 2,3-dioxygenase production therefore leads to starvation of cells for tryptophan, which has a much more devastating effect on rapidly dividing cells, such as microbial pathogens and tumour cells As a result the enzyme has apparent antimicrobial and antitumour properties Another consequence of inducing indoleamine 2,3-dioxygenase activity is an increase in the production of kynurenine metabolites, including quinolinic acid, which may be responsible for the side effects observed when γ-interferon is administered to humans

Indoleamine 2,3-dioxygenase is monomeric haem containing glycoprotein, with a molecular weight of 41 000 There are few mechanistic data on the reaction and no isotope effects have been measured However a similar mechanism to that of tryptophan 2,3-dioxygenase is likely as they are both haem-dependent enzymes The enzyme is highly autoxidisable, especially in the presence of O_2 and substrate It requires superoxide or a reductant such as ascorbic acid in combination with methylene blue (an artificial dye), not only to initiate activity but also to maintain it at its maximum level *In vivo* the role of the dye is thought to be assumed by either a flavin (*e g* $FMNH_2$) or tetrahydrobiopterin, for which there is likely to be a specific binding site Evidence for an additional binding site was obtained by examination of the interaction of the enzyme with 3-substituted indoles (12),[10] which serve as effectors Indole-3-ethanol (12, R = CH_2OH) and indole itself enhance the enzyme's affinity for D-tryptophan but not L-tryptophan, while having opposing effects on V_{max} for both substrates Changes were observed in the CD spectra of the protein when the indoles bound to either the ferrous enzyme or its complexes with O_2, CO, and NO The effects were independent of the presence of the substrate It thus appeared that the indoles did not bind either to the haem or at the substrate binding site This suggested the existence of a further binding site, close to the substrate, the physiological role of which was proposed to be binding of the flavin or tetrahydrobiopterin cofactor

2 1 3 Inhibition of Dioxygenases

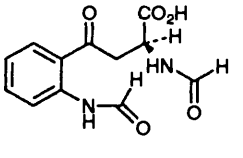
A wide range of tryptophan analogues have been examined as inhibitors of both tryptophan 2,3-dioxygenase and indoleamine 2,3-dioxygenase Tryptophan 2,3-dioxygenase is competitively inhibited by the indoleamines such as tryptamine, 5-hydroxytryptophan and melatonin, which are able to act as substrates for indoleamine 2,3-dioxygenase, as well as some phenyl derivatives including 2,5-dihydro-L-phenylalanine Derivatives of β-carboline (13), including the natural product norharman (13, $R_1 = R_2 = H$), are also potent competitive inhibitors [11] These showed some selectivity between enzymes from different sources A further class of competitive inhibitors were the indole derivatives, *e g* indole-3-ethanol (12, R = CH_2OH) and 3-acetamidoindole Interestingly, these compounds were effectors rather than inhibitors for indoleamine 2,3-dioxygenase (see above) [11]

Indoleamine 2,3-dioxygenase inhibition has been studied in more detail and the structural requirements for binding at the active site have been elucidated [12] Methylation of the indole nitrogen or replacement by both oxygen and sulfur gave analogues that were potent competitive inhibitors [13] Modification of the amino acid group eliminated both substrate and inhibitor activity Reduction of the double bond of the indole ring also eliminated inhibitor activity None of the other metabolites on the kynurenine pathway showed any inhibitory activity With the β-carboline derivatives (13), non-competitive inhibition was observed, in contrast to the competitive inhibition with tryptophan 2,3-dioxygenase Norharman (13, $R_1 = R_2 = H$) was the most potent with a K_i of 3 3 μmol l^{-1}, compared to the K_M for L-tryptophan of 13 μmol l^{-1} Further, more detailed, studies revealed that these compounds bound to the haem iron as N-donor ligands and in fact competed with the oxygen [14] There are no reports of effective irreversible inhibitors of either tryptophan 2,3-dioxygenase or indoleamine 2,3-dioxygenase

2.2 Kynurenine Formamidase

The immediate product from the oxidative cleavage of tryptophan is *N*-formylkynurenine (2), which is rapidly hydrolysed to kynurenine (3) (Scheme 1) by kynurenine formamidase (EC 3 5 1 9), with the concomitant release of formate The enzyme from all sources is essentially the same However, there are some differences in substrate specificity and sensitivity to inhibitors In particular, some enzymes are sensitive to sulfydryl directed reagents while others are not The enzyme has a rather broad substrate specificity and is capable of hydrolysing a number of aryl formamides including D- and L-enantiomers of *N*-formylkynurenine, *N*-formyl-3-hydroxykynurenine and *N*-formylanthranilate Kynurenine formamidase will not hydrolyse N^{α}-formylkynurenine and this forms the basis of an assay for the enzyme If N^1 N^{α}-diformylkynurenine (14) is employed as substrate the product is N^{α}-formylkynurenine which accumulates in the medium and its concentration can be monitored In crude preparations, kynurenine itself cannot be monitored as it is rapidly metabolised

Although there is little work on the mechanism or inhibition of kynurenine formamidase, it has been observed to be inhibited very strongly by organophosphorus and methylcarbamate insecticides [15] These compounds were known to affect the formation of several metabolites on the kynurenine pathway and further studies revealed that the formamidase was the site of action In mice, the liver enzyme was almost completely inhibited by the organophosphorus acid triester, diazinon (15), at the 10 mg kg^{-1} level Diazinon and related compounds show toxicity due to cholinesterase inhibition, but also have a degree of non-cholinergic toxicity Inhibition of kynurenine formamidase was thought to be important in understanding the latter pathway The inhibition was essentially irreversible, although a small degree of activity could be recovered if the enzyme was chromatographically separated from the inhibitor The structural requirements for optimum inhibition were examined using a number of organophosphorus compounds (16) [15] Phosphorothioates (16, X = S) with a pyrimidin-4-yl substituent at R_2 were found to be the most potent inhibitors, with other aromatic or aliphatic substituents being less effective Replacement of sulfur by oxygen reduced the inhibitory activity Although the mechanism of the inhibition reaction was not fully understood, phosphorylation of an active site serine was proposed to be the cause of the inactivation The aryl phosphorothioates may also be thought of as mimics of the tetrahedral transition state for the hydrolysis reaction, which



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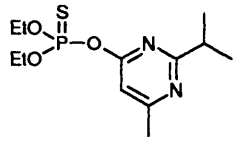

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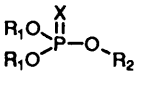


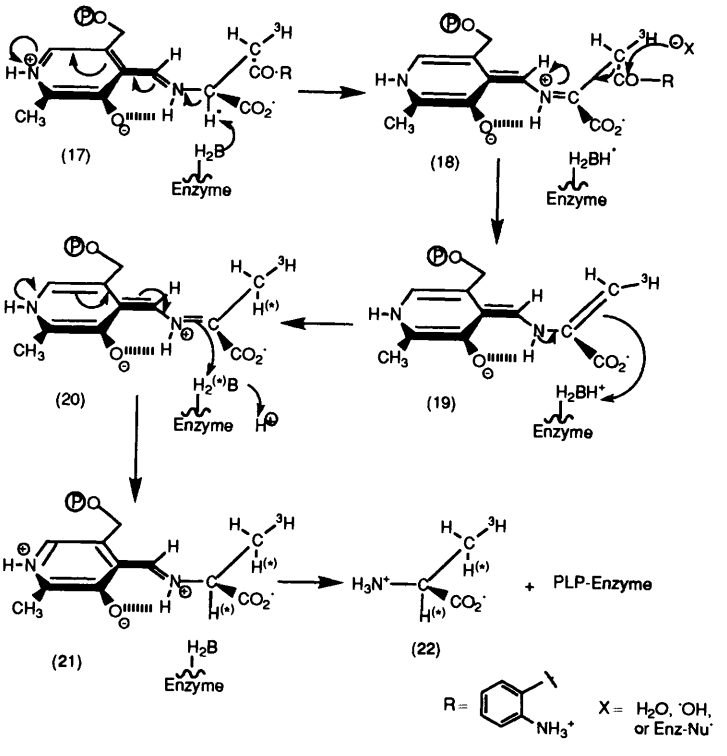
(16)
R_1 = Alkyl, X = S or O,
R_2 = Alkyl or Aryl

should result in tight binding to the active site of the enzyme and contribute to the efficiency of the inactivation process.

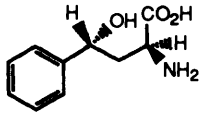
2.3 Kynureninase

Kynureninase (EC 3.7.1.3), a pyridoxal 5′-phosphate (PLP) dependent enzyme, catalyses a unique reaction in metabolism, the hydrolytic β,γ-cleavage of aryl substituted γ-keto-α-amino acids. The natural substrates are kynurenine (3) and 3-hydroxykynurenine (6), which are hydrolysed to give anthranilic acid (5) and 3-hydroxyanthranilic acid (7) respectively. The other product in both cases is L-alanine. The enzyme belongs to a rare category of pyridoxal 5′-phosphate dependent enzymes which catalyse β,γ-cleavage reactions, which includes L-aspartate-β-decarboxylase and L-selenocysteine-β-lyase. The enzyme has been purified from bacterial and mammalian sources and the relative rates of cleavage of the two substrates have been observed to vary with enzyme preparations from different sources. For example the pseudomonad enzyme hydrolyses kynurenine five times faster than 3-hydroxykynurenine, while the liver enzyme hydrolyses the hydroxy derivative twice as rapidly as kynurenine. Specificity studies demonstrated that the enzyme would tolerate some substitutions in the benzene ring of the substrate and retain reasonable activity. The *ortho*-amino group was not an absolute requirement as desaminokynurenine was still a substrate, although at a much reduced rate. Replacement of this group by either a nitro or hydroxy group, or its formylation, also gave active substrates. Reduction of the carbonyl group to the alcohol gave a compound which was still cleaved by kynurenine (see below).

The stereochemical course of the reaction with regard to C-3 has been elegantly determined by Floss, Soda and coworkers.[16] They prepared (2*S*,3*R*)- and (2*S*,3*S*)-[3-^{3}H]-kynurenine from the corresponding stereospecifically tritiated tryptophan species by enzymic methods. These were converted into alanine by kynureninase in deuterium oxide, and chiral analysis of the alanine methyl group revealed the steric course of the replacement of the anthranilyl group by hydrogen. The reaction was found to occur with retention of configuration. It was also observed that tritium from the α-position of the substrate was recycled and appeared at both C_α and C_β of the product. A mechanism has been proposed to account for the results (Scheme 3). The PLP was proposed to bind to the apoenzyme *via* the ϵ-amino group of an active site lysine residue to give a Schiff's base (the internal aldimine), which is common to most other pyridoxal phosphate dependent enzymes. Kynurenine then binds to the enzyme and a transaldimination reaction takes place, displacing the active site lysine and forming a Schiff's base between the PLP and kynurenine (17). Cleavage of the C_α–H bond then generates the ketimine intermediate (18). The Dunathan postulate states that the bond to be cleaved is held perpendicular to the delocalised π-system of the co-enzyme in order to maximise orbital overlap. Attack of an enzymic nucleophile or water molecule (X) then takes place at the γ-carbonyl group and the β,γ-carbon–carbon bond is cleaved to give anthranilate and an α-aminoacrylate derivative (19). For the above stereoelectronic reasons the C_β–C_γ bond should be orientated perpendicular to the plane of the PLP π-system. However it may lie either on the same, or opposite side, to the C_α–H bond. The α-aminoacrylate is then protonated at the β-carbon giving (20) and the stereochemical results show that this is stereospecific and occurs with retention of configuration. Thus the departing anthranilyl group and the enzyme base responsible for reprotonation must be on the same face of the PLP–substrate complex. Reprotonation at the α-carbon gives the Schiff's base of the product (21) and finally a further transaldimination takes place to release the alanine (22). The observation of recycling of the labelled α-proton of the substrate back into both the α and β positions of the product implies that reprotonation at C_β is mediated by the same base that removes the α-proton. These two results support an active site conformation in which the α-hydrogen and the anthranilyl group at C_β are *syn* orientated. It was assumed, but not proven, that they were both on the *Si* face relative to C-4′ of the co-enzyme, as in all other PLP dependent enzymes so far examined.



Scheme 3



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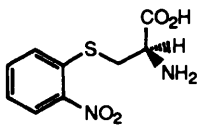

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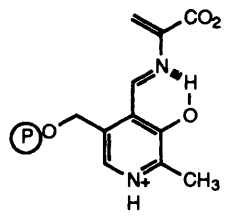

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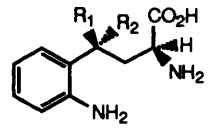


(26a, R_1 = H, R_2 = OH)
(26b, R_1 = OH, R_2 = H)

The identity of the active site base has not been established Kishore[17] observed alkylation of an active site carboxy group using mechanism based inhibitors of kynureninase, implying that this was involved in catalysis However the partitioning of tritium between C_α and C_β implied that a polyprotic base, such as a lysine residue, was more likely [16] There is much better evidence for the α-aminoacrylate intermediate, provided by trapping experiments [18] Hydrolysis of kynurenine in the presence of benzaldehyde gave high yields of 2-amino-4-hydroxy-4-phenylbutyric acid (23) The α-aminoacrylate intermediate which has nucleophilic character at the β-carbon therefore has a sufficiently long lifetime to enable the anthranilic acid to debind from the active site, and the benzaldehyde to bind and undergo reaction Only aromatic aldehydes were effective, presumably because they best mimicked the substrate and bound most tightly to the active site Later work revealed that the reaction was stereospecific and only the (4*R*, 2*S*) isomer (23) was formed [19] Interestingly catalysis of the reverse reaction has not been observed and neither has trapping of the putative β-carbanion generated from alanine The identity of the group responsible for attack on the β-carbon is still unknown This could be either a water molecule or an enzymic nucleophile In the first case this would involve a single step, with a water molecule being bound in a suitable position at the active site to attack the carbonyl group Presumably this would involve general base catalysis, with an active site base removing a proton from the water The alternative nucleophile-mediated mechanism would involve two steps Initially an acyl-enzyme intermediate would be formed, which requires subsequent hydrolysis to give the product As yet these two mechanisms have not been distinguished, although inhibition studies have provided some circumstantial evidence (see below) for the bound-water mediated mechanism

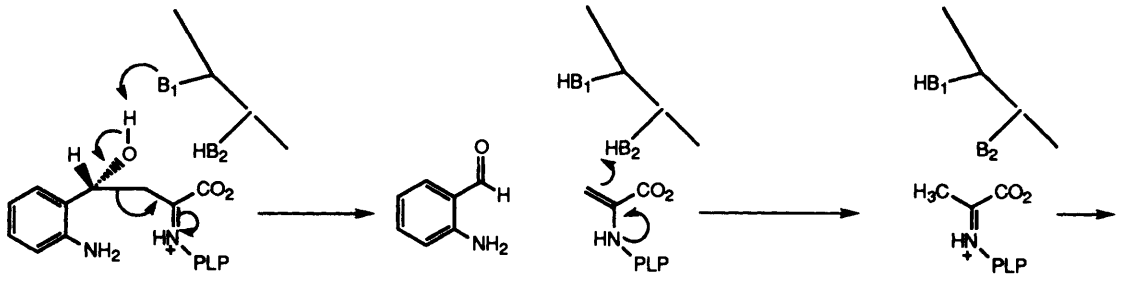
Incubation of kynureninase with alanine or ornithine results in inactivation of the enzyme due to an abortive transamination reaction This converts the pyridoxal phosphate to pyridoxamine phosphate which is then unable to form an aldimine with another amino acid substrate molecule This reaction, however, is very slow and only occurs at less than 0 4% of the rate of the β,γ-cleavage reaction Essentially it represents an alternative fate for the α-aminoacrylate intermediate, and it may in fact act as a regulatory mechanism *in vivo*

2 3 1 *Inhibitors of Kynureninase*

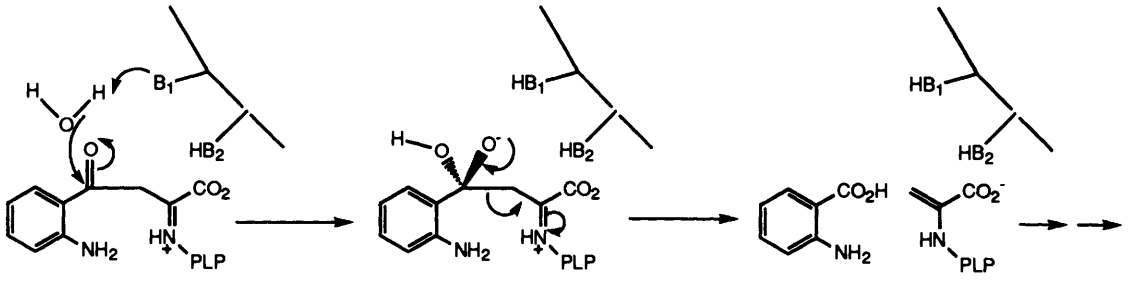
A range of β-substituted amino acids, a class of compounds known to inhibit PLP dependent enzymes,[17] have been shown to inhibit kynureninase Examples include β-chloro-L-alanine and L-serine-*O*-sulfate The most potent, however, was L-*S*-(*o*-nitrophenyl)-cysteine (24), presumably as a result of the strong binding interaction between the aromatic moiety and the active site Kynureninase catalyses a β-elimination reaction of these compounds to give an enzyme bound aminoacrylate intermediate (25) In this case the intermediate is electrophilic at the β-carbon and so may be attacked by nucleophilic residues at the enzyme active site This results in covalent attachment of the inhibitor to the protein and inactivation of the enzyme In the physiological reaction the aminoacrylate intermediate produced following β,γ-cleavage has the opposite reactivity and is nucleophilic at the β-carbon (19) The β-elimination activity of kynureninase has also been observed in its reaction with cysteine conjugates Indeed a purified cysteine conjugate β-lyase enzyme was in fact found to be kynureninase

Both diastereoisomers of dihydrokynurenine were examined as inhibitors [19] The (4*R*,2*S*) diastereoisomer (26a) was a potent competitive inhibitor (K_I 1 4 μmol l $^{-1}$) but was also a substrate for the enzyme, undergoing a retro-aldol cleavage to give the aldehyde and alanine (Scheme 4) The (4*S*,2*S*) diastereoisomer was an even more potent competitive inhibitor (K_I 0 3 μmol l $^{-1}$) but did not act as a substrate It was proposed that the tight binding arose because both the alcohols were able partially to mimic the tetrahedral intermediate (27), which would arise if the mechanism involved attack of a bound water molecule As this can be used as a model for the transition state, these compounds can be thought to be acting as transition state analogues This provides some evidence for the operation of this mechanism, rather than the alternative attack of an enzymic nucleophile The stereospecificity of the retro-aldol cleavage is also interesting The earlier stereochemical studies[19] implied that a single polyprotic base mediated the protonation and deprotonation at both the α- and β-carbons However, there must also be a second base present, which is capable of removing the proton from the alcohol to facilitate the retro-aldol cleavage This is positioned such that it is only able to remove the proton from the (4*R*,2*S*) diastereoisomer and not the (4*S*,2*S*) diastereoisomer In the physiological reaction, the role of this second base could be removal of a proton from a water molecule to assist in attack of the carbonyl group (Scheme 5)

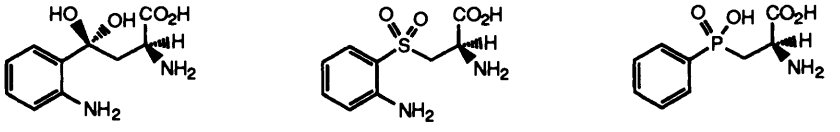
Other selective inhibitors have been designed using the tetrahedral intermediate (27) as a model for the transition state These compounds mimic the structure of the intermediate but are chemically stable and therefore potential tight binding competitive inhibitors Such a strategy is now widely accepted in the design of enzyme inhibitors, having particular application in the inhibition of protease enzymes Firstly, Phillips and Dua have prepared a sulfone analogue (28) from cysteine *via* aromatic nucleophilic substitution [20] This was found to have a K_I of 70 μmol l $^{-1}$ Secondly, a difluoro substituted analogue of desaminokynurenine (29) has been elegantly synthesised [21] In aqueous solution this will exist predominantly as the hydrate (30) and so mimic the tetrahedral



Scheme 4



Scheme 5



(27) (28) (31)

(29) (30)

transition state However as yet there are no biological data available Thirdly, we have also recently prepared a phosphinic acid based analogue (31), using β-lactone-type methodology [22] It is notable in the latter two cases that the inhibitors lack the aromatic amino group that is present in kynurenine However, it is known that desaminokynurenine is still a reasonable substrate for kynureninase and so a loss of binding is accepted in return for increased ease of synthesis

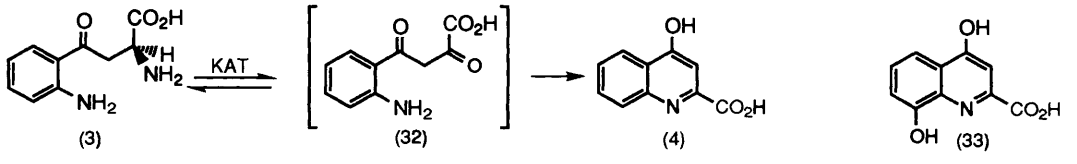
2.4 Kynurenine Aminotransferase (KAT)

Kynurenine aminotransferase (KAT, EC 2 6 1 7) is a PLP dependent enzyme that catalyses the transamination of L-kynurenine (3) (Scheme 6), employing α-ketoglutarate as the amino group acceptor This reaction is unusual for a transamination because it is actually irreversible Rapid cyclisation of the initial product (32) *via* attack of the aromatic amino group on the newly formed carbonyl group produces, after loss of water, a very stable aromatic species, kynurenic acid (4) The stability of kynurenic acid means that its formation is irreversible and this drives the whole reaction sequence to the right, preventing the reverse of the initial transamination from taking place Indeed if model reactions between PLP and kynurenine are examined, very high yields of kynurenic acid are obtained

The identity of KAT enzymes has not always been clear The PLP dependent amino acid transaminases often have rather broad specificities and so assignment of their physiological role often depends on how they are examined For example one proposed KAT was found to be identical to α-aminoadipate transaminase (EC 2 6 1 39), when the two activities were eventually observed exactly to copurify However, there is no doubt that some enzymes have been isolated and characterised whose primary function is transamination of kynurenine KAT enzymes have been obtained from various sources, including yeasts, bacteria and mammals A range of amino acids have been observed to act as substrates, with the relative activities varying for each enzyme In most cases transamination of 3-hydroxykynurenine is observed giving xanthurenic acid (33) Also a variety of oxo-acids will act as amino acceptors, with α-ketoglutarate generally the most effective

Both KAT and kynureninase have very similar affinities for PLP however kynureninase activity is greatly reduced because of vitamin B_6 deficiency, while KAT is essentially unaffected This is thought to be due to localisation of KAT at the inner mitochondrial membrane, which seems to protect it from vitamin B_6 deficiency Kynureninase, however, is a cytosolic enzyme and more vulnerable

More recently, KAT from human brain has been investigated [23] In human brain, two enzymes were in fact discovered, which were denoted as KAT I and KAT II The type I enzyme was purified to homogeneity and characterised Kinetic analysis gave K_M values of 2 0 and 10 0 μmol l^{-1} for L-kynurenine and pyruvate respectively, similar to values obtained with KAT from other sources The enzyme was substantially inhibited by glutamine, phenylalanine and tryptophan It was, however, unusual in other respects Neither α-aminoadipate nor 3-hydroxykynurenine were substrates, the pH optimum was very high (pH 9 5–10) and the enzyme was larger than most other KATs Antibodies raised to the KAT I protein also confirmed that was immunologically distinct from KAT II and the rat enzyme The alternative KAT II enzyme was more similar to other KAT enzymes, although interestingly it was not inhibited by any common amino acids It was thus proposed that KAT II was primarily responsible for the synthesis of kynurenic acid under normal physiological conditions, whereas KAT I may become more important under pathological conditions Disease states certainly exist where the levels of the amino acids that inhibit KAT I are reduced, and its activity could play a more important role For example



(3) (32) (4) (33)

Scheme 6

reduced glutamine and phenylalanine levels have been observed in the brains of Huntington's disease patients, and reduced tryptophan levels in certain other virological conditions, including AIDS

The mechanism of KAT has attracted little attention, although the irreversible nature of the transamination offers some interesting possibilities for study, which are not available with other aminotransferases Although much work has been carried out on the competitive inhibition of KAT with amino acids, few irreversible inhibitors have been examined or discovered, except aminooxyacetic acid, a non-specific transaminase inhibitor This effectively inhibited KAT from rat brain with a K_i of 5 μmol l^{-1} However, there is still a need for much more specific inhibitors of KAT to allow the *in vivo* importance of kynurenine acid to be assessed

2.5 Kynurenine 3-Hydroxylase

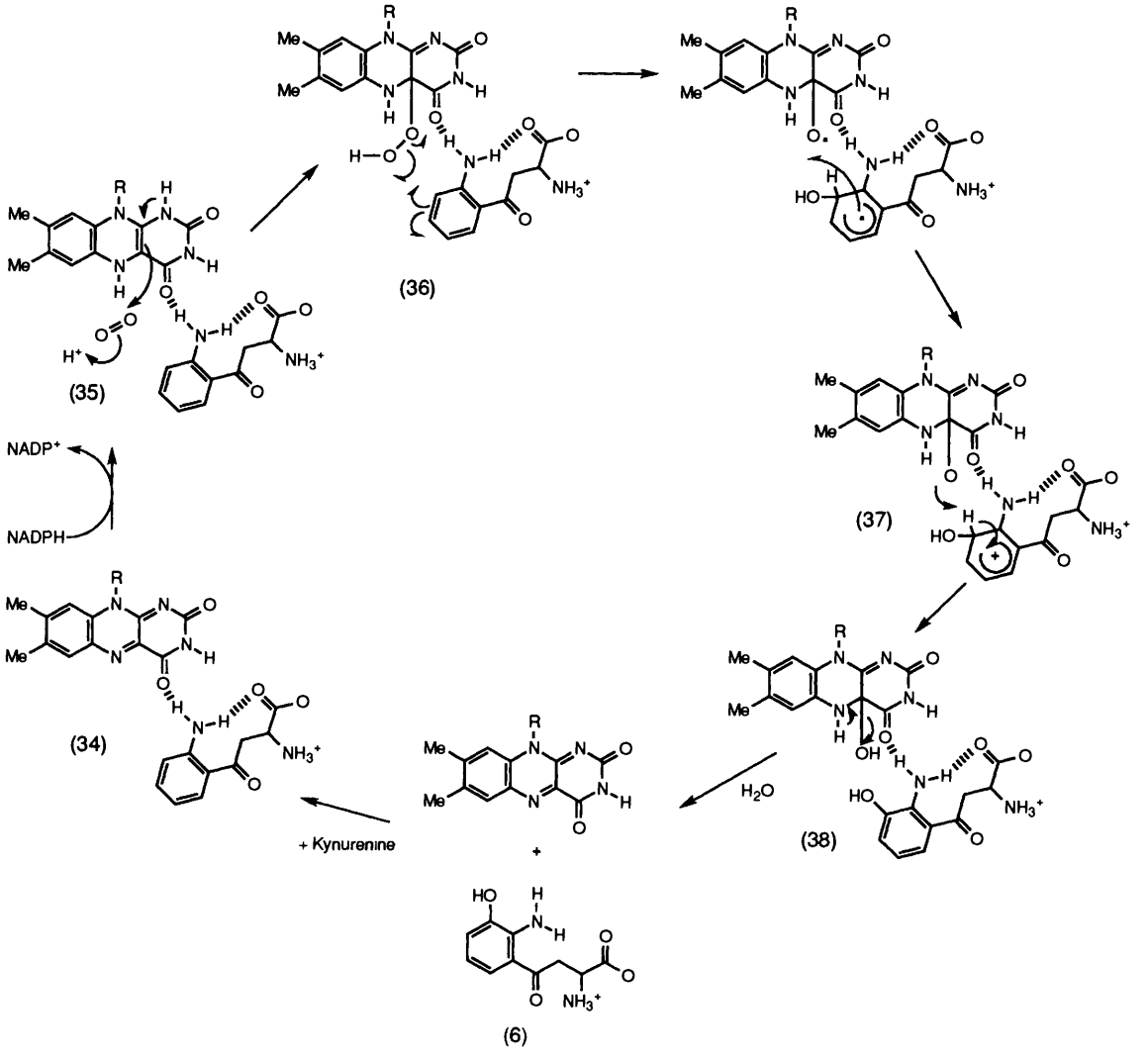
Kynurenine 3-hydroxylase (EC 1 14 13 9) catalyses the oxidation of L-kynurenine (3) at the 3-position of the aromatic ring to give 3-hydroxykynurenine (6) (Scheme 1) The enzyme is dependent on FAD (flavin adenine dinucleotide) and employs either NADH or NADPH as an electron donor With NADPH as cosubstrate, the K_M for kynurenine is slightly lower and the value of V_{max} higher, than with NADH It has been demonstrated that the oxygen incorporated into the kynurenine is derived from molecular oxygen In mammalian liver the enzyme is present in the outer membrane of the mitochondria, and although it has been purified it is unstable and of little use for mechanistic studies The purified enzyme is an oligomer with 4 moles of flavin per monomeric unit

Recently, a sensitive, precise assay has been developed for the enzyme, using L-[^{3}H]kynurenine, which has enabled studies to be carried out on crude preparations [24] Measurement of the kinetics for the enzyme catalysed reaction demonstrated that there was sequential binding of the nicotinamide cofactor and kynurenine Using stereospecifically deuteriated NADH and NADPH the stereochemistry of hydride transfer was determined A large primary deuterium isotope effect was observed only when the *pro-R* hydrogen was labelled This showed that it was this hydrogen being transferred The isotope effect was dependent on the concentration of kynurenine, which indicated that the NADH was binding before the kynurenine

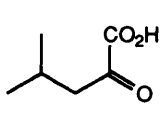
A mechanism has been proposed for the reaction, taking account of the available data on the enzyme and by analogy with the flavoprotein phenolic hydroxylases (Scheme 7) [25] Starting with a complex of the flavin and substrate (34), the first reaction is reduction of the flavin by the NADH This reduced flavin (35) then reacts with molecular oxygen to give the hydroperoxyflavin intermediate (36) The aromatic ring of the kynurenine undergoes electrophilic attack at the 3-position by the hydroperoxy group, *via* an electron transfer type mechanism, to give the cationic intermediate (37) Loss of a proton, with the isoalloxazine acting as a base, results in re-aromatisation Finally, dehydration of the hydroxyflavin (38) regenerates the original flavin cofactor and the 3-hydroxykynurenine product (6) is released The catalytic cycle may then begin again

2 5 1 Inhibition of Kynurenine 3 Hydroxylase

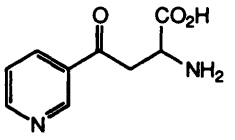
Kynurenine 3-hydroxylase from yeast is inhibited by the α-oxoacids of branched chain amino acids In particular α-oxoisocaproate (39), derived from leucine, was found to be a



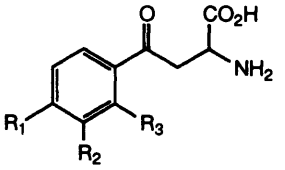
Scheme 7



(39)



(40)



(41; $R_1 = R_2 = H$, $R_3 = NO_2$)
(42; $R_1 = R_3 = H$, $R_2 = NO_2$)
(43; $R_2 = R_3 = H$, $R_1 = NO_2$)

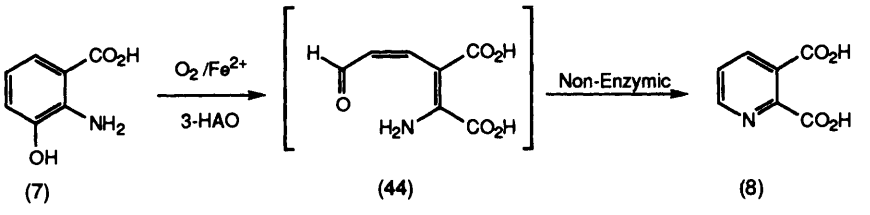
competitive inhibitor of both kynurenine (K_I = 4.2 μmol l^{-1}) and NADPH (K_I = 8.3 μmol l^{-1}). In studies to develop novel neuroprotective agents, analogues of kynurenine were prepared, including nicotinoylalanine (40) and the three isomers of nitrobenzoylalanine (41—43), and evaluated as inhibitors of kynureninase and kynurenine 3-hydroxylase.[25] By far the most potent competitive inhibitor was *m*-nitrobenzoylalanine (42), which had an IC_{50} of 0.9 μmol l^{-1}. Values for the *ortho* (41) and *para* (43) isomers were 2000 and 300 μmol l^{-1} respectively, while nicotinoylalanine (40) had an IC_{50} of 900 μmol l^{-1}. Molecular modelling studies were carried out in order to rationalise the high selectivity observed. As there are no experimental data concerning the active site structure of the enzyme, a computer model of the potential binding interactions was constructed. When *m*-nitrobenzoylalanine was bound at the model active site it was found that one of the oxygen atoms of the nitro group closely mimicked the oxygen atom of the molecular oxygen that was involved in a hydrogen bonding network. This interaction was proposed to be responsible for the high affinity and selectivity observed for *m*-nitrobenzoylalanine. The affinity for nicotinoylalanine was weaker owing to the lack of any oxygen-mimicking group, while the other two inhibitors, although they possessed a nitro group, were unable to adopt a similar orientation and therefore bound less tightly.

2.6 3-Hydroxyanthranilic Acid Oxidase (3-HAO)

In mammals, quinolinic acid is exclusively synthesised by the action of 3-hydroxyanthranilic acid oxidase (3-HAO), an iron dependent enzyme. It is an important target for drug action, as its inhibition provides the most direct method for reducing quinolinic acid levels. The initial product of the enzyme catalysed reaction, however, is not quinolinic acid but α-amino-β-carboxymuconic acid ω-aldehyde (44) (Scheme 8), which undergoes spontaneous non-enzymic cyclisation to give quinolinic acid (8). The intermediate was identified spectrophotometrically and shown to produce quinolinic acid even after protein precipitation with 90% ethanol, implying that the second step did not involve the enzyme.

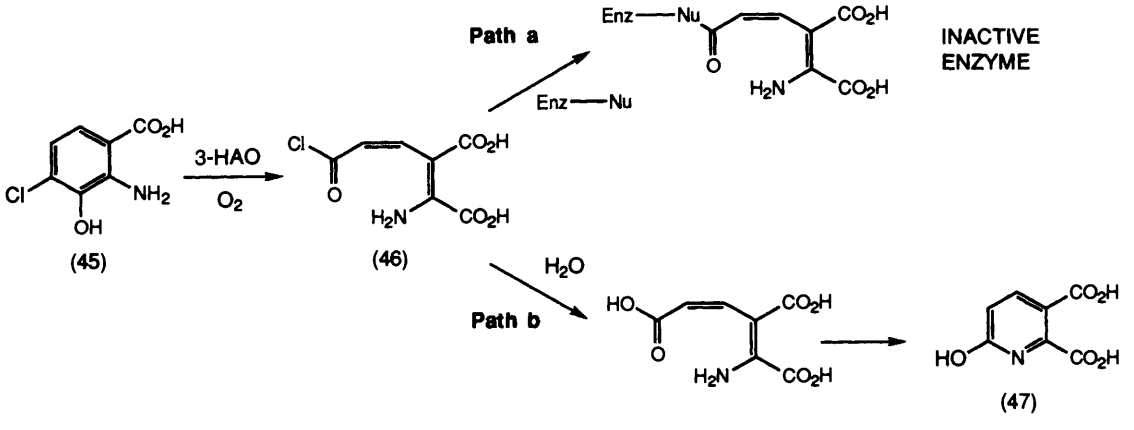
3-HAO requires both Fe^{2+} ions and reduced sulfydryl groups for its activity. Early attempts to purify 3-HAO were hampered by the apparent lability of purified enzyme preparations. However, more recently the rat liver enzyme was purified to homogeneity.[26] The protein was a monomer of 38 000 molecular weight. The K_M for the substrate was 3 μmol l^{-1} and for Fe^{2+} was 16 μmol l^{-1}. Partially purified 3-HAO from rat brain was also shown to be identical to the rat enzyme. The sequence of the rat enzyme was then employed to produce probes so that the human enzyme could be cloned and overexpressed. Further studies are awaited on the human enzyme.

Halogenated derivatives of 3-hydroxy anthranilic acid have been found to be very potent inhibitors of 3-HAO. The first example was 4-chloro-3-hydroxyanthranilic acid (45). This was first observed as a metabolite of the sweetener 6-chloro-D-tryptophan, which is converted through to this product by the enzymes of the kynurenine pathway. Initially the inhibition was thought to be irreversible and it was proposed that the inhibitor was converted through to the acid chloride (46) by 3-HAO, which then acylated the enzyme causing inactivation (Scheme 9, Path a).[27] However, later work revealed that the inhibition was in fact competitive and reversible.[28] The K_I values obtained were 190, 6 and 4 nmol l^{-1} for the 4-fluoro, 4-chloro and 4-bromo analogues, respectively. It was proposed that the inhibitors were metabolised by the enzyme giving 6-hydroxyquinolinic acid (47) (Scheme 9. Path b) as the final product, although this was not identified.



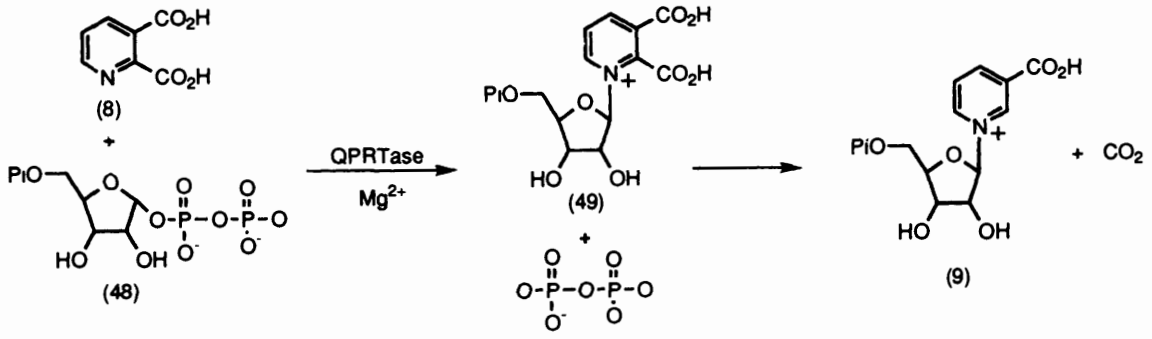
(7) (44) (8)

Scheme 8



(45) (46) (47)

Scheme 9



Scheme 10

2.7 Quinolinate Phosphoribosyltransferase (QPRTase)

Quinolinate phosphoribosyltransferase (QPRTase, EC 2 4 2 19) catalyses the conversion of quinolinic acid (8) to nicotinic acid mononucleotide (9) and carbon dioxide (Scheme 10), with the phosphoribosyl group being provided by 5-phosphoribosyl-1-pyrophosphate (48) There are a whole family of enzymes that catalyse phosphoribosyltransfer reactions,[29] which produce products that are important intermediates in amino acid and nucleotide biosynthesis However, QPRTase is unique in that it actually catalyses two chemical reactions The initial product is quinolinic acid mononucleotide (49), which must undergo a subsequent decarboxylation to give the final product It has been shown that only a single protein is involved, in contrast to the metabolism of orotic acid where a similar sequence of phosphoribosyltransfer and decarboxylation is catalysed by two separate enzymes

QPRTase is widely distributed in nature and has been isolated and purified from bacterial, plant and mammalian sources The enzymes have molecular weights of around 170 000, but are made up of a varying number of subunits Magnesium ions are required for activity in all cases There is evidence that an active site cysteine residue is required for catalysis and also essential are lysine and histidine residues Currently there is no structural information on QPRTase although the enzyme has been previously crystallised

Conflicting results have been obtained concerning the kinetic mechanism of QPRTase Essentially, there are two main possibilities Firstly the 5-phosphoribosyl-1-pyrophosphate and quinolinic acid may both bind at the enzyme active site simultaneously, giving a ternary complex, and the transfer reaction then takes place in one step Secondly, there could be a two-step process In this case 5-phosphoribosyl-1-pyrophosphate binds first and reaction takes place to eliminate phosphate and leave a reactive phosphoribosyl intermediate at the active site The quinolinic acid then binds and reacts with this intermediate to give the mononucleotide Currently, there is evidence for both mechanisms, depending on the source of the QPRTase, and no overall consensus

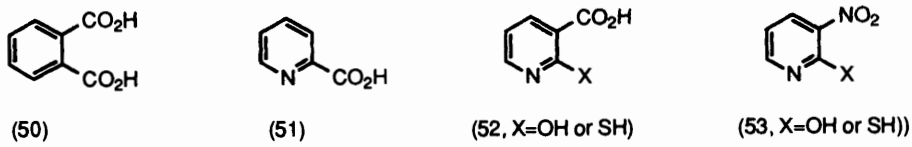
2 7 1 Inhibition of QPRTase

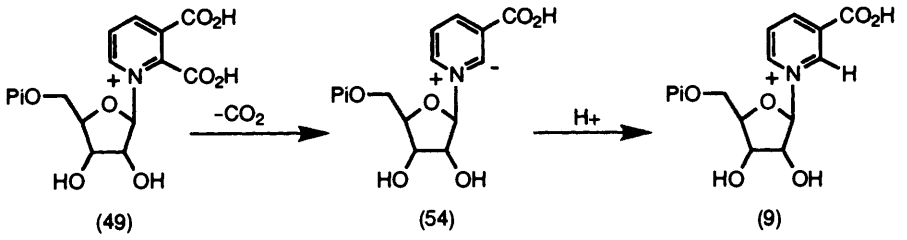
Phthalic acid (50) is a good competitive inhibitor of QPRTase from all sources, while picolinic acid (51) is also a potent inhibitor, but only for some of the enzymes, leaving others virtually unaffected None of the other pyridine carboxylic acids or dicarboxylic acids show any significant inhibition The methyl esters of picolinic acid and phthalic acid also did not inhibit, implying that the free acid is required at the 2-position for binding at the active site Nicotinic acid derivatives containing a hydroxy or a thiol group in the 2-position (52) were observed to be good competitive inhibitors of QPRTase The corresponding 3-nitro compounds (53) were even more effective [30] The inhibitory effect correlated with the degree of ionisation of the group at the 2-position, implying that negative charge at this position was an important contributor to tight binding to the enzyme Although all of the above pyridine analogues are capable of forming mononucleotides, as they contain an appropriately positioned nitrogen atom, no evidence has yet been obtained for the formation of these products A variety of pyridine nucleotides have been examined as potential inhibitors but they have been largely ineffective

2 7 2 Chemical Mechanism of QPRTase

The chemical mechanism of QPRTase is not well understood and even the order of the two chemical reactions is only assumed and has not been proven The phosphoribosyltransfer reaction is thought to occur in a similar fashion to that catalysed by other phosphoribosyltransferases, although there is actually no consensus concerning the mechanism of these enzymes They catalyse the addition of a phosphoribosyl moiety to a nucleophilic nitrogen, with overall inversion of stereochemistry at the sugar centre,[29] implying that reaction occurs *via* a direct displacement This is compatible with the ternary complex kinetic mechanism observed for some enzymes, where both reactants bind to the active site simultaneously However, the ^{14}C and ^{3}H isotope effects indicate the presence of a carbocation like (S_N1 type) transition state This carbocation intermediate must be shielded in the enzyme active site, such that attack by the nucleophilic nitrogen can only occur from one face, to give the observed stereochemistry This mechanism is more consistent with the two-step kinetic mechanism, as the carbocation can be proposed to be the enzyme-bound ribosylphosphate intermediate The alternative covalently bound intermediate, from initial attack by an active site nucleophile, would imply that reaction should proceed with overall retention of stereochemistry *i e* by two consecutive S_N2 inversions This problem remains unresolved at present

The putative second step, the decarboxylation reaction, has been assumed to have an analogous mechanism to that observed for the decarboxylation of pyridine carboxylic acids in chemical studies [31] Quinolinic acid (8) decarboxylates in aqueous hydrochloric acid (pH 1) at 95 °C, with a half life of *ca* 3 days and is the most rapidly decarboxylated of all the pyridine carboxylic acids studied Picolinic acid (51) decarboxylates 500 times slower, although *N*-methylation results in a 200-fold rate enhancement A mechanism for the reaction was proposed involving loss of carbon dioxide to give a nitrogen ylide, which was then protonated to give the product The intermediate ylide could be trapped using a suitable electrophile, in this case acetone A similar scheme can be envisaged for the decarboxylation of quinolinic acid mononucleotide (49), *via* the nitrogen ylide (54) (Scheme 11) As the spontaneous decarboxylation had been previously observed, the proposal was made that the enzyme played no part in this reaction and it occurred





(49) (54) (9)

Scheme 11

after the mononucleotide had been released from the active site. However, this seems very unlikely as the conditions employed in the chemical studies were very harsh in terms of pH and temperature, and very far removed from the physiological conditions under which the QPRTase catalysed reaction takes place. Some involvement of QPRTase would seem to be necessary, with suitable binding interactions at the active site to stabilise the anionic intermediate (54).

Currently there is only indirect evidence for the involvement of the ylide in the enzyme catalysed reaction. Inhibitor studies with 2,3-disubstituted pyridines (52,53), showed that the effectiveness of the inhibitor increased as the pK_a of the group at the 2-position decreased.[30] Binding thus appeared to improve as the amount of anionic character increased and this was proposed to be mimicking the ylide formed during the normal reaction. The substituent at the 2-position of the inhibitor was either a hydroxy group or a thiol group, so that the negative charge would be one bond away from the pyridine ring. This would seem to be as an appropriate mimic of the carboxylate group, known to be required for binding at the active site, as it was for the ylide. In both cases the negative charge is one bond distant from its position in the species it is supposed to mimic. Therefore, as yet there is no compelling evidence for the ylide intermediate in the reaction, only analogy with the uncatalysed reaction.

3 Neurochemistry

Where once the kynurenine metabolites of tryptophan were essentially biochemical curiosities, they have now risen to occupy a very prominent position in research on the causes and treatment of several of the major disorders of the central nervous system (CNS). Quinolinic acid and kynurenic acid are the two metabolites that have elicited the majority of the interest.

3.1 Quinolinic Acid

In 1981 quinolinic acid was discovered to be a selective agonist for the *N*-methyl-D-aspartic acid (NMDA) receptor.[32] As a consequence it possesses neurotoxic activity resulting from overstimulation of the receptor. Intrastriatal injections of the compound in experimental animals produce lesions which closely resemble those resulting from Huntington's disease, while intrahippocampal applications lead to convulsions and associated lesions similar to that observed in temporal lobe epilepsy.[33] When the presence of quinolinic acid was observed in the human brain it also became speculatively linked with the aetiology of several neurological diseases (see below). Another potential role for quinolinic acid is as an endogenous modulator of the NMDA receptor. However, concentrations of quinolinic acid in the human brain range from 100 nmol l^{-1} to 1 μmol l^{-1} and its relatively low affinity for the receptor make this seem unlikely. Recent studies have suggested that local concentrations at the synapse could be much higher and these are not reflected in the mean levels. These higher concentrations may then be sufficient to have some influence on receptor function.

The source of quinolinic acid found in the brain is still the object of some discussion. The enzymes of the kynurenine pathway are mainly localised in systemic tissues, in particular the liver, and most of the tryptophan in the CNS is either converted to indolamines or used in protein synthesis. Also quinolinic acid is unable to enter the brain because of its polar nature and the lack of an active transport system. However, 3-hydroxyanthranilic acid and the enzyme 3-hydroxyanthranilic acid oxidase (3-HAO) are both present in rat and human brain, and provide the most probable source.[33] Also present in the brain is QPRTase, the catabolic enzyme for quinolinic acid. The activity of 3-HAO is *ca.* 100-fold greater than that of QPRTase implying that the latter enzyme is rate limiting, although it is not clear if the relative activities are critical in maintaining quinolinic acid concentrations. Administration of 3-hydroxyanthranilic acid results in increased quinolinic acid concentrations implying that 3-HAO is not normally saturated. The two enzymes are both found in the astrocytes, but 3-HAO is more closely associated with the glutamatergic synapses such that the quinolinic acid released is in an excellent position to influence NMDA receptor function.

3.1.1 The Importance of Quinolinic Acid in Neurological Diseases

Quinolinic acid was first proposed to be an important factor in Huntington's disease, because it produced nerve cell damage very similar to that caused by the disease. The observed levels of the metabolite, however, were the same in patients with the disease as in those without. This did not bring an end to the quinolinic acid story because further work showed that its levels did increase in a range of other neurological diseases. In some very detailed studies, Heyes and coworkers have examined quinolinic acid concentrations in patients with inflammatory brain disorders.[5] The first disease investigated was AIDS, where up to two thirds of all patients suffer from neurological problems, including dysfunction of cognition, movement and sensation. In over 100 patients, quinolinic acid levels were found to be increased by between 10- and 1000-fold, and the degree of dementia correlated very well with the quinolinic acid concentration. Since this early work, patients with inflammatory brain disease resulting from a wide range of different causes have been studied. These causes include Lyme disease, poliovirus, ischemia (stroke), autoimmune diseases, sepsis and head trauma. In all cases elevated quinolinic acid levels have been observed.

The extra quinolinic acid has been proposed to originate from macrophages. These are cells employed by the non-specific immune system to neutralise foreign substances or cells and they are able to infiltrate the brain following immune system activation.[34] Macrophages have been shown to be capable of the biosynthesis of quinolinic acid, and the enzyme indoleamine 2,3-dioxygenase, which catalyses the first step in the kynurenine pathway, is actually activated by γ-interferon released during immune stimulation.[9] It has also been shown that quinolinic acid levels drop, and cognitive processes improve, when AIDS patients take the drug AZT. This drug inhibits HIV virus reproduction causing a decrease in the immunological response and therefore also in quinolinic acid synthesis.

3.2 Kynurenic Acid

Kynurenic acid is the other important neurologically active metabolite.[33] It is a broad spectrum antagonist of excitatory amino acid receptors. However it has a particularly high affinity for the strychnine-insensitive glycine recognition site of the NMDA receptor and is likely to act preferentially as a NMDA receptor antagonist. Kynurenic acid has been identified as a natural constituent of the brain and as a result it is a potential endogenous neuroprotective

agent against naturally occurring excitatory neurotransmitters such as glutamate, aspartate, sulfur containing amino acids and, most interestingly, quinolinic acid Its concentration in brain tissue varies from 6 nmol l^{-1} in mice to 1 μmol l^{-1} in humans However, it has yet to be demonstrated that the kynurenic acid concentration at the synapse is sufficient significantly to affect receptor function Increases in the concentration of kynurenic acid have been shown to lead to pharmacological effects consistent with NMDA antagonist activity One intriguing possibility that has been much debated is that kynurenic acid and quinolinic acid concentrations must be finely balanced in order to maintain the NMDA receptor activity at its optimum level It has already been discussed how increased quinolinic acid levels can cause overstimulation of the receptor, but these could also result from decreases in the levels of kynurenic acid Indeed recent work has shown that this may be the case in Huntington's disease where reduced kynurenic acid levels have been observed [35] This seemed to be the result of impaired biosynthesis

Like quinolinic acid, kynurenic acid cannot enter the brain and must be biosynthesised *in situ* Kynurenine, however, can readily cross the blood–brain barrier and is the main source of kynurenic acid *via* the enzyme kynurenine aminotransferase (KAT) KAT is also preferentially localised in the astrocytes, but the system is complex as two distinct KAT enzymes have been observed in human brain, both of which have different properties (see Section 2 4) As yet the relative localisation of KAT and 3-HAO has not been elucidated and so it is not known for certain whether synthesis of both metabolites can occur in the same astrocytes, although it would seem quite likely Interestingly no catabolic enzyme has yet been detected for kynurenic acid and so diffusion from the brain must be responsible for termination of its action [33]

4 Modulation of the Kynurenine Pathway

Pharmacological control of the CNS in a clinically useful way is a very important goal in medicinal chemistry, a particular target being control of the excitatory pathways responding to L-glutamate Modulation of the kynurenine pathway represents one strategy towards achieving this goal The activity of the NMDA receptor can be controlled by altering the relative concentrations of quinolinic acid, an agonist, and kynurenic acid, an antagonist Two approaches have been employed Firstly, inhibitors have been developed for the enzymes of the kynurenine pathway, and secondly kynurenic acid analogues have been developed that are more selective and more potent antagonists of the strychnine-insensitive glycine recognition site of the NMDA receptor

4.1 Enzyme Inhibitors

Analogues of kynurenine are attractive as potential inhibitors of kynurenine pathway enzymes The most studied of these is nicotinoylalanine (40), which is reported to inhibit both kynureninase and kynurenine 3-hydroxylase When administered to mice, following a dose of tryptophan, this produced a 19-fold increase in kynurenic acid concentration in the brain, compared to tryptophan alone [36] This dose also reduced the incidence of seizures following electroshock treatment, consistent with NMDA antagonist activity However, the doses were rather high for useful applications although the work did at least demonstrate the potential of the strategy Recently more potent inhibitors for kynurenine 3-hydroxylase have been developed (see Section 2 5) The most effective was *m*-(nitrobenzoyl)alanine (45), which had an IC_{50} of 0 9 ± 0 1 μmol l^{-1} *in vitro* [25] When this was administered to rats significant increases in the concentration of kynurenine and kynurenic acid were observed in the brain, blood and liver The kynurenic acid concentration was also increased in the hippocampal extracellular fluid, in a dose-dependent, long-lasting manner As would be expected from increases in kynurenic acid levels, anticonvulsant and sedative effects were obtained

If compounds are to be prepared which will reduce quinolinic acid levels *in vivo* the most obvious enzyme to inhibit is 3-hydroxyanthranilic acid oxidase (3-HAO), the enzyme directly responsible for its synthesis 4-Chloro-3-hydroxyanthranilic acid (45) is a potent inhibitor of the enzyme and its *in vivo* effects have been examined When the compound was administered to rats and the production of quinolinic acid in the hippocampus was assessed, a dose-dependent decrease was observed [37] The effect was reversible and the IC_{50} was 32 μmol l^{-1} Attenuation of quinolinic acid synthesis was also observed in mouse brain and blood and in poliovirus infected rhesus macaques following systemic immune activation Detailed studies using monocytes showed that following γ-interferon stimulation quinolinic acid biosynthesis was inhibited by 4-chloro-3-hydroxyanthranilic acid [38] and also by inhibitors of indolamine 2,3-dioxygenase, namely 6-chlorotryptophan and norharman (13, $R_1 = R_2 = H$) The latter had a similar, although less potent, effect, giving IC_{50} values of 0 11, 58 and 51 μmol l^{-1}, respectively The indolamine 2,3-dioxygenase inhibitors also attenuated kynurenine synthesis with IC_{50} values of 51 and 43 μmol l^{-1}, respectively, while 4-chloro-3-hydroxyanthranilic acid had no effect

These data show that inhibitors of the kynurenine pathway enzymes are indeed able to modulate quinolinic acid and kynurenic acid levels They thus have potential as anticonvulsant agents and in the treatment of various neurodegenerative diseases

5 Conclusions

In conclusion it can be seen that the kynurenine pathway of tryptophan metabolism presents a number of exciting challenges for bioorganic chemists The enzymes on the pathway catalyse some unusual and interesting transformations These range from the oxidative cleavage of tryptophan, *via* the unusual β,γ cleavage catalysed by kynureninase to QPRTase, which apparently catalyses two separate reactions Mechanistic studies are required in order to understand better the chemistry of these enzyme catalysed reactions

The neurological importance of the pathway and its metabolites is still an issue of some debate Although it is clear that quinolinic acid levels are elevated in patients with inflammatory brain diseases, it is still to be demonstrated that this is a cause rather than a consequence of the neurological problems The role of chemists here is to produce potent and specific inhibitors for the enzymes on the pathway, not only as potential therapeutic agents, but also as pharmacological tools to elucidate the importance of the pathway in greater detail Although some progress has been made in this area there is a great deal of work still to be done

5 References

1 G W Beadle, H K Mitchell and J F Nyc, *Proc Natl Acad Sci USA* 1947, **33**, 155
2 J K Yeh and R R Brown, *J Nutr*, 1977, **107**, 261
3 T W Stone, ed, 'Quinolinic Acid and the Kynurenines,' CRC Press, Boca Raton, Florida, 1989
4 T W Stone, *Pharmacol Rev*, 1993, **45**, 309
5 M P Heyes, *Biochem Soc Trans*, 1993, **21**, 83
6 G Shutz, E Chow and P Feigelson, *J Biol Chem*, 1972, **247**, 5333
7 J M Leeds, P J Brown, G M McGeehan, F K Brown and J S Wiseman, *J Biol Chem*, 1993, **268**, 17781
8 M Nakagawa, H Watanabe, S Kodato, H Okajima, T Hino, J L Flippen and B Witkop, *Proc Natl Acad Sci USA*, 1977, **74**, 4730
9 J M Carlin, Y Ozaki, G I Byrne, R R Brown and E C Borden, *Experientia*, 1989, **45**, 535
10 M Sono, *Biochemistry*, 1989, **28**, 5400
11 N Eguchi, Y Watanabe, K Kawanishi, Y Hashimoto and O Hayaishi, *Arch Biochem Biophys*, 1984, **232**, 602
12 A C Peterson, A J LaLoggia, L K Hamaker, R A Arend, P L Fisette, Y Ozaki, J A Will, R R Brown and J M Cook, *Med Chem Res*, 1993, **3**, 473, A C Peterson, M T Migawa, M J Martin, L K Hamaker, K M Czerwinski, W Zhang, R A Arend, P L Fisette, Y Ozaki, J A Will, R R Brown and J M Cook, *Med Chem Res*, 1994, **3**, 531
13 S G Cady and M Sono, *Arch Biochem Biophys*, 1991, **291** 325
14 M Sono and S G Cady, *Biochemistry*, 1989, **28**, 5392
15 J Seifert and J E Casida, *Pest Biochem Phys*, 1979, **12**, 273 J Seifert and T Pewnim, *Biochem Pharm*, 1992, **44**, 2243, T Pewnim and J Seifert, *Eur J Pharm*, 1993, **3**, 237

16 M M Palcic, M Antoun, K Tanizawa, K Soda and H G Floss, *J Biol Chem*, 1985, **260**, 5248
17 G M Kishore, *J Biol Chem*, 1984, **259**, 10669
18 G S Bild and J C Morris, *Arch Biochem Biophys*, 1984, **235**, 41
19 R S Phillips and R K Dua, *J Am Chem Soc*, 1991, **113**, 7385
20 R K Dua, E W Taylor and R S Phillips, *J Am Chem Soc*, 1993, **115**, 1264
21 J P Whitten, C L Barney, E W Huber, P Bey and J R McCarthy, *Tetrahedron Lett*, 1989, **30**, 3549
22 F C Ross, P D Leeson and N P Botting, unpublished results
23 H Baran, E Okuno, R Kido and R Schwarz, *J Neurochem*, 1994, **62**, 730
24 J S Wiseman and J S Nichols, *Anal Biochem*, 1990, **184**, 55
25 R Pellicciari, B Natalini, G Costantino, M R Mahmoud, L Mattoli, B M Sadeghpour, F Moroni, A Chiarugi and R Carpenedo, *J Med Chem*, 1994, **37**, 647
26 E Okuno, C Kohler and R Schwarz, *J Neurochem*, 1987, **49**, 771
27 C J Parli, P Krieter and B Schmidt, *Arch Biochem Biophys*, 1980, **203**, 161
28 J L Walsh, W P Toss, B K Carpenter and R Schwarz, *Biochem Pharm*, 1991, **42**, 985
29 W D L Musick, *CRC Crit Rev Biochem*, 1981, **11**, 1
30 L Kalikin and K C Calvo, *Biochem Biophys Res Commun*, 1988, **152**, 559
31 G E Dunn, G K J Lee and H Thimm, *Can J Chem*, 1972, **50**, 3017
32 T W Stone and M N Perkins, *Eur J Pharmacol*, 1981, **72**, 411
33 R Schwarcz, *Biochem Soc Trans*, 1993, **21**, 77
34 K Saito, J S Crowley, S P Markey and M P Heyes, *J Biol Chem*, 1993, **268**, 15496, K Saito, T S Nowak, K Suyama, B J Quearry, M Saito, J S Crowley, S P Markey and M P Heyes, *J Neurochem*, 1993, **61**, 2061
35 D Jauch, E M Urbanska, P Guidetti, E D Bird, J P G Vonsattel, W O Whetsell and R Schwarcz, *J Neurol Sci*, 1995, **130**, 39
36 J H Connick, G C Heywood, G J Sills, G G Thompson, M J Brodie and T W Stone, *Gen Pharmacol*, 1992, **23**, 235
37 J L Walsh, H Q Wu, U Ungerstedt and R Schwarcz, *Brain Res Bull*, 1994, **33**, 513
38 K Saito, C Y Chen, M Masana, J S Crowley, S P Markey and M P Heyes, *Biochem J*, 1993, **291**, 11