AN INVESTIGATION INTO THE BIOCHEMICAL CHANGES IN TOURETTE SYNDROME WITH A POTENTIAL FOR PHARMACOLOGICAL MANIPULATION

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Dedicated to my daughter Kulshini

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Biochemical Changes In Tourette Syndrome And Associated Conditions With A Potential For Pharmacological Manipulation

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Summary

Kynurenine (KYN) is the first stable metabolite of the kynurenine pathway, which accounts for over 95% of tryptophan metabolism. Two previous studies by this research group reported elevated plasma KYN in Tourette syndrome (TS) patients when compared with age and sex matched controls and another study showed that KYN potentiated 5-HT_{2A}-mediated head-shakes (HS) in rodents. These movements have been suggested to model tics in TS. This raised the questions how KYN acts in eliciting this response and whether it is an action of its own or of a further metabolite along the kynurenine pathway. In the liver, where most of the kynurenine pathway metabolism takes place under physiological conditions, the first and the rate limiting enzyme is tryptophan-dioxygenase (TDO) which can be induced by cortisol. In extrahepatic tissues the same step of the pathway is catalyzed by indoleamine-dioxygenase (IDO), which is induced by cytokines, predominantly interferon-γ (INF-γ). Plasma neopterin, which shows parallel increase with KYN following immune stimulation, was also found elevated in one of these studies positively correlating with KYN.

In the present work animal studies suggested that KYN potentiates and quinolinic acid (QUINA) dose dependently inhibits the 5-HT_{2A}-mediated HS response in mice. The potentiating effect seen with KYN was suggested to be an effect of KYN itself. Radioligand binding and phosphoinositide (PI) hydrolysis studies were done to explore the mechanisms by which kynurenine pathway metabolites could alter a 5-HT_{2A}-receptor mediated response. None of the kynurenine pathway metabolites tested showed direct binding to 5-HT_{2A}-receptors. PI hydrolysis studies with KYN and QUINA showed that KYN did not have any effect while QUINA inhibited 5-HT_{2A}-mediated PI hydrolysis.

Plasma cortisol determination in TS patients with elevated plasma KYN did not show elevated plasma cortisol levels, suggesting that the increase of plasma KYN in these TS patients is unlikely to be due to an increased TDO activity induced by increased cortisol. Attention deficit hyperactivity disorder (ADHD) is commonly associated with TS. Salivary cortisol detected in a group of children primarily affected with ADHD showed significantly lower salivary cortisol levels when compared with age and sex matched controls. Plasma tryptophan, KYN, neopterin, INF-γ and KYN/tryptophan ratio and night-time urinary 6-sulphatoxymelatonin (aMT6s) excretion measured in a group of TS patients did not show any difference in their levels when compared with age and sex matched controls, but TS patients failed to show the expected positive correlation seen between plasma INF-γ, neopterin and KYN and the negative correlation seen between plasma KYN and night-time urinary aMT6s excretion seen in healthy controls.

The relevance of the kynurenine pathway, melatonin secretion and cortisol to Tourette Syndrome and associated conditions and the mechanism by which KYN and QUINA alter the 5- HT_{2A}-receptor mediated HS response are discussed.

Key words: Tourette Syndrome, kynurenine, quinolinic acid, head-shakes, Interferon-γ, neopterin, cortisol.

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ABBREVIATIONS

AA	anthranilic acid
aMT6s	6-sulphatoxymelatonin
ADHD	Attention deficit hyperactivity disorder
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate
APA	American Psychiatry Association
ASOT	antistreptolysin O titre
BBB	blood brain barrier
B_{max}	maximum number of binding sites
CD	Conduct disorder
CNS	central nervous system
CSF	cerebrospinal fluid
DOI	1-(2,5-dimethoxy-4-iodophenyl)2-amino-propane
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
DSMIIIR	Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition, revised
EAA	excitatory amino acid
ELISA	enzyme linked immunosorbent assay
GABA	γ-aminobutyric acid
GABHS	Group A β haemolytic Streptococci/Streptococcal
GTP	guanosine triphosphate
GTPC1	guanosine triphosphate cyclohydrolase-1
5-HT	5-hydroxytryptamine
5-HTP	5-hydroxytryptophan
5-HIAA	5-hydroxyindoleacetic acid
i.c.v.	intracerebroventricular
IDO	indoleamine-2-3-dioxygenase
I-1-P	inositol-4-monophosphate
IL	interleukin
INF	interferon
i.p.	intraperitoneal
IP3	inositol 1,4,5-trisphosphate
K_d	dissociation constant
K_i	inhibition constant
KYN	kynurenine

KYNA	kynurenic acid
LH	leutinising hormone
NA	nicotinic acid
NAD	nicotinamide adenine dinucleotide
NMDA	N-methyl-D-aspartate
OCD	Obsessive compulsive disorder
OCB	obsessive compulsive behaviour
OCD/B	Obsessive compulsive disorder/obsessive compulsive behaviour
ODD	Oppositional defiant disorder
3-OHAA	3-hydroxy-anthranilic acid
3-OHKYN	3-hydroxykynurenine
QUINA	quinolinic acid
PA	picolinic acid
PANDAS	Paediatric autoimmune neuropsychiatric disorder associated with Streptococcal infection
PET	Positron emission tomography
PI	phosphatidylinositol 4,5-bisphosphate
s.c.	subcutaneous
SEM	standard error of the mean
SPECT	single photon emission computed tomography
TNFα	tumour necrosis factorα
TH	T helper
TS	Tourette syndrome
YGTSS	Yale Global Tic Severity Scale
XA	xanthurenic acid

GENERAL INTRODUCTION

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1. General

Tourette syndrome (TS) is the most severe of a group of human tic disorders. It was first described by the French Physician Gilles de la Tourette in 1885 and it was named after him. At present the name is shortened for convenience and is commonly known as TS. Though it was known as a relatively rare neuropsychiatric disorder it is being more frequently diagnosed over recent years (Kurlan, 1997; Robertson & Stern, 1997). It shows comorbidity with a number of other neuropsychiatric conditions. Several studies have shown a genetic link to its causation. However the aetiology of the disorder is not clear and the present treatments, mainly with neuroleptics have considerable disadvantages resulting in high rejection rates (Robertson *et al.*, 1990). Biochemical changes detected in TS may direct towards an aetiological mechanism, which will help to suggest more specific and efficient forms of treatment. The work reported in this thesis explores some of the biochemical changes in TS and considers the implications they might have on research into the aetiology and treatment of TS.

2. Tourette Syndrome

Gilles de la Tourette syndrome is named for George Gilles De La Tourette, who published his first description of the condition in 1885. TS is one of the four tic disorders classified in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric association (APA), 1995). The others are chronic motor or vocal tic disorder, transient tic disorder and tic disorder not otherwise specified. TS is described as a relatively rare neuropsychiatric disorder, with a prevalence of 4-5 individuals per 10,000 population (APA, 1995), but considering the more recent studies, Kurlan (1997) has suggested it to be a more common disorder. TS has been widely reported in diverse racial and ethnic groups. It is 1.5-3 times commoner in males than in females (Apter *et al.*, 1992). TS is characterised by multiple motor and one or more vocal tics of more than a years duration and an age of onset before 18 years. The age of onset of TS ranges from 2-15 years and a mean age of seven years is most commonly reported (Robertson, 1989). In 96% of cases symptoms have appeared by the age of eleven years (Comings & Comings, 1985).

The following diagnostic criteria are recommended by the APA in the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (APA, 1995):

* Both multiple motor and one or more vocal tics have been present at some time during

the illness, although not necessarily concurrently.

* The tics occur many times a day (usually in bouts) nearly every day or intermittently throughout a period of more than one year, and during this period there was never a tic free period of more than 3 consecutive months.
* The disturbance causes marked distress or significant impairment in social, occupational or other important areas of functioning.
* The onset is before 18 years.
* The disturbance is not due to the direct physiological effects of a substance (stimulants) or a general medical condition (e.g. Huntington's disease or postviral encephalitis).

2.1 Characteristics of tics in TS

A tic is a sudden, rapid, recurrent, nonrhythmic, stereotyped motor movement or vocalisation (APA, 1995). The most frequent initial symptom is tics involving the eyes (eye blinking) occurring in 38-59% of cases (Lees *et al.*, 1984; Comings & Comings, 1985). Other initial symptoms may be motor tics involving head or face or vocal tics in the form of throat clearing: 12-37% (Comings & Comings, 1985; Regeur *et al.*, 1986). 2-6% of the GTS patients may present initially with coprolalia (a complex vocal tic involving the uttering of obscenities) or a tic in the form of mouth opening (Comings & Comings, 1986). When considering the cumulative lifetime tic symptoms, those involving the face are the most frequent (94-97%), and others may be involving the head, neck and shoulder (89-92%), upper arms (51-81%), legs (40-55%) and body (41-54%) (Robertson, 1989). In about 50% of patients, more complex movements such as touching of people or objects, stamping, jumping, hitting or striking, smelling of the hands or objects and complexities of gait have been shown (Shapiro *et al.*, 1978; Lees *et al.*, 1984; Shapiro *et al.*, 1973; Robertson, 1989). In most cases the onset of vocal tics usually occur later than the motor tics. The common vocal tics are grunting, coughing, throat clearing, barking, snorting, explosive noises, screaming, word accentuation, humming, hissing, clicking, colloquial emotional exclamations, low and high pitched noises and inarticulate sounds (Robertson, 1989). Coprolalia was first described by Gilles de la Tourette in 1885, but does not occur in all TS patients and is not a diagnostic criterion. Coprolalia usually has an age of onset of 13-14.5 years and later disappears in up to a third of patients. Copropraxia (inappropriate obscene gestures) has also been demonstrated in TS patients (Lees *et al.*, 1984; Robertson *et al.*, 1988).

TS symptoms appear to be aggravated by anxiety, stress, boredom, fatigue and excitement. Stress has been proposed to be a precipitating factor of the illness (Robertson, 1989) and chronic stress is present in some sufferers (Eisenberg *et al.*, 1959; Stevens,

1964; Faux, 1966). However sleep, alcohol, orgasm, fever, relaxation or concentration on an enjoyable task usually lead to a transient disappearance of symptoms (Robertson, 1989). Tics are shown to be present during sleep (Glaze *et al.*, 1982; Incagnoli & Kane, 1983).

2.2 Other neuropsychiatric conditions and genetics associated with TS

Studies conducted on TS considering it as a disorder of both movement and behaviour, have shown comorbidity with attention deficit hyperactivity disorder (ADHD), obsessive-compulsive behaviours (OCB), self-injurious behaviours, learning disorders and depression (Robertson *et al.*, 1993; Sandor, 1995; Abwender *et al.*, 1996). The most commonly associated symptoms of TS are obsessions and compulsions. Hyperactivity, distractibility and impulsivity are also relatively common. There is a high incidence of obsessive compulsive disorder (OCD) in individuals having TS with estimates ranging from approximately 35-50%. The incidence of TS in OCD is however about 5-7%. About 20-30% of individuals with OCD have reported current or past tics. The rate of OCD in first degree biological relatives of individuals with TS is higher than that in general population (APA, 1995).

Both TS and ADHD are best visualised as spectrum disorders: that is studies of both the patients and their relatives show that TS and ADHD are associated with a wide range of comorbid disorders, including drug and alcohol abuse, OCB, inappropriate sexual behaviour, conduct and oppositional defiant disorder, depression, anxiety, sleep, learning and other disorders (Biederman *et al.*, 1991; Comings, 1995).

Cohen *et al.*, (1982) have suggested a multifactorial aetiology to the phenotypic expression of TS, probably with a complex interaction of genetic, neuroanatomical, environmental, behavioural and emotional factors. Family (Eldridge *et al.*, 1977; Kidd *et al.*, 1980; Pauls *et al.*, 1990), twin (Price *et al.*, 1985; Walkup *et al.*, 1988) and adoption (Shapiro *et al.*, 1988) studies have demonstrated a genetic basis for TS. Swedo and Kiessling (1993,1994) have suggested group A β haemolytic Streptococcal (GABHS) involvement in TS together with a genetic vulnerability to the disorder. They have suggested the hypothesis that when genetically vulnerable children are exposed to a GABHS infection, antibodies are produced that mistakenly recognise cells within the basal ganglia and cause an inflammatory response, which may be manifested as TS.

Several groups have conducted segregation studies to determine the mode of inheritance of TS (Walkup *et al.*, 1996). Some of these studies have been consistent with autosomal dominant inheritance with reduced penetrance (Devor, 1984; Pauls *et al.*, 1990; Curtis *et*

al., 1992; Cavallini *et al.*, 1995) and some studies could not rule out multifactorial polygenic inheritance (Kidd & Paul, 1982; Comings *et al.*, 1984).

A number of genes have been tested as candidate genes of TS, using association and linkage strategies. Comings *et al.*, (1991) reported an increase in the frequency of the A1 allele of a Taq1 restriction fragment-length polymorphism at the dopamine D_2 receptor locus (DRD2) in patients with TS, ADHD, alcoholism, post-traumatic stress disorder and autism, compared with normal controls. But later studies have failed to replicate this finding in TS (Gelertner *et al.*, 1994; Brett *et al.*, 1995). An association between homozygosity of one allele of the Msc1 polymorphism in the first exon of dopamine D_3 gene and TS has also been reported (Comings *et al.*, 1993). This finding could not be replicated by the linkage and association studies done later (Brett *et al.*, 1993). The Dopamine D_4 receptor gene (DRD4) has been excluded from having a link to TS assuming an autosomal dominant model and locus homogeneity (Pakstis *et al.*, 1991), but after identifying polymorphisms in the gene, Grice *et al.*, (1996) showed a transmission distortion of a DRD4 allele. Studies done later using an autosomal dominant model with reduced penetrance and model free method have failed to show any evidence of DRD4 having a link to TS (Barr *et al.*, 1998; Edwards *et al.*, 1992). Other genes of the dopaminergic system tested as candidates having a linkage to TS include the dopamine receptor D_5 (Brett *et al.*, 1995; Barr *et al.*, 1998), dopamine receptor D_1 (Brett *et al.*, 1995), dopamine β hydroxylase (Brett *et al.*, 1995; Comings *et al.*, 1996), tyrosine hydroxylase (Brett *et al.*, 1995; Barr *et al.*, 1998; Comings *et al.*, 1995), tyrosine 46 (Brett *et al.*, 1995; Comings *et al.*, 1996) and dopamine transporter (Comings *et al.*, 1996). These works have not shown clear evidence of linkage of any of these genes to TS. 5-HT1A and tryptophan oxygenase genes have been tested and excluded from having a linkage (Brett *et al.*, 1995). The $5ht_7$ gene also has been tested, but no evidence of linkage found (Gelernter *et al.*, 1995). Even though several positive findings with candidate genes have been reported, they are not convincingly replicated. Therefore despite an intense international effort having been made to test the genes involved in the aetiology of TS, convincing evidence for a linkage to TS is not yet present.

2.3 Pathophysiology of TS

The pathophysiology of TS is not clear. It is important to detect the neuroanatomical location of the dysfunction and neurochemical abnormalities involved in TS, to find more specific and efficient therapies against it with less adverse effects.

PET with [^{18}F] fluorodeoxy – D - glucose has shown increased glucose metabolism in the basal ganglia of TS patients together with differences in the frontal and temporal

lobes bilaterally (Chase *et al.*, 1984). Later reduced glucose metabolism has been shown in the striatum, cingulate cortex and the insular cortex of TS patients (Chase *et al.*, 1986). Further studies have shown altered metabolism in several brain areas (striatum, thalamus, prefrontal cortex, parietal cortex, cingular cortex, insula and parietal association area) in TS patients making it more difficult to localise a single area (Stoetter and Braun, 1991).

The role of the amygdaloid complex in TS has been suggested as playing a key role in TS (Danitza, 1991). Improvement of tics and OCB has been shown after limbic leucotomy in a patient with TS, OCD and obsessional slowness. The preoperative PET (positron emission tomography) scan showed hypermetabolism in the caudate nuclei, which normalised after operation. It was proposed that the beneficial effect of this operation be mediated by disruption of abnormal neural activity in the basal ganglion-thalamocortical loops (Sawle *et al.*, 1992). SPECT (single-photon emission computed tomography) done on five pairs of monozygotic twins concordant for the diagnosis of TS, but discordant for symptom severity has shown greater binding of [I^{123}] Iodobenzamide (highly selective ligand for D_2 receptors with high affinity (Singhaniyom *et al.*, 1988)) to D_2 receptors in the caudate nucleus in all five of the more affected TS patients, compared with their less affected siblings (Wolf *et al.*, 1996). Functional brain imaging studies on persons with OCD have shown the involvement of the head of the caudate nucleus together with its projections from orbitofrontal and cingulate cortices (Insel, 1992). Considering these findings together Wolf *et al* (1996) further suggested that TS and OCD are overlapping neurobehavioral conditions. But a preliminary SPECT analysis of D_2 receptors in TS patients using [I^{123}] Iodobenzamide as the ligand has failed to show an increase in receptor binding (George *et al.*, 1991).

Dysfunction of central neurotransmitter systems have been implicated in the pathophysiology of TS based upon the response of patients to specific medications, studies of neurotransmitters or their metabolites in the CSF and analysis of post-mortem brain tissue. So far dopaminergic, serotonergic, noradrenergic, gamma aminobutyric acid and opioid systems have shown abnormalities.

The dopaminergic hypothesis, which proposes a dopaminergic over-activity as underlying the symptomatology seen in TS, is an area that has received the greatest amount of support so far. The dopamine hypothesis may suggest an excessive amount of dopamine or an increased sensitivity to it. The major metabolite of dopamine, homovanilic acid has shown to be lower in the CSF of TS patients (Cohen *et al.*, 1978). Later normal concentrations of dopamine, homovanilic acid and 3,4-dihydroxy-phenylacetic acid were shown in post-mortem striatal samples of TS patients, when compared with that of healthy individuals (Singer *et al.*, 1991). These findings suggest

that there is no excessive production or release of dopamine in TS patients. The brain imaging studies mentioned above showing the involvement of the caudate nucleus in TS show abnormalities in D_2 receptor binding. Further evidence of abnormalities in striatal dopamine neurotransmission in TS has been found by examining dopamine transporters with SPECT, where an increase in presynaptic dopamine transporters in the striatum has been shown (Malison *et al.*, 1995). The non-specific dopamine D_2 receptor antagonists such as haloperidol, pimozide and fluphenazine suppress motor and phonic tics in most TS patients (Jankovic, 1984). Improvement of symptoms have been shown without disabling side effects in four TS patients, by treating with a D_2 receptor antagonist: piquindone, designed via a three dimensional model of dopamine (Sarita *et al.*, 1996). D_2 receptor density has been shown to be normal (Brook *et al.*, 1992) or variable (low, normal and elevated) in unmedicated TS patients (Singer *et al.*, 1992) in two different studies making it difficult to determine the role of D_2 receptors in TS.

There is limited evidence for the involvement of the central noradrenergic system in TS. The α_2–adrenergic agonist clonidine has been shown to be effective in the treatment of TS (Leckman *et al.*, 1985) and is used as a therapy at present in a minority of patients. Reduced noradrenaline levels in the ventral thalamus (Anderson *et al.*, 1992) and reduced excretion of noradrenergic metabolites in urine (Baker *et al.*, 1991) have been shown in TS patients. Noradrenergic metabolites in the CSF have been shown to be unchanged (Butler *et al.*, 1979).

The GABAergic system has been implicated in TS as tics have been suppressed by treatment with the benzodiazepine, Clonazepam (Gonce & Barbeau, 1977). But the blood and CSF levels of GABA have been shown to be normal in TS patients (Van Woert *et al.*, 1982). A highly specific marker for GABAergic interneurons, glutamate decarboxylate activity, has been shown to be normal in the post-mortem brains of TS patients (Singer *et al.*, 1990).

In post-mortem studies dynorphin A[1-17] immunoreactivity was shown to be decreased in striatal fibres projecting to the globus pallidus externa in patients with TS (Haber *et al.*, 1986). Dynorphin A[1-8] has been shown to be increased in the cerebrospinal fluid of patients and the concentration of opiate has shown a correlation with the severity of obsessive compulsive symptoms, but not with tic severity (Leckman *et al.*, 1988). Sandyk and Bamford (1988) have identified the striatum (caudate nucleus and putamen) and the limbic-hypothalamic network as the sites of the abnormalities in TS and suggested a relationship between the 5-HT and the opioid systems: when a disequilibrium occurs in their interactions, the behavioural and psychological manifestations of TS occur.

Altered serotonin function has been suggested as a cause of many neuropsychiatric conditions including TS (Schwitzer & Friedhoff, 1988; Comings, 1990b). Head shakes and wet dog shakes in rodents have been classified as 5-HT_{2A} receptor mediated central actions (Kennett & Curzon, 1991). It has also been suggested that head shakes and wet-dog shakes in animals may model human tics seen in TS (Handley and Dursun, 1992). The metabolite of 5-HT, 5-HIAA has been shown to be lower in the CSF of TS patients (Butler *et al.*, 1979). Reduced 5-HT and 5-HIAA concentrations have been shown in several cortical regions of the post-mortem brains from TS patients along with an increase in 5-HT_{1A} and 5-HT_{2A} receptor binding, with the 5-HT_{2A} receptor binding most increased in the primary motor cortex (Akbari *et al.*, 1993).

Comings (1990a) demonstrated lower levels of blood tryptophan in TS patients, and he suggested higher synthesis or hyper-inducibility of the enzyme tryptophan oxygenase, which is involved in the metabolism of tryptophan (Knox & Auerbach, 1955). Based on the lower blood tryptophan levels observed in TS patients, Comings (1990a) also suggested that TS patients may have increased blood kynurenine concentrations, correlating with lower tryptophan and 5-HT levels, as greater proportion of tryptophan would be diverted towards kynurenine production. The kynurenine pathway (see figure 1) which accounts for about 90% of tryptophan metabolism has several neuroactive metabolites (Lapin, 1978; Stone & Perkin, 1981; Shwarcz *et al.*, 1984). Of the kynurenine pathway metabolites, a generalised increase of plasma kynurenine levels have been shown in TS patients when compared with healthy controls (Dursun *et al.*, 1994; Gaynor *et al.*, 1997). In the liver the first enzyme of the kynurenine pathway is the cortisol-inducible tryptophan dioxygenase (Knox & Auerbach, 1955), while in extrahepatic tissues this is replaced by the cytokine-inducible indoleamine dioxygenase (Takikawa *et al.*, 1986; Yoshida *et al.*, 1986). Cytokynes also induce neopterin formation (Huber *et al.*, 1984). Of the cytokines mainly interferon-γ (INFγ) is known to cause induction of IDO and the pteridine synthesising enzyme, GTP-cyclohydrolase 1 causing parallel increases of kynurenine and neopterin in clinical studies done on various disease conditions (Weil *et al.*, 1983). INFγ has been shown to cause induction of IDO activity in murine cloned macrophages and microglial cells, causing detectable increases in 3-hydroxyanthranilic acid and quinolinic acid levels, supporting the notion that activated invading macrophages may constitute one of the major sources of cerebral neuroactive kynurenines during inflammation (Alberti-Giani *et al.*, 1996). See section on cytokines for more details on their role in immune functions.

Immune mediation of TS has become increasingly implicated in the pathophysiology of at least a subgroup of the clinical phenotype. Gaynor *et al* (1997) have suggested the

activation of cellular immune processes within the nervous system as a possible explanation for the rise in plasma kynurenine levels, associated with increased plasma neopterin levels observed in TS patients. A role for streptococcal infection as one infectious provocation to the nervous system in TS and OCD was supported by the findings of several American research groups (Allen *et al.*, 1995; Giedd *et al.*, 1996; Tucker *et al.*, 1996). Several researchers continue systematic efforts at showing at least a subgroup of TS patients with post-streptococcal aetiology. Several neuropsychiatric disorders including TS have been classed as Paediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infections (PANDAS), by the National Institute of Mental Health, Child Psychiatry Branch (1997). Studies by Swedo, Kiessling and Singer have shown significant increases in the incidence of antineuronal antibody positivity and the levels of serum antineuronal antibodies against parts of basal ganglia, together with at least one marker of streptococcal infection present, in children with TS, tic disorders and obsessive-compulsive symptoms (Keissling *et al.*, 1993,1994; Swedo *et al.*, 1997; Singer *et al.*, 1998). See section on immune reactions and cytokines for further details on the immune hypothesis.

Melatonin, which is the major hormone of the pineal gland and a metabolite of 5-HT, may have a relationship with TS. Tumour in the pineal gland has been associated with TS (Lakke and Wilmink, 1985). Symptoms of TS are found to be seasonal, tending to wane during summer months, a period in which melatonin secretion is elevated compared with the winter months (Goetz, 1986). Melatonin shows interactions with several neurotransmitter systems including 5-HT (Eison et al., 1995; Miguez *et al.*, 1996; Miguez *et al.*, 1997; Golombek *et al.*, 1996). Acute administration of melatonin and related agonists has shown inhibition of DOI-induced head-shakes in rats (Eison *et al.*, 1995). Melatonin in supraphysiological concentrations has caused competitive inhibition of TDO, suggesting that melatonin could inhibit catabolism of tryptophan resulting in greater availability of tryptophan for uptake into the brain (Walsh *et al.*, 1994).

2.4 Treatment issues in TS

At present neuroleptics are the most effective and most frequently used drugs for the treatment of TS and other tic disorders. Haloperidol was the first neuroleptic drug approved by the Food and Drug Administration, USA (FDA) in 1969 for the treatment of adults with TS and in 1978 for the children with TS. Its effectiveness in the treatment of tics is well documented (Corbett *et al.*, 1969; Corbett and Turpin, 1990). However adverse effects of it limit its use and new drugs with less adverse effects and greater efficacy are needed. Pimozide, another neuroleptic was approved by the FDA for use in

TS in 1984. With these two neuroleptics a rejection rate of up to 80% has been observed due to unacceptable side effects (Robertson *et al.*, 1990). The selective D_2 antagonist, sulpiride has shown a net clinical benefit (Robertson *et al.*, 1990) and is used at present for the treatment of TS. Risperidone, which is an atypical neuroleptic with potent 5-HT_2, D_2 and noradrenergic antagonism is shown to be effective in the treatment of TS (Bruun and Budman, 1996; Lombroso *et al.*, 1995; Shulman *et al.*, 1995) and is used by several clinicians at present for the treatment of TS. Clonidine appears to be effective in a smaller proportion of TS patients than is haloperidol and the extent of improvement is shown to be less (Leckman *et al.*, 1985; Shapiro & Shapiro, 1982), but it is shown to reduce the tic frequency (Cohen *et al.*, 1980). The Ca^{++} channel blocker, nifedipine has been shown to be effective in TS (Goldstein, 1984; Berg, 1985). Transdermal nicotine patch alone and together with neuroleptics have shown reduction of tic severity in TS patients (Silver *et al.*, 1996; Dursun *et al.*, 1994). Nicotine acts on cholinergic nicotinic-specific receptors and has effects on several neurotransmitter systems (Nisell *et al.*, 1997; Lendvai *et al.*, 1996; Summers *et al.*, 1996). Nicotine is also known to have effects on the immune responses (Geng *et al.*, 1995; Madretsma *et al.*, 1996). A review by Sanberg *et al.*, (1997) suggests that nicotine could serve as an effective adjunct to neuroleptic therapy in TS. One of the major limitations of drug trials in TS seems to be due to measurements having to be taken from patients who express a dynamic phenotype that is constantly waxing and waning in severity (review by Dure and Tucker, 1997).

Successful interventions with botulinum toxin have been shown for refractory vocal tics in TS (Solloway *et al.*, 1996). Immunologic hypotheses, where antibodies against parts of the brain have been suggested as causing an inflammation, which may be manifested as tics of TS (see the account on immune reactions below), have rationalised therapeutic trial with intravenous immunoglobulin G or plasmapheresis (Allen *et al.*, 1995; Swedo, 1994).

Some non-pharmacological approaches to the management of TS have been tried. Behaviour therapy although practised, studies reporting efficacy tend to be small and long-term effects have not been adequately assessed (Carr & Bailey, 1996). Neurosurgical approaches to the management of TS have been reviewed by Rauch *et al.*, (1995) documenting 36 cases in the literature where some form of ablative procedure was performed. But the majority of these cases were not comprehensively assessed before or after surgery, or there was a lack of documentation regarding concurrent pharmacological interventions. Therefore Rauch *et al.* (1995) suggest that there may be a rationale for surgical management of TS and controlled studies must be performed to decide on benefits. Laser therapy has been tried on children with TS and shown a decrease in the

doses of neuroleptics required in the treatment group, compared with a control group (Bondarenko *et al.*, 1997).

2.5 Animal models of TS

In order to validate an animal model, one must provide face validity (similarities between the disease state and the model including pharmacological considerations), construct validity (theoretical) and predictive validity (the prediction of drug efficacies in the clinic based on the animal model), before such a model may be accepted as modelling a human disease state (Abrahamson & Selingman, 1977; McKinney & Bunny, 1969).

In being sudden, rapid, recurrent, non-rhythmic and stereotyped, the head-shakes and the wet-dog-shakes seen in animals fulfil the definition of motor tics as applied to humans (APA, 1995). The head-shake tic was first described in mice by Corne *et al.*, (1963) and proposed as a model for the study of central 5-HT activity. The head shakes occurring in rodents have been characterised as a 5-HT_{2A} receptor mediated behaviour (Kennett and Curzon, 1991; Kennett *et al.*, 1994). 5-HTP or citalopram (a 5-HT re-uptake inhibitor) administration has induced a shudder of the head and trunk in rats which resembled the shaking behaviour observed in dogs when wet and has been termed the wet-dog shake (Bernard and Pycock, 1977; Arnt *et al.*, 1984). Head shakes and wet-dog shakes occur in animals spontaneously at a low rate (Wei, 1981) and are induced to occur at a high rate by agents increasing 5-HT_{2A} receptor activation (Green *et al.*, 1983).

Currently construct validity cannot be assessed for a model of TS as the exact aetiology of TS is not fully understood (Handley & Dursun, 1992). Predictive validity has not yet been carried out to any great extent. Handley and Dursun (1992) have proposed that head shakes and wet-dog shakes occurring in rodents may model tics occurring in TS, since they display face validity for the tics observed in TS.

3. Significance of kynurenine pathway and its metabolites in neuropsychiatric disorders.

Tryptophan is an essential amino acid for humans and many other animals, as it cannot be synthesised in the body in adequate amounts to meet the requirements for protein synthesis (Rose *et al.*, 1957; Osborne & Mendel, 1914). Therefore the plasma tryptophan arises primarily from the diet and intake of less than the required amount promptly results in a negative nitrogen balance and significant reductions in tryptophan metabolites in blood and urine (Vivian *et al.*, 1966). The kynurenine pathway (figure 1) constitutes the major route of L-tryptophan metabolism (>90%) in mammals and essential products include nicotinamide and its nucleotide conjugates, which are cofactors in the reactions of

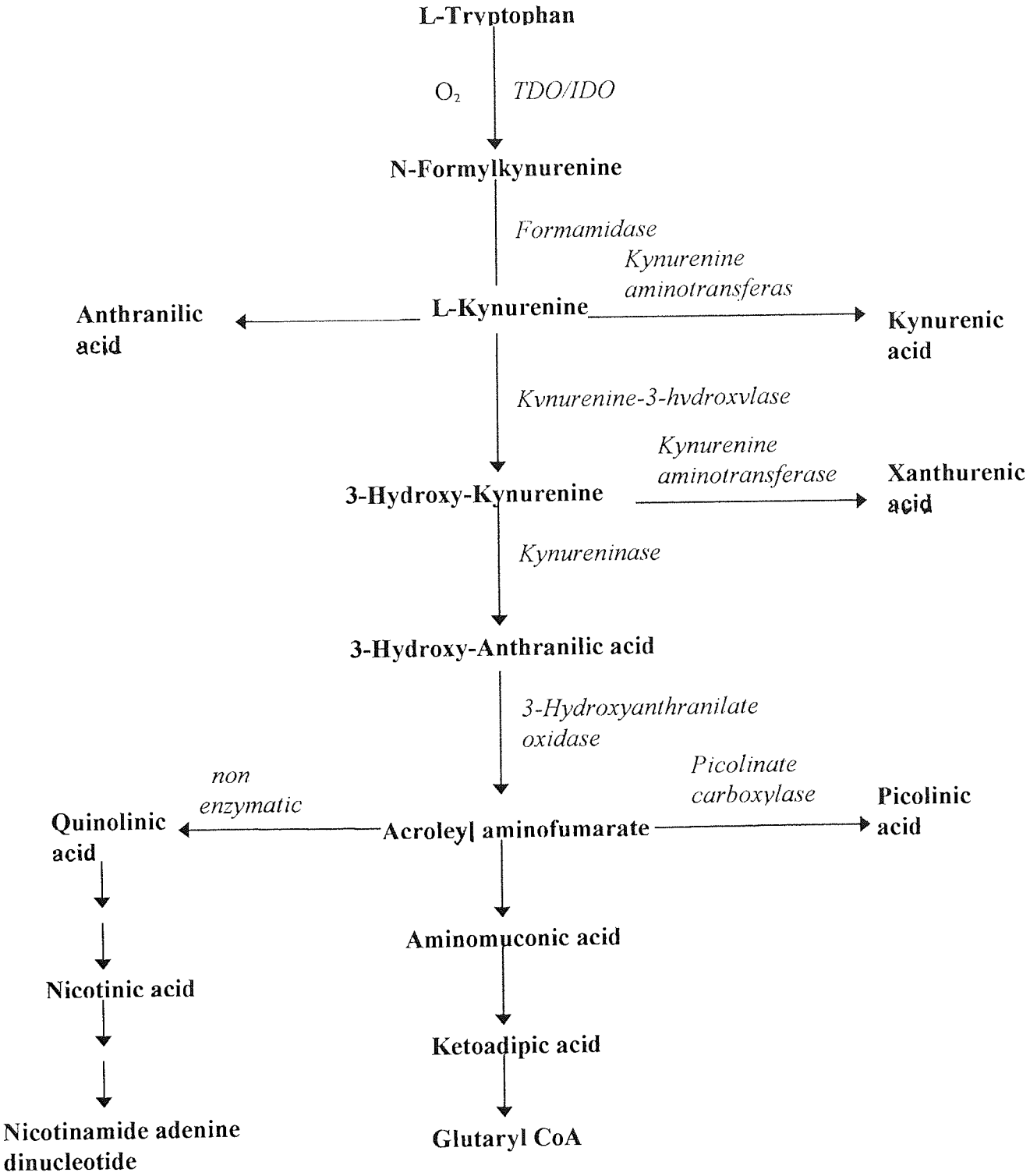

Figure 1. Schematic representation of the kynurenine metabolic pathway of tryptophan leading to the production of nicotinamide adenine dinucleotide. A number of biologically active metabolites produced along the pathway and enzymes catalysing the important stages of the pathway are shown.

glycolysis and pentose phosphate shunt (Beadle *et al.*, 1947; Wolf, 1974). However several intermediates of the kynurenine pathway have also been found to possess significant biological effects, particularly in the central nervous system (for reviews, see Schwarcz, 1993; Heyes, 1993; Stone & Connick, 1985). These metabolites are collectively known as kynurenines.

The liver is a major site of systemic kynurenine pathway metabolism, and substrate flux is predominantly regulated by the first enzyme tryptophan 2,3-dioxygenase (TDO) (Knox & Auerbach, 1955), which has a high substrate specificity for L-tryptophan (Higuchi & Hayaishi, 1967). The activity of this enzyme is induced by its substrate tryptophan (Schimke *et al.*, 1965), corticosteroids (Knox *et al.*, 1970; Schutz *et al.*, 1973) and nicotinic acid (Saino, 1997). TDO is a haem-dependent enzyme (Badawy and Evans, 1975). Administration of progestogens is shown to be associated with increased oxidative metabolism of tryptophan as progesterone induces the synthesis of the rate-limiting enzyme of haem synthesis, δ-aminolaevulinate synthetase (Rifkind *et al.*, 1970). TDO is also induced after morphine, theophylline or salicylate administration and may be regulated by β-adrenoceptors (Badawy & Morgan, 1982; El-Sewedy, 1989).

However in several extrahepatic tissues such as brain and macrophages the first enzyme of the kynurenine pathway is indoleamine 2,3- dioxygenase (IDO) (Yamamoto & Hayaishi, 1967). IDO activity in the brain is seen in the microglia, astrocytes and invading macrophages (Heyes *et al.*, 1992b; Saito *et al.*, 1993; Heyes *et al.*, 1993). This has a much broader substrate profile for indoleamine-containing compounds and is also active in metabolizing 5-hydroxytryptophan (5-HTP), tryptamine, 5-hydroxytryptamine (5-HT) and melatonin (Hayaishi, 1975). IDO is a superoxide-dependent enzyme and therefore the activity of it is dependent on the presence of free radical-generating systems, such as ascorbate and methylene blue or xanthine and xanthine oxidase and the activity is inhibited by superoxide dismutase (Hirata & Hayaishi, 1971). Several studies have shown induction of IDO by cytokines, mainly interferon-γ (INF-γ) (Takikawa *et al.*, 1986; Yoshida *et al.*, 1986). It is shown that INF-γ, interferon-α (INF-α), interferon-β (INF-β) and interleukin-2 induce IDO in human peripheral blood mononuclear cells, degrading tryptophan (Bitterlich *et al.*, 1988; Goldstein *et al.*, 1989) and antibodies to these interferons specifically neutralized the induction of IDO activity by their respective interferons (Carling *et al.*, 1987). Increases in kynurenine (KYN), kynurenic acid (KYNA) and quinolinic acid (QUINA) levels in CSF and serum have been shown in acute septiceamia (Heyes & Lackner, 1990). It has been suggested that this increase in kynurenine pathway metabolites in septicaemia may be secondary to increased cytokine production in response to infection. Treatment of patients with type 1 (INF-β and INF-

α2A) and type 11 (INF-γ) interferons have shown significant decrease in serum tryptophan levels accompanied by a marked increase in urinary KYN excretion (Brown *et al.*, 1987; Byrne *et al.*, 1986b; Datta *et al.*, 1987a) and serum neopterin and biopterin levels (Brown *et al.*, 1987; Datta *et al.*, 1987b; Goldstein *et al.*, 1989).

Mammals have the ability to synthesize unconjugated pteridines such as neopterin and biopterine from guanosine triphosphate (GTP). GTP cyclohydrolase 1 (GTPC1), which cleaves purine is the key enzyme of this pathway, which is present mainly in macrophages and monocytes and it is induced by cell-mediated immune responses *in vitro* and *in vivo* (Huber *et al.*, 1983; 1984). Plasma neopterin and biopterin levels are shown to be increased in a number of clinical conditions linked to immune stimulation, and neopterin is widely accepted as a marker of cell mediated immunity (Wachter *et al.*, 1983; Huber *et al.*, 1985; Werner *et al.*, 1985). These increases in neopterin are shown to correlate with increases in plasma KYN levels (Weil *et al.*, 1983). Several experiments have shown increased neopterin production in human peripheral monocyte/macrophage systems in response to direct stimulation by INF-α, INF-β and INF-γ, with INF-γ being the most potent stimulus (Bitterlich *et al.*, 1988; Troppnair *et al.*, 1988). Werner *et al.*, (1987) have shown that several human cells can be induced by INF-γ to degrade tryptophan via the kynurenine pathway and to synthesize neopterin as INF-γ induces both IDO and GTPC1 (Schoedon *et al.*, 1989; Alberati-Giani *et al.*, 1996). These enzyme regulatory functions of INF-γ are shown to be due to regulation of specific genes and over 200 such genes are shown to be regulated INF-γ (review by Boehm *et al.*, 1997).

Even though TNFα alone was shown to be ineffective in peripheral blood mononuclear cells and macrophages in inducing IDO and GTPC1, it has shown significant potentiation of the actions of all 3 interferons in macrophages and fibroblasts in inducing tryptophan breakdown and neopterin production (Werner-Felmayer *et al.*, 1989). Endogenous interferon production in response to stimulation of immune processes by viral infections (Eyster *et al.*, 1983), endotoxin producing bacterial infections, tissue transplant rejections or autoimmune diseases (Talal, 1985; Cleaveland *et al.*, 1988) have been shown. Release of cytokines, such as interferons is an important effect of the interaction of macrophages with pathogens (see the account on cytokines below).

Increased plasma KYN levels associated with increased plasma neopterin levels have been shown in TS and with this observation also an immune mediation in TS has been suggested (Gaynor *et al.*, 1997), in addition to the inclusion of TS as a PANDAS (refer to section on pathophysiology of TS).

KYN is present in human and rodent brain at an average concentration of 1 μM (Joseph, 1978). KYN can be synthesized within the brain from L-tryptophan or it can be

transported across the BBB by the large neutral amino acid uptake system (Fukui *et al.*, 1991). It is estimated that 40% is synthesized in the brain, while the remaining 60% come from the periphery (Gal & Sherman, 1980). 1-(2,5-dimethoxy-4-iodophenyl)2-amino-propane (DOI), 5-HT or 5-HTP induced head shakes in mice, which are suggested to model tics in TS (Handley and Dursun, 1992) have been potentiated by low doses of systemic KYN (Handley & Miskin, 1977; Mcreary & Handley, 1995), raising the question, whether KYN or a further metabolite of it influences tics in TS.

KYN has convulsant effects (Lapin, 1981a). A physiological role of KYN in the modulation of N-methyl-D-aspartate (NMDA) sensitivity has been implied on an observation of it having activity on some hippocampal neurons similar to that of glycine (Stone 1991). An anxiogenic effect of KYN has been shown in rodents (Lapin, 1984; 1989; Lapin *et al.*, 1990). Increased plasma KYN levels have been shown in humans at the peak of anxiety induced by caffeine (Orlikov & Ryzov, 1991). Increased plasma KYN concentrations have also been shown in patients suffering from endogenous anxiety, with the KYN concentrations correlating with the severity of anxiety and these concentrations have returned to normal after successful treatment. Further to this, decreased plasma KYN levels were shown in the same study in patients suffering from depressive illness, without these decreases correlating with the severity of depression, but the KYN levels normalized after cessation of depressive symptoms as in anxiety (Orlikov *et al.*, 1994). Increased KYN levels have been shown in the cortex, striatum and amygdala after central ischaemia (Jellinger & Riederer, 1981). Tryptophan, KYN, KYNA, xanthurenic acid (XA), QUINA, anthranilic acid (AA) and picolinic acid (PA) have shown analgesic effects in rats (Heyliger *et al.*, 1998). Mendelson *et al.*, (1987) have suggested that KYN has a physiological role in the modulation of female sexual behaviour from observations on rat behaviour. Potentiation of noradrenaline mediated hyperthermic activity and prolongation of amphetamine-induced stereotypies are shown to be caused by KYN, 3-hydroxyanthranilic acid (3-OHAA), AA and PA (Lapin, 1989).

3-Hydroxy kynurenine (3-OHKYN) is neurotoxic (Eastman & Guilarte, 1989) and it is indicated that hydrogen peroxide or free radicals are involved in this toxicity (Eastman & Guilarte, 1990; Okuda *et al.*, 1996). 3-OHKYN is also an endogenous convulsant (Gal & Sherman, 1972). High levels of plasma 3-OHKYN levels have been shown in patients suffering from hepatic coma (Pearson & Reynolds, 1991) and the ratio of 3-OHKYN to KYN appears to be elevated in the basal ganglia of patients with Parkinson's disease (Ogawa *et al.*, 1992).

QUINA and KYNA have opposite effects on the NMDA, excitatory amino acid receptors, the former acting as a selective agonist and the latter as a broad spectrum

antagonist (Stone & Perkin, 1981; Perkin and Stone, 1982; Stone & Connick, 1985). The ability of the endogenous QUINA to act at NMDA receptors raised the possibility that it might be involved in neurodegenerative disorders of the CNS. Excessive stimulation of glutamate receptors, especially those sensitive to NMDA or kainate can lead to accumulation of Ca^{++} in neuronal cytoplasm, with consequent activation of cellular proteases which cause cell damage or even death. It is not clear whether this cell death is necrotic or apoptotic (Ignatowicz *et al.*, 1991). QUINA is similarly able to increase intracellular Ca^{++} (Tsuzuki *et al.*, 1989; Lu *et al.*, 1991). The importance of QUINA as a neurotoxin was first evident from work by Lapin (1978), when he demonstrated that the administration of QUINA to mice caused convulsions. But Heyes *et al.* (1990b) did not find a difference in brain and CSF QUINA levels of patients, relative to controls with intractable, complex partial seizures. Neurotoxicity induced by QUINA was shown to occur preferentially in the neocortex, striatum and hippocampus and they were axon-sparing lesions (Schwarcz *et al.*, 1983; Schwarcz and Kohler, 1983; McGeer and Singh, 1984). QUINA was shown to be present in normal post-mortem human brain at levels similar to those of rat and other species without great variability among different brain regions (all were micromolar concentrations) (Wolfensberger *et al.*, 1984; Moroni *et al.*, 1984a). QUINA concentrations were shown to increase with aging in mice (Moroni *et al.*, 1984b), and as an extention of this study, brain QUINA levels in patients with senile dementia of the Alzheimer type have been assessed, but no evidence for a change of QUINA content in the cerebral cortex has been found in comparison with control subjects (Moroni *et al.*, 1986). Data from intrastriatal injection of QUINA to rats have suggested that QUINA may play a role in the pathogenesis of Huntington's disease (Schwarcz *et al.*, 1983), but later studies have shown that QUINA is not elevated in either CSF or the brain tissue of patients with Huntington's disease (Reynolds *et al.*, 1988; Schwarcz *et al.*, 1988; Sofic *et al.*, 1989; Heyes *et al.*, 1991c). However KYNA was reported to be decreased in CSF from Huntington's patients (Beal *et al.*, 1990), suggesting that unopposed excitatory transmission by glutamate and QUINA may play a role in Huntington's disease. Decreased KYNA concentrations were also found in the post-mortem brains from Huntington's disease patients (Beal et al., 1990). Heyes *et al.* (1989a) detected elevated CSF QUINA levels in AIDS patients, with higher levels in those presenting with AIDS dementia (Heyes *et al.*, 1991a) and the highest levels in AIDS patients with opportunistic infections (Heyes *et al.*, 1991a,b). Elevated QUINA levels have been found in a number of other infective and inflammatory conditions (Heyes *et al.*, 1992a). Heyes and Nowak (1990) found elevated QUINA in the hippocampus and striatum after ischaemia in gerbils and this increase was shown to be

due to a combined increase in the enzymatic activities of IDO, kynureninase, kynurenine-3-hydroxylase and 3-hydroxy anthranilate oxygenase in the hippocampus (Saito *et al.*, 1992A). Finding of macrophage infiltration in the brain areas damaged by ischaemia has suggested that these cells were the source of the increased enzyme activity (Heyes *et al.*, 1992b). Glutaric aciduria is a recessively inherited neurometabolic disorder, where there is a glutaryl-CoA dehydrogenase deficiency and is characterised by encephalopathic crises and severe extrapyramidal symptoms (Leonard *et al.*, 1996). It is proposed that increased amounts of kynurenine pathway metabolites including QUINA may appear in the body in this condition and some of the CNS effects seen may be due to QUINA (Heyes, 1987).

Both *in vitro* and *in vivo* experiments have established effects of QUINA on other neuroactive agents. Lehmann *et al.* (1983) reported QUINA induced acetylcholine release from striatal slices. Lapin (1972, 1989) found enhancement of the hypothermic effect of nicotine by KYN, QUINA and nicotinic acid. Release of noradrenaline (Fink *et al.*, 1990; Pitaluga and Raiteri, 1992), cholecystokinin (Bandopadhyay and Belleroche, 1991), GABA (Reynolds *et al.*, 1989) and dopamine (Mount et al., 1991; Krebs et al., 1991) by NMDA have been shown in the CNS tissues and it is suggested that endogenous QUINA may also cause similar effects if present in sufficient concentrations (Stone, 1993). QUINA is also an effective inhibitor of monoamine oxydase type B in humans (Naoi *et al.*, 1987). Following QUINA administration into either the striatum or hippocampus, there is an increase of 5-HT turnover as reflected in the concentration of the primary metabolite, 5HIAA (Aldinio *et al.*, 1985). Intracisternal administration of QUINA to female rats induces the secretion of luteinising hormone (LH) by its action at NMDA receptors and this action has been abolished by disturbance of 5-HT neurons, either by the use of the 5-HT neurotoxin, 5,7-dihydroxytryptamine, the 5-HT antagonist methysergide or the 5-HT synthesis inhibitor p-chlorophenylalanine (Johnson *et al.*, 1985a,b). Systemic injection of QUINA failed to show such effect (Mason *et al.*, 1983). Further experiments in which QUINA was injected directly into the hypothalamus detected release of LH as well as prolactin and growth hormone (Nemeroff *et al.*, 1985), suggesting that action of QUINA on the release of these hormones is a local one. NMDA receptors play a critical role in learning and some forms of associative memory in animals (Tsien *et al.*, 1996). In a multicentre, placebo-controlled, double-blind randomized study of 91 Alzheimer's patients, a 10-week regimen of D-cycloserine, a partial agonist at the glycine recognition site of NMDA receptors, appeared to improve recall in an implicit memory test (Schwarz *et al.*, 1996b). Therefore QUINA, which acts as a selective agonist at the NMDA receptors may have a role in learning and memory

functions and may play a beneficial role in neuropsychiatric disorders associated with impaired learning and memory.

Due to their polar nature, neither KYNA nor QUINA appear to be able to enter the brain efficiently under physiological conditions (Hillered *et al.*, 1992). Rothe *et al.*, (1993) have shown increased penetration of Mg^{++} from plasma into the brain after intracerebroventricular injection of QUINA and have suggested that QUINA may increase permeability of the blood brain barrier.

KYNA concentration in the brain varies substantially between species, ranging from approximately 6nM in mice and 1μM in humans and it can be synthesized in the brain (Swartz *et al.*, 1990). Brain KYNA increases with age (Moroni *et al.*, 1991). KYNA shows a diurnal rhythm in its appearance in the brain and a role of KYNA on biological rhythms and sleep induction or maintenance is suggested on this observation (Moroni *et al.*, 1992). Perkin and Stone in 1982 showed KYNA to be effective in blocking responses to QUINA, NMDA and quisqualic acid in cortical neurons. Later experiments done on spinal cord, hippocampus, neocortex and caudate nucleus showed KYNA to be an effective antagonist to QUINA, NMDA, kainic acid and quisqualate (Peet *et al.*, 1986; Herrling, 1985; Curry *et al.*, 1986). KYNA acts as an antagonist at the glycine allosteric site and the agonist recognition site on the NMDA-receptor complex as well as non NMDA excitatory amino acid receptors at much higher concentrations (Foster *et al.*, 1984; Birch *et al.*, 1988; Danysz *et al.*, 1989). In line with its ability to antagonise excitatory amino acid (EAA) receptors, KYNA shows anticonvulsant and neuroprotective properties in several relevant animal models (Lapin, 1985; Ghribi *et al.*, 1994; Speciale *et al.*, 1996). These properties of KYNA make it a suitable candidate for therapeutic trials as an anticonvulsant and/or a neuroprotectant against many insults to the brain. Intracerebroventricular injection of KYNA to rats causes stereotyped behaviours (shaking, sniffing, backward walking, lying, turning, gnawing) and marked muscular hypotonia and ataxia in a dose related manner (Vecsei & Beal, 1990). Increased brain KYNA levels have been reported in Down's syndrome (Baran *et al.*, 1996).

Picolinic acid (PA) is another metabolite of the kynurenine pathway, which has shown neuroprotective effects on striatal neurons and cholinergic neurons in the nucleus basalis against QUINA-induced neurotoxicity (Kalisch, 1994). PA also has anticonvulsant and muscle relaxant activities in rodents (Tonohiro *et al.*, 1997). It antagonized KYN-induced seizures in mice more efficiently than KYNA, XA or AA when administered systemically (Lapin, 1983).

The diverse biological effects shown by the kynurenine pathway metabolites and their interactions with other neuroactive substances make it interesting to explore their role in TS and related behaviours.

4. An overview of immune reactions and cytokines with relevance to TS

Following any infection, the innate immune mechanisms that are not antigen-specific act immediately by which means most infective agents are detected and destroyed within hours. Surface epithelia comprise a mechanical, chemical and microbiological barrier to infection. The alternative pathway of complement activation provides a non-adaptive first line of defence against many microorganisms. Macrophages mature continuously from circulating monocytes and leave the circulation to migrate into tissues throughout the body. They are found in especially large numbers in connective tissue and along blood vessels in the liver and spleen. These large phagocytic cells play a key role in all phases of host defense. When pathogens cross an epithelial barrier, they are recognized by phagocytes in the subepithelial connective tissue, with three important consequences. The first is trapping, engulfment and destruction of the pathogen by tissue macrophages, thereby providing an immediate innate cellular immune response. This process may be sufficient to prevent an infection from becoming established. The second important effect of the interaction of macrophages with pathogens is the secretion of cytokines by the phagocytes. It is thought that the pathogen induces cytokine secretion by binding to the same receptor used for engulfment. The macrophages are important in the induction of the adaptive immune response, and their released cytokines play an additional role in determining the form of the adaptive immune response. Adaptive immunity (cellular and humoral) occurs when pathogens evade non-adaptive mechanisms of host defence and establish a focus of infection. The antigens of the pathogen are transported to local lymphoid organs by migrating antigen-presenting cells, or trapped there by resident cells. This trapped antigen is processed and presented to antigen-specific naive T cells that continuously recirculate through the lymphoid organs. T cell priming and the differentiation of armed effector T cells occur here, and the armed effector T cells either leave the lymphoid organ to effect cellular immunity (cell-mediated) in sites of infection or remain in the lymphoid organ to participate in humoral immunity (antibody-mediated) by activating antigen binding B cells (Beverley *et al.*, 1996). As a rule, humoral immune responses function predominantly in the elimination of soluble antigens and the destruction of extracellular microorganisms, while cell-mediated immunity is more important for the elimination of intracellular organisms such as viruses. This compartmentalization of the immune response is an oversimplification, and there is

significant interplay between the humoral and cellular immune responses with some humoral responses depending on cytokine production by T lymphocytes, while some cell-mediated immune mechanisms depending on antibodies for target selection (Goust and Bierer, 1998).

TS has been classed as a paediatric autoimmune neuropsychiatric disorder associated with streptococcal infection (PANDAS) and this is based on an antibody-mediated autoimmune mechanism (see section on pathophysiology of TS). Failure of the immune system to tolerate self-tissues may result in pathological processes known as autoimmune diseases. Autoimmunity is involved in a variety of apparently unrelated diseases. The autoimmune pathological process may be initiated and/or perpetuated by autoantibodies, immune complexes containing autoantigens, and autoreactive T lymphocytes. Each of these immune processes plays a preponderant role in certain diseases or may be synergistically associated, particularly in multiorgan, systemic autoimmune diseases. Autoantibodies may be directly involved in the pathogenesis of the disease or may serve simply as disease markers without a pathogenic role. American researchers who suggest an autoimmune pathogenesis for TS associated with GABHS infection suggest on their findings, that the antineuronal antibodies produced in response to GABHS infection cross react with parts of the basal ganglia and cause adverse changes in them and that these antibody-mediated changes are responsible for the causation of neuropsychiatric manifestations. A similar autoimmune mechanism has been shown in the pathogenesis of rheumatic carditis, which is a sequel of a pharyngeal GABHS infection, by demonstrating antibodies against cardiac tissues developed in response to GABHS infection (Zabriskie & Freimer, 1966; Dell *et al.*, 1991). However both cell-mediated and humoral responses are exaggerated in Rheumatic fever (Mandal *et al.*, 1996).

Cytokines are soluble proteins produced by leucocytes and many other cells, which act as chemical communicators between cells. Most cytokines are secreted, but some can be expressed on the membrane, and others are held in reservoirs in the extracellular matrix. Cytokines bind to specific receptors on the suface of target cells, which are coupled to intracellular transduction and second messenger pathways (Collard & Gearing, 1994). The effects of cytokines are extremely diverse and they influence not only the immune responses but inflammatory processes and haematopoiesis The precise role of individual cytokines in the immune and inflammatory responses is difficult to determine because so many cytokines are produced by immune cells, when activated and each one of them can exert different and overlapping effects on multiple targets (Goust & Bierer, 1998). In all cases a cell can only respond to a cytokine if it expresses the appropriate receptor. Thus the activation of both T cells and B cells is critically dependent upon receiving signals

delivered by appropriate cytokines binding to receptors on their cell membranes. Nomenclature of cytokines is complicated. Classification of cytokines into lymphokines and monokines is historic. Interleukins are cytokines made by leukocytes that affect primarily other leukocytes. Chemokines are especially involved in initiating inflammation. Interferons are cytokines discovered originally through their ability to inhibit the replication of viruses inside cells. Some interferons (INF-α, INF-β) may be synthesized by a cell as a direct consequence of its infection with virus and some interferons (INF-β and INF-γ) are produced by lymphocytes in response to their stimulation by antigen and they have functions that extend beyond being anti-viral (Staines *et al.*, 1993). Cytokines that influence inflammatory reactions are known as proinflammatory cytokines and they include INF-γ, TNF-α, IL-1, IL-8 and migration inhibition factor (Goust & Bierer, 1997). Work by Mossman and colleagues (1989) identified two groups of cytokines produced by different T helper lymphocytes, TH1 and TH2. TH2 cytokines such as IL-4, IL-5, IL-6, IL-10 and IL-13 generally promote B cell function, specifically favouring IgE and IgA responses. The TH1 cytokines such as INF-γ and IL-2 can also have some antibody promoting effects but favour an IgG2A response. These two cytokine groups are also antagonistic in that INF-γ inhibits TH2 cytokine production probably via effects on antigen presenting cells (Trichieri *et al.*, 1993). Cytokine production is shown to differ in adults and children with low production of some (IL-2 and IL-4) and high production of others (IL-6), following immune stimulation (Lilic *et al.*, 1997).

Individually cytokines are potent molecules which *in vitro* can cause changes in cell proliferation, differentiation and movement at nanomolar to picomolar concentrations. Injection of cytokines into animals and humans locally or systemically have profound effects on leucocyte migration and function, haemopoietic cell numbers, temperature regulation, acute phase responses, tissue remodeling and cell survival (Beverly *et al.*, 1996).

If TS is proved to be, an autoimmune disorder where autoantibodies are directly involved in the pathogenesis, or a disorder where immune reactions play a part by means of altered cytokine production, novel immunotherapies may become useful in the treatment of TS.

5. 5-Hydroxytryptamine (5-HT)/Serotonin

5-HT seems to be the most closely involved monoamine neurotransmitter with neuropsychopharmacology. It was originally discovered in the chromaffin cells of the intestinal mucosa as enteramine (Vialli & Erspamer, 1933). Several years later 5-HT was shown in the serum as a vasoactive substance by Rapport *et al.* (1947), which was

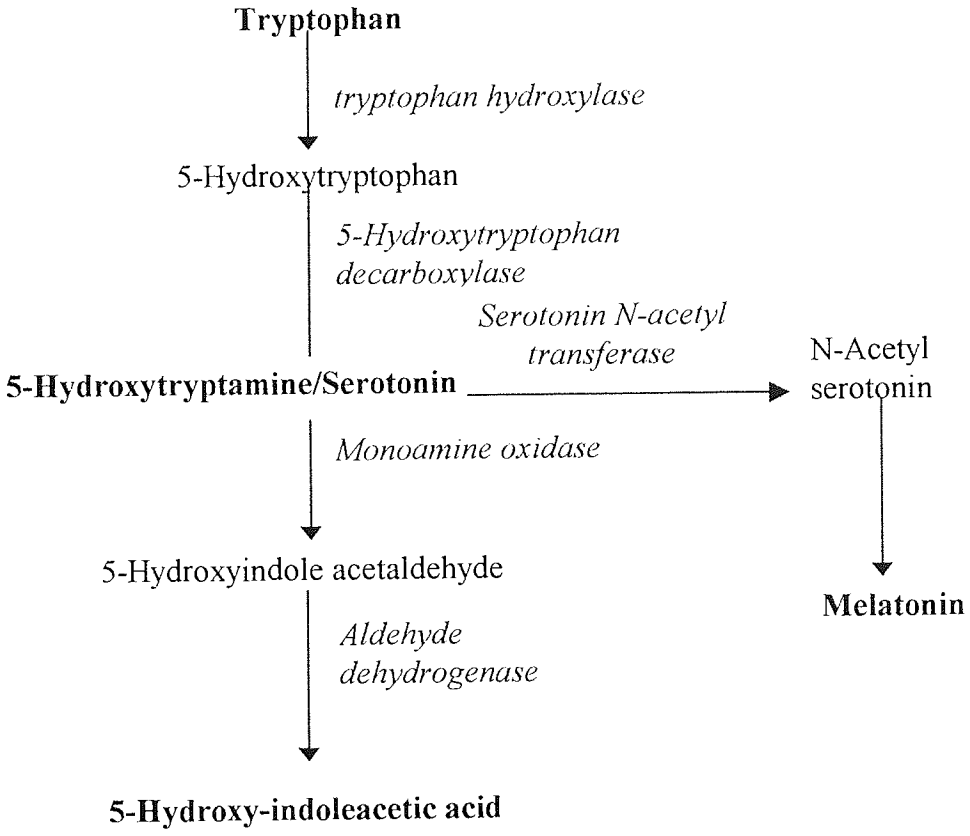

Figure 2. The metabolic pathway of tryptophan leading to the synthesis of serotonin and the catabolic pathways of serotonin leading to the formation of 5-hydroxy-indole acetic acid and melatonin

therefore called serotonin. Twarog and Page (1953) demonstrated the presence of 5-HT in the mammalian brain, and Amin *et al.* (1954) showed that it has a heterogenous distribution in the dog brain. These findings led to the suggestion that 5-HT might act as a neurotransmitter in the CNS (Bogdanski *et al.*, 1956; Brodie & Shore, 1957). It was first synthesized by Hamilton and Fisher in 1951.

5-HT is a metabolite of tryptophan and in the peripheral tissues only about 1% of dietary tryptophan becomes converted to 5-HT (Udenfriend *et al.*, 1955). Only about 1-2% of the 5-HT in the whole body is found in the brain (Cooper *et al.*, 1996). Since 5-HT does not cross the blood brain barrier (BBB) the brain cells have to synthesize it. Therefore the first step in brain synthesis of 5-HT is cerebral uptake of tryptophan. Serum albumin binds tryptophan and the availability of tryptophan to cross the BBB is determined by the amount of plasma free tryptophan. The entry of tryptophan across the BBB is controlled by a specific large neutral amino acid carrier and this system seems to be shared by tyrosine, phenylalanine, valine and isoleucine. Therefore the entry of tryptophan through this system is determined by the ratio of level of tryptophan to all the other neutral amino acids in the plasma (Fenstrom & Wurtman, 1972). Conversion of tryptophan to 5-HT is accomplished in a two-step enzymatic process (Figure 2). Firstly tryptophan is hydroxylated by tryptophan hydroxylase to 5-hydroxy-tryptophan (5-HTP) and this step requires molecular oxygen and the co-factor tetrahydrobiopterin. This step is accepted to be the rate-limiting step in the synthesis of 5-HT in the brain (Moir & Eccleston, 1968). Dose dependent increase of 5-HTP has been shown in the rat brain following intracerebroventricular administration of tetrahydrobiopterin (Miwa *et al.*, 1985; Sawada *et al.*, 1986). On post-mortem brain examinations done on patients with senile dementia of the Alzheimer type and controls, reduced tryptophan hydroxylase activity together with low tetrahydrobiopterin levels have been shown (Sawada *et al.*, 1987). The inhibition of the activity of the rate limiting enzyme of tetrahydrobiopterin biosynthesis, GTPC1 by systemic administration of an inhibitor to it has been shown to cause reduction of 5-HTP production in the brain (Suzuki *et al.*, 1988). Neopterin is the precursor of tetrahydrobiopterin and the purine cleaving enzyme GTPC1 controls the synthesis of tetrahydrobiopterin (Hesslinger *et al.*, 1998). 5-HTP is decarboxylated to 5-HT by the same pyridoxal phosphate dependent aromatic amino acid decarboxylase as that involved in the synthesis of catecholamines (Sandler & Ruthven, 1969).

5-HT is catebolised by oxidative deamination, catalysed by monoamine oxidase and aldehyde dehydrogenase, and the main excretory product is 5-hydroxyindoleacetic acid (5-HIAA) (Gorkin, 1966; Bosnan, 1978). In the pineal gland and some other tissues 5-HT is N-acetylated and then O-methylated, to form melatonin (Axelrod & Weissbach, 1960;

Klein & Weller, 1970). Figure 2 shows the metabolic pathways of 5-HT leading to the production of 5-HIAA and melatonin. Melatonin has a number of important biological functions and is attracting increasing attention as a potential substance playing a role in neuropsychiatric disorders (see below for details of melatonin).

Serotonin containing neurones are restricted to clusters of cells (nuclei) lying in or near the midline raphe region of the midbrain and pons. Dahlstrom and Fuxe (1965) have described nine such nuclei (B1-B9). These show extensive projections (see figure 3) into various parts of the brain (Dahlstrom & Fuxe 1965; Azmitila & Segal, 1978; Steinbusch, 1981). Due to these widespread projections gross manipulation of the central 5-HT function may modulate a range of behavioural and physiological events associated with different areas of the brain.

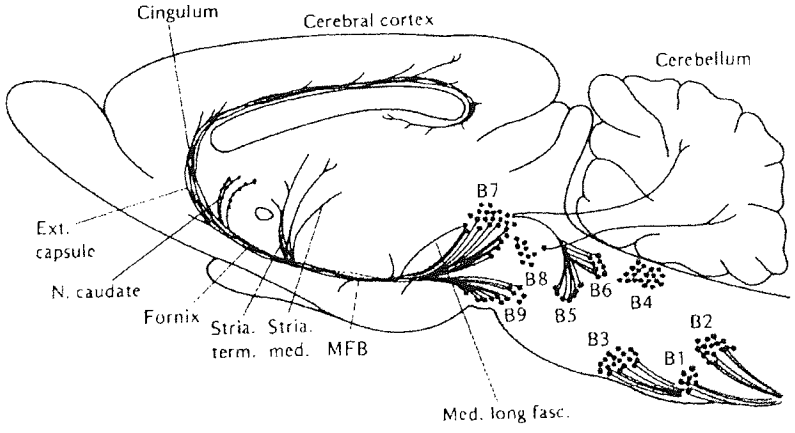

Figure 3. Schematic diagram illustrating the distribution of the main 5-HT-containing pathways and the 5-HT containing midline raphe nuclei in the rat brain (adapted from Breese, 1975).

5.1 5-HT receptors and functions of the central nervous system influenced by 5-HT

5-HT is involved in a number of physiological events and to some extent affects various functions of the central nervous system (CNS). Table 1 shows some of the important CNS functions affected by 5-HT.

Table 1. CNS functions affected by 5-HT (adapted from review by Zifa and Fillion, 1992).

Function	References
sleep	Koella, 1988
thermoregulation	Feldberg & Myers, 1964; Jacob & Girault, 1979; Myers, 1981
learning and memory	McEntee & Crook, 1991
pain	Tenen, 1967; LeBars, 1988
aggressive behaviour	Sheard, 1969; DiChiara *et al.*, 1971
sexual behaviour	Hoyland et al., 1970; Meyerson & Malmnas, 1978; Fernandez-Guasti *et al.*, 1987; Gorzalka *et al.*, 1990
feeding	Blundell,1984; Marazziti *et al.*, 1988; Curzon, 1990
neuroendocrine regulations	Wesemann & Weiner, 1990
motor activity	Schneider & McCann, 1970; Tuomisto & Mannisto, 1985; Fuller, 1990
biological rhythms	Gershon & Baldessarini, 1980; Sternbach, 1991

A role of 5-HT on the immune system has also been shown by several studies in addition to its other diverse actions in the CNS (Mossner & Lesch, 1988). An inverse relationship between brain 5-HT concentrations and antibody synthesis has been reported (Denoino *et al.*, 1970; Idova & Devoino, 1972).

Specific 5-HT receptors mediating physiological and pathological events have been shown. Evidence for the existence of 5-HT receptors was first presented by Gaddum and Picarelli (1957), who experimented with the isolated guinea pig ileum. The earliest evidence for selective, high-affinity and saturable binding of [^{3}H]5-HT was presented by Bennet and Snyder (1975;1976) and Fillion *et al.* (1976). Presence of two classes of 5-HT receptors: 5-HT$_1$ and 5-HT$_2$ were first proposed by Peroutka and Snyder (1979). At present 5-HT receptors are classified into 7 classes and agreed by the subcommittee on 5-HT receptors of the International Union of Pharmacology committee for receptor nomenclature (NC-IUPHAR, 1997). These receptors are characterized according to the 'finger print' criteria: operational (drug related characteristics: selective agonists, selective antagonists and ligand binding affinities), transductional (receptor-effect coupling events: intracellular transductional mechanisms) and structural (gene and receptor structural sequences for their nucleotide and amino acid components respectively) characteristics (Humphrey *et al.*, 1993). Table 2 shows the operational characteristics of different classes of 5-HT receptors. Table 3 shows major central locations and gene chromosomal localization of 5-HT receptors.

The transduction aspect of receptor mechanisms suggests selective interaction of the receptor with unique membrane or cytosolic components of the cell that then carry the

message imparted by the drug (Kenakin *et al.*, 1992). There are two major signal transduction pathways linked with 5-HT receptors (Harting, 1989) as shown in table 4. Both these pathways require a guanine nucleotide triphosphate binding protein (G protein) to link the receptor to the effector molecule. In addition to coupling with adenylate cyclase 5-HT_{1A} receptors are linked directly to voltage sensitive K^+ channels via G proteins (see table 4). Several neurotransmitters, including 5-HT, hormones and other extracellular stimuli impart information to their target cells by stimulating cell-surface receptors that activate phospholipase C (phosphoinositidase C), this hydrolyses the bond between the diacylglycerol and inositol 1,4,5- trisphosphate (IP3) portions of phosphatidylinositol 4,5-biphosphate (Kirk *et al.*, 1981; Michell *et al.*, 1981). The IP3 produced during stimulation of the receptors diffuses into the cytosol leaving diacylglycerol behind in the membrane. IP3 mobilizes Ca^{++}. A wide variety of cells contain ATPase-loaded Ca^{++} in some element of the endoplasmic reticulum system and the IP3 produced during stimulation of the receptors binds to a receptor on these membranes (Spat *et al.*, 1986a,b) and opens a membrane Ca^{++} channel through which Ca^{++} flows out of this store into the cytoplasm (Streb *et al.*, 1984; Burgess *et al.*, 1984; Joseph *et al.*, 1984; Dawson & Irvin, 1984; Berridge & Irvin, 1984).

Table 2. Operational characteristics of 5-HT receptors (Hoyer *et al.*, 1994; Alexander & Peters, 1997).

Table 3. Major central locations and gene chromosomal localizations of 5-HT receptors (adapted from, Hoyer *et al.*, 1994 and Alexander & Peters, 1997).

Receptor	subtype	central location	gene chromosomal localization
5-HT_1	5-HT_{1A}	hippocampus, parts of the limbic system, septum, amygdala, raphe nuclei hypothalamus, neocrtex, substantia gelatinosa of the spinal cord	*HTR1A*/5q11.2-q13 (Kobilka *et al.*, 1987)
	5-HT_{1B}	substantia nigra, globus pallidus	*HTR1B*/6q13 (Demchyshyn *et al.*, 1992)
	5-HT_{1D}	substantia nigra, globus pallidus	*HTR1D*/1p34.3-36.3 (Libert *et al.*, 1989)
	5-ht_{1E}	cortex	*HTR1E*/6q14-15 (Levy *et al.*, 1994)
	5-ht_{1F}	dorsal reaphe, hippocampus, cortex, striatum	*HTR1F* (Saudou & Hen, 1994)
5-HT_2	5-HT_{2A}	cortex, hippocampus, facial motor nuclei, olfactory tubercles, claustrum	*HTR2A*/13q14-21 (Sparkes *et al.*, 1991)
	5-HT_{2B}	cortex	*HTR2B*/2q36.3-2q37.1 (Le-Coniat *et al.*, 1996)
	5-HT_{2C}	choroid plexus, substantia nigra, globus pallidus	*HTR2C*/Xq24 (Milatovich *et al.*, 1992)
5-HT_3		dorsal vagal nerve, solitary tract nerve, trigeminal nerve, area postrema, limbic system, spinal cord	*HTR3*/11 (Miyake *et al.*, 1995; Belelli *et al.*, 1995; Isenberg *et al.*, 1993)
5-HT_4		colliculus, hippocampus	*htr4* (Gerald *et al.*, 1995)
5-HT_5	5-HT_{5B}	cortex	*htr5b*/2q11-q13 (Matthes *et al.*, 1993)
	5-ht_{5A}	cortex	*HTR5A*/7q36 (Matthes *et al.*, 1993)
5-ht_6		cortex, striatum, nucleus accumbence	*HTR6*/1p35-36 (Kohen *et al.*, 1996)
5-HT_7		thalamus, hypothalamus, amygdala	*HTR7*/10q23.3-24.3 (Lucas and Hen, 1995)

Table 4. Trasduction mechanisms of 5-HT receptors:

Receptor	Transduction system	G protein effector	Reference
5-HT_{1A}	negatively couples to adenylyl cyclase via regulatory G proteins, opening K+ channels	Gi/o	De Vivo & Maayani, 1985; Weiss *et al.*, 1986; Andrade *et al.*, 1986
5-HT_{1B} 5-HT_{1D} 5-ht_{1E} 5-ht_{1F}	negatively couples to adenylyl cyclase	Gi/o	Bouhelal *et al.*, 1988; Hoyer & Schoeffter, 1988; Schoeffter *et al.*, 1988
5-HT_{2A} 5-HT_{2B} 5-HT_{2C}	couples with phospholipase-C and stimulates phosphatidylinositol turnover with accumulation of inositol-1-phosphate in the presence of Li^+, resulting in Ca^{++} mobilisation.	Gq/11 Gq/11	Conn & Sanders-Bush, 1984 Kursar *et al.*, 1992 Conn & Sanders-Bush, 1986 Conn *et al.*, 1986
5-HT_3	ligand-gated ion channel		Derkach *et al.*, 1989
5-HT_4	stimulation of adenylyl cyclase and elevation of cAMP	Gs	Hoyer *et al.*, 1994
5-HT_5	not known	Gs	
5-ht_6	stimulation of adenylyl cyclase	Gs	Mosma *et al.*, 1996
5-HT_7	stimulation of adenylyl cyclase	Gs	Mosma *et al.*, 1996

5.3 Relevance of 5-HT to TS and other neuropsychiatric disorders

Altered serotonergic function is implicated in a number of pathological conditions, including neuropsychiatric disorders. Table 5 shows the neuropsychiatric disorders in which 5-HT is suggested to play a role.

Table 5. Neuropsychiatric disorders in which 5-HT is thought to play a role:

Neuropsychiatric disorder	Reference
Affective disorders	Schildkraut, 1965; Meltzer & Lowry, 1987; Cowen, 1988; 1990; Curzon, 1988; Kalus *et al.*, 1989; Plaznik *et al.*, 1989; Meltzer, 1990
Aggressive behaviour	Pucilowski and Kostowski, 1983; Miczek and Donat, 1989; Olivier *et al.*, 1989; Coccaro, 1989; Wetzler *et al.*, 1991
Anxiety disorders	Iverson, 1984; Gardner, 1985; 1986; Johnsson & File, 1986; Kahn *et al.*, 1988; Nutt & George, 1990
Autism	Cook, 1990; Anderson *et al.*, 1990
Eating disorders	Garattini & Samanin, 1976; Tamai *et al.*, 1990

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OCD	Zohar & Insel., 1987; Insel *et al.*, 1990
Schizophrenia	Csernansky *et al.*, 1992
Sleep disorders	Koella, 1988
Substance abuse	Tollefson, 1989; Sellers *et al.*, 1992
Suicidal behaviour	Roy & Linnoila, 1988; Coccaro & Astill, 1990; Mann *et al.*, 1990
Tourette syndrome	Schweitzer & Friedhoff, 1988; Comings, 1990b

Since the head shake behaviour in rodents, suggested to model TS (see animal models of TS), is clearly a 5-HT_{2A} receptor-mediated behaviour response and is widely used as a measure of 5-HT_{2A} receptor activity (Lucki *et al.*, 1984; Kennett & Curzon, 1991), it may be useful to investigate the effects of substances which have shown changes in TS patients, on this receptor activity and substances which alter 5-HT_{2A} receptor activity in TS patients.

6. Melatonin

Melatonin, 5-methoxy-N-acetyl-tryptamine, a metabolite of 5-HT (see figure 2 for the metabolic pathway by which melatonin is synthesized from 5-HT) is the main secretory product of the pineal gland and was initially thought to be synthesized exclusively in the pineal gland (Ebadi, 1984). Even though melatonin is produced predominantly by the pineal gland in mammals, the retina (Gern *et al.*, 1978), the harderian gland (Bubenik *et al.*, 1976), the intestines (Vakkuri *et al.*, 1985), certain blood cells (Finocchiaro *et al.*, 1988) and some regions of the brain (Brown *et al.*, 1983) are other sources of this hormone. In mammals, including humans and other vertebrates, melatonin synthesis and secretion exhibit a circadian rhythm: maximum levels during darkness and almost undetectable levels during day-light (Arendt, 1985). Furthermore, melatonin displays annual fluctuations in animals and humans, suggesting the existence of a seasonal rhythm (Aredt *et al.*, 1979; Martikainen *et al.*, 1985; Bojkowski and Aredt, 1988). These seasonal fluctuations are claimed to entrain essential biological phenomena such as reproduction (Reiter, 1980; Bittman *et al.*, 1983), sexual maturation (Vaughan *et al.*, 1978; Waldhauser *et al.*, 1984;1986), hibernation (Stanton, 1984), thermoregulation (Ralph *et al.*, 1979), immunomodulation (Maestroni *et al.*, 1987; Maestroni, 1993) and behaviour (Wurtman, 1986). Age plays a role in the regulation of melatonin production, thus in advanced age the magnitude of the nocturnal increase in melatonin is much less than that in young animals including humans (Reiter, 1989; Miguez *et al.*, 1998).

High affinity melatonin receptor sites were characterized and localized in the mammalian central nervous system using the radioligand, [^{125}I]-iodomelatonin (Vakkuri *et al.*, 1984; Duncan *et al.*, 1988; 1989). At present melatonin receptors are classified as MEL1A, MEL1B and other receptors or binding sites coupled to phosphoinositide hydrolysis and G proteins by the NC-IUPHAR (1997). The full range of physiological actions of melatonin is not yet completely known. Melatonin is reported to have anxiolitic (Golombek *et al.*, 1933), sedative (Romijin, 1978) and anticonvulsant (Champney & Champney, 1992) effects on humans and animals. Both *in vivo* and *in vitro* experiments have proved melatonin to be a neuroprotective substance. Protection from kainate-mediated neurotoxicity without effects on the NMDA-mediated neurotoxicity was shown by Giusti *et al.* (1995) and Lipartiti *et al.* (1996), and later Daya and Potgieter (1998) showed melatonin playing a protective role in the NMDA agonist, QUINA-induced neurotoxicity in the rat hippocampus. Bonilla *et al.* (1997) demonstrated reduction in the death rate and evolution of the disease in mice infected with Venezuelan equine encephalomyelitis virus by treatment with melatonin. Melatonin is effective as a free radical scavenger, electron donor and an antioxidant both *in vivo* and *in vitro* (Tan *et al.*, 1993; Pieri *et al.*, 1994; Reiter *et al.*, 1994;1995; Pierrefiche *et al.*, 1993; Cagnoli *et al.*, 1995). These properties of melatonin may be contributing to its protective actions on tissues.

Several studies have shown effects of melatonin on neurotransmitter systems. Thus, it has been reported that exogenous melatonin systemically administered significantly increased the hypothalamic concentrations of aspartic acid and GABA and decreased the hypothalamic concentrations of β-endorphin and 5-HT (Xu *et al.*, 1995). Chuang and Lin (1994) also showed inhibition of brain 5-HT release in rats, by systemic administration of melatonin at pharmacological doses. Administration of melatonin and analogs have altered the sensitivity to 5-HT_{2A} agents in the regulation of sleep-wakefulness and 5-HT induced behaviour states in the rat (Dugovic *et al.*, 1989; Eison *et al.*, 1995). Pinealectomy and exogenous melatonin have elicited a variety of changes in 5-HT metabolism (Miguez *et al.*, 1991; 1994; 1996) and 5-HT synaptic function (Miguez *et al.*, 1995) in discrete hypothalamic areas, which could be related with the behavioural actions shown by melatonin. In addition to the effects of melatonin on 5-HT, inhibition of meltonin-induced behavioural changes (decreased locomotor activity and rearing, and increased grooming and sniffing) by 5-HT and induction of behavioural responses similar to those after treatment with melatonin by 5-HT antagonists were shown in rats by

Gaffori and Van Ree in 1985. Melatonin has also shown antidopaminergic effects (Tenn & Niles, 1997). Following the demonstration of interactions of melatonin with neurotransmitter systems, its involvement in the aetiology and as a potential therapeutic agent in neuropsychiatric disorders has been suggested (Durlach-Nisteli & Van Ree, 1992; Brown, 1995; Webb & Puig-Domingo, 1995). Sandyk and Kay (1991) have hypothesized that disturbances in melatonin secretion may also be relevant to the pathophysiology of TS, on a number of similarities between TS and melatonin secretion (see introduction in Chapter 5 for details). Melatonin is successfully used as a therapeutic agent for the treatment of rhythm disorders, such as those manifested in jet lag, shift work or blindness for quite a long time (Arendt & Deacon, 1997).

7. Aims of the project

The initial aim of this project was to investigate the mechanisms by which KYN potentiated 5-HT_{2A}-mediated head shakes in mice, in order to determine whether altered kynurenine pathway metabolism influences the tics in TS. This would include animal experiments to examine the effects of further metabolites of KYN and the time course effect of KYN on 5-HT_{2A}-mediated head shakes in mice and *in vitro* experiments (radioligand binding and PI hydrolysis studies) to determine the effects of the kynurenine pathway metabolites on 5-HT_{2A} receptor function. Dr. Handley's research group suggested that the head shakes in animals model tics in TS and demonstrated potentiation of 5-HT_{2A}-agonist induced head shakes in mice by KYN.

Two further studies by her research group also showed increased plasma KYN levels in TS patients when compared with age and sex matched controls. These two studies showed marked difference in the magnitude of the increase in plasma KYN levels in TS patients with reference to age and sex matched controls. Therefore the main aim of the clinical studies carried out in this project were to further explore KYN and the mechanisms by which KYN might be altered in TS by measurement of plasma KYN, cortisol, INF-γ, neopterin and tryptophan in TS patients and matched controls.

Cortisol determination in TS patients showed an interesting pattern with the ADHD

severity in the TS patients who had associated ADHD and therefore it was also intended to examine cortisol secretion in a group of subjects primarily affected with ADHD.

Since melatonin has shown inhibitory effects on TDO activity and 5-HT_{2A}-agonist induced head shakes, a further aim of the clinical studies was to examine melatonin secretion in TS patients by measuring the night-time urinary excretion of melatonin's chief metabolite, 6-sulphatoxy melatonin.

Furthermore with the aim of investigating the role of GABHS infections in TS, ASOTs were determined in the group of TS patients and their matched controls recruited in Clinical Study 1, and examined the effect of GABHS immunity on DOI induced head shakes in mice.

The results from the animal, *in vitro* and clinical studies may direct us to identify new areas to research that lead to the finding of more efficient and acceptable treatments for TS and associated conditions.

EXPERIMENTAL METHODS

EXPERIMENTAL METHODS

1. Animals, animal husbandry and laboratory conditions

All the experiments were carried out on Aston bred male mice of MF1 strain. Mice weighing between 18-22g were used for behavioural experiments and 30-40g were used for *in vitro* experiments. Subsequent to weaning mice were kept in groups of 20 in polypropylene cages in the animal house at an ambient temperature of 21-23°C. They were fed a rodent breeder diet (SDS, LILLICO) and received tap water *ad libitum*. The animals were exposed to a 12-hour light-dark cycle.

2. Injection Techniques

2.1. Subcutaneous (s.c.) Injection

S.C. injections were made by insertion of a hypodermic needle under the loose skin at the scruff of the neck. The injection volume was 5ml/kg.

2.2. Intraperitoneal (i.p.) Injection

I.P. injections were made by inserting a hypodermic needle through the abdominal wall pointing towards the diaphragm. Care was taken not to damage the internal organs by holding the animal with its head lower than the abdomen and not penetrating too deeply.

2.3. Intracerebroventricular (i.c.v.) Injection

This method has been described by Brittain and Handley (1967). A 0.25ml Tuberculin syringe was used with a 26-gauge needle cut to the length of 3mm. The injection volume was 20μl. The mouse was immobilised by holding the head firmly on a flat surface by holding the skin at the scruff of the neck. The needle was placed vertically on the midline of the skull and drawn backwards until a depression was felt. This depression is the junction between the two parietal and the interparietal bones, an area which is not ossified in the young mouse. The skull was penetrated at this depression for delivering the drug solution or the vehicle in to the ventricular system.

3. General methods for the analysis of DOI induced head shakes

Head shake rates were determined as described by Boulton and Handley (1973) using groups of 4 mice (2 test mice and 2 control mice) and always observing a test and a control mouse at the same time. Groups of 4 mice were habituated in sawdust lined glass aquaria (25x20x20cm) for at least one hour prior to the experiments. All mice were given 1.0mg/kg of the $HT_{2A/2C}$ agonist DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-amino propane) i.p. 5 min before videotape recording for 10 min. All treatment details were displayed at the end of each recording. A head shake was defined as a rapid rotational flick of the head and these

were counted later from the video recordings with the experimenter unaware of the treatment each mouse received. These experiments were repeated to give at least six paired determinations for each category. All the experiments were carried out between 13.00 and 17.00 hours against a background noise level of 45±5db.

4. Determination of the effects of kynurenine pathway metabolites on DOI-induced head shakes in mice.

Male MF1 mice weighing between 18-22g taken at random from the same stock cage were habituated in groups of 4 for 1 hour in the observation aquaria prior to the experiments. Drugs dissolved in Krebs physiological solution at pH 7.4 or Krebs alone were given s.c. or i.c.v. Doses were randomised between groups such that two mice in each group received the vehicle (Krebs) as appropriate controls (two test mice and two control mice in a group). Effects were observed at different pre-treatment times. DOI-induced head shakes were determined as described above in 3.

5. Investigation of the effects of kynurenine pathway metabolites on $5HT_{2A}$ receptor binding

MF1 male mice weighing 30-40g were killed by cervical dislocation, the brains were immediately removed and cortices were dissected on ice. Dissected cortices were immediately stored at -20°C.

5.1. Tissue preparation

Membranes for [^{3}H]ketanserin binding were freshly prepared essentially as described by Leysen et al., (1982). Frozen cortical tissue was placed in ice-cold 0.25M sucrose (1:30w/v) and homogenised with a motor-driven teflon pestle (8 strokes at 120rpm). Myelin and cell debris were removed by centrifugation at 1000g for 10min. The supernatant was stored on ice and the pellet was rehomogenised in 0.25M sucrose (1:15w/v) and centrifuged at 750g for 10min. Combined supernatants were diluted with 50mM Tris-HCl buffer, PH 7.7 (at 25°C) to 1:80 w/v and centrifuged at 35000g. The final pellet was resuspended in Tris buffer equivalent to 25mg wet weight of tissue per ml). All centrifugations were carried out at 4°C.

5.2. Binding assays

Assays were carried out in Skatron 1.4ml plastic tube strips and all incubations were done at 37°C for 15min in a water bath.

5.2.1. Saturation binding experiments were done with a range of 8 concentrations (0.03-3.5nM) of the radioligand ([^{3}H] ketanserin (NEN), specific activity 80.9Ci/mMol) to determine the K_d (dissociation constant) and B_{max} (maximum number of binding sites). 400µl of freshly prepared membrane, 50µl of [^{3}H] ketanserin at 8 concentrations and 50µl of 10%

ethanol or 50µl of methysergide dissolved in 10% ethanol were incubated together. Non-specific binding was defined with 50µM methysergide (RBI).

5.2.2. Displacement experiments were done with a 0.5nM (K_d of [^{3}H] ketanserin determined by the saturation binding experiments) fixed concentration of the radioligand.

The first set of displacement experiments were done with ten concentrations (10^{-10}-10^{-4}M) of the metabolites: kynurenine, 3-OH kynurenine, kynurenic acid, quinolinic acid, xanthurenic acid and 3-OH anthranilic acid to determine the % displacement of the ligand by the kynurenines. 400µl of freshly prepared membrane, 50µl of [^{3}H] ketanserin, 50µl of 10% ethanol (total) or 50µM methysergide (non spesific) or a kynurenine dissolved in water or polyethylene glycol were incubated together.

The second set of displacement experiments were done to determine the K_i (inhibition constant) values of methysergide, alone (control) and in the presence of the above concentrations of kynurenine, kynurenic acid and quinolinic acid. 400µl of freshly prepared membrane, 50µl of [^{3}H] ketanserin, 50µl of Tris buffer, 50µl of kynurenine or the vehicle and 50µl of methysergide at 10 concentrations with the IC_{50} (concentration of the inhibitor that inhibits 50% of the specific binding) falling in the middle of the range were incubated together.

Membrane bound radioactivity was recovered by filtration under vacuum through Skatron 11734 filters using a Skatron cell harvester. Filters were rapidly washed with ice-cold 50mM Tris-HCl, pH 7.4 (wash setting 9,9,0) and radioactivity (decays per min (DPM)) were determined by liquid scintillation counting using Packard Ultima Gold MV scintillant, in a calibrated scintillation counter.

These ligand binding experiments were done at the Research and Development laboratory of the Knoll Pharmaceuticals, Nottingham under the kind guidance of Dr. S. C. Cheetham .

6. Investigation of the effects of kynurenine pathway metabolites on 5-HT$_{2A}$ mediated phosphoinositide hydrolysis

Phosphoinositide hydrolysis assays were carried out with a modification of the methods described by Conn and Sanders-Bush (1985). MF1 male mice weighing 30-40g were killed by cervical dislocation, the brains were immediately removed and cortices were dissected on ice. Dissected cortices were cross-chopped on a Mcllwain tissue chopper (350µm x 350µm) and placed in warm (37°C), oxygenated Krebs solution. The tissues were incubated for 30 min and during this incubation tissue slices were washed thrice with Krebs. Aliquots of 25µl of tissue slices were next incubated with 100µl of a 4µCi/ml solution of [^{3}H]-myo-inositol (Amersham: 115 Ci/mmol) for 2 hours. After this prelabeling incubation 25µl of Krebs containing 10mM LiCl and 10µM pargyline were added followed by 25µl of the metabolite

tested dissolved in Krebs (tests) or Krebs alone (controls). Samples were then returned to the water bath and incubated for 30 min. Samples were next removed from the water bath and different concentrations (10^{-8}-10^{-4}M) of the 5-$HT_{2A/2C}$ receptor agonist: α methyl-5-HT (Ragunathan and Sourkes, 1990) were added sequentially into the series of both test and control tubes. Samples were again incubated for 45 min. The assays were performed in capped, 5ml flat bottomed tubes under periodical supply of 95%O_2/5%CO_2 and all incubations were done at 37°C in a shaking water bath. The final assay volume was kept at 300µl. Reactions were terminated by adding 0.9ml of a mixture of chloroform/methanol (1:2 v/v). After standing at room temperature for 15 min 1 assay volume of chloroform and 1 assay volume of distilled water were added to all samples. Samples were stored at -20°C until assayed. Samples were then thawed and vigorously vortexed for 1 min. Phases were then separated by centrifugation for 10 min at 1500rpm. 0.75ml aliquots of the upper aqueous phase were applied to columns containing 0.6 ml gravity packed Dowex-1 anion exchange resin (Sigma-Aldrich Chemie, Germany) in the formate form. Columns were washed with 2 x 4ml of distilled water and 2 x 4ml of 60mM ammonium formate/5mM disodium tetraborate respectively. Finally [^{3}H]-inositol-1-phosphate was eluted from the columns with 4ml of 200mM ammonium formate/0.1M formic acid directly into 20ml scintillation vials, 10ml of Optiphase 'Hiphase' (Fisher chemicals, England) liquid scintillation cocktail was added and radioactivity (counts per min (cpm)) was determined by liquid scintillation counting for 3 min in a scintillation counter with high efficiency.

7. Detection of the effect of Group A β-haemolytic Streptococcal immunity on DOI-induced head shakes in mice.

7.1. Preparation of Group A β-haemolytic Streptococci (GABHS) for injection

GABHS was grown on blood agar plates as shown by figure 5.1. in Chapter 3. They were harvested by suspending streaks of grown Streptococci in brain heart infusion (Oxoid LTD., England) and centrifuging at 10,000g for 10min. The pellet of cells was washed with saline and resuspended in saline. Then killing was done by 254nM u.v. irradiation and cells were resuspended in saline to have 10^9 cells/ml of saline. This preparation was tested on 20-25g mice for any immediate adverse reactions and found to be safe for i.p. injection. This preparation was kindly prepared by Dr. Peter Lambert in the Microbiology Department at Aston University.

7.2. Immunisation of mice with GABHS

12 MF1 male mice weighing 20-25g were kept in groups of 3 in sawdust lined polypropylene cages in a separate room in the animal house under the same animal husbandary conditions as described above, the day before the commencement of the experiment. On day 1 of the

experiment all 6 mice marked as test animals were given 0.1ml of the GABHS preparation i.p. in the morning (9.00 hrs) and in the afternoon (15.00 hrs). The same was repeated on day 3 and 5, for each test animal to receive 6 doses of the GABHS preparation. The animals were kept under observation for their general well being (weight gain, sleep-wake pattern, alertness, grouping, faecal passage and posture) and movements. On day 21 all the test animals were given a booster dose (0.1ml) of the same GABHS preparation at 9.00 hrs. All the control animals were given 0.1ml of saline i.p. in parallel with all the GABHS doses given to the test animals.

7.3. Determination of DOI head shakes

On day 30 the mice were put in groups of 4 (2 test and 2 control mice in each) into aquaria and the DOI induced head shakes determined as described in 3 above.

7.4. Detection of immunity development in mice against Group A β-haemolytic Streptococcal preparation.

After the determination of DOI head shakes, blood was taken from all mice by intracardiac puncture under terminal anaesthesia. The intracardiac puncture was kindly carried out by Mr. M. Gamble. Plasma was separated from the blood as described in 6 under clinical studies and the evidence for the development of immunity against GABHS were tested by an ELISA method as described below.

2 polystyrene microtitre plates were coated for 18 hrs at 5°C with the GABHS preparation used for immunising the mice, suspended in bicarbonate buffer at pH 9.6. Unbound sites on the plates were blocked by the Tween buffer (TTBS) for 3 hrs at 5°C. All 12 mouse plasma samples were diluted 100 times with TTBS (10μl plasma in 1ml TTBS). 100μl of TTBS was pippetted into the first 2 wells on the left-hand corner of the first plate to detect non-specific binding. 200μl of each diluted plasma sample was pippeted into the wells in 12 different rows and doubling dilutions were done serially from each pippetted sample on the microtitre plates with TTBS by pippetting 100μl of TTBS into each well and adding 100μl of the diluted plasma serially. This way the plates were reacted with 6 dilutions of each of the 12 plasma samples. Plasma was incubated for 18 hrs at 5°C with intermittent shaking. At the end of 18hrs the plates were decanted and washed with TTBS repeating the process 3 times for a total of four washes. 100μl of Goat anti-mouse IgG-HRP enzyme conjugate (Sigma-Aldrich LTD., England) in TTBS (diluted 2000 times: 25μl enzyme in 50ml TTBS) were then added into each well and allowed to react for 3hrs at 5°C with intermittent shaking. At the end of 3hrs the plates were decanted and washed 3 times as before with TTBS. After the washing 100μl of the substrate for the IgG-HRP enzyme was pippetted into each well and left at room temperature for 15 min with continuous shaking on a plate shaker. The reaction was stopped by pippetting 100μl of 0.18 M sulphuric acid. The absorbance was detected by a

spectrometer set at 450nm. This assay was kindly designed by Dr. Peter Lambert and was carried out under his guidance.

8. Clinical Studies

Clinical study 1 was designed by Dr. S. L. Handley, Dr. H. E. G. Rickards and Prof. J. A. Corbett, primarily to investigate whether the kynurenine pathway metabolites and neopterin were elevated in TS. Based on some of the findings of the clinical study 1 and the current findings and suggestions on TS, the clinical studies 2 and 3 were designed by me together with Dr. S. L. Handley to determine the state of plasma INF-γ, neopterin, KYN, tryptophan and melatonin secretion in TS and cortisol in ADHD. Permission from the Aston University and South Birmingham ethical committees' were obtained to carry out these studies and the patients and controls were recruited according to the guidelines laid by them. Birmingham Educational Authority approval was obtained for recruiting the child volunteers from local schools as healthy controls for the study 3.

8.1 Recruitment of patients and controls

Study 1

72 male and female TS patients who fell into a wide age range (mean age: 21±1.3 years), both children and adults were recruited in this study. They were patients diagnosed by Professor Mary M. Robertson or Dr. H. E. G. Rickards at the National Hospital, Queen Square, London or Queen Elizebeth Psychiatry Hospital (QEPH), Birmingham, according to the DSMIIIR or DSM-1V criteria previously and attending follow-up clinics at these hospitals. All these patients were assessed for tic severity using the Yale Global Tic Severity Scoring system (Leckman *et al.*, 1989) by Professor Mary M. Robertson or Dr. H. E. G. Rickards. The TS patients on whom associated ADHD were diagnosed have been given a ADHD severity score according to the number of DSM criteria present (some patients have been assessed according to the DSMIIIR and some according to the DSM-IV). All these patients were recruited by Dr. H. E. G. Rickards.

Students and staff of Aston university who volunteered to take part in this study were recruited by Dr. S. L. Handley and Mr. C. M. Gaynor as age and sex matched adult controls. The child controls were recruited from children undergoing cold orthopaedic surgery and who attended Medical Day Unit for allergy testing at Birmingham Children's Hospital, by Dr. H.E.G. Rickards.

Study 2

32 male and female TS patients between 16-45 years of age, diagnosed to have TS by the Consultant Neurospychiatrist, Dr. H. E. G. Rickards according to DSM-IV criteria and attending QEPH, in Birmingham for follow up care were recruited as patients for this study.

The patients' details (age, sex, ethnic group, age of onset, associated conditions and medications) were taken from the clinic records and also a history for the presence of conditions, associated with TS, such as OCD/OCB, ADHD/AD/HD and others (see introduction for associated conditions), current smoking status and medications taken for other physical illness was taken by me. Patients who were suffering from any other neuropsychiatric disorder not recognised to be associated with TS or chronic physical illness were not recruited in the study. None of these patients were on any medication other than for TS or associated conditions at the time they were recruited or for 2 weeks prior. The tic severity of all the patients were assessed by me in the clinic using the Yale Global Tic Severity Scoring system.

Students and staff who volunteered to take part in the study were recruited as age, sex and ethnic group matched healthy controls by Dr. S.L. Handley and me. A brief history was taken from all the healthy controls to make sure they were not suffering from physical or mental illness, were not on any medication and not had a family history of TS or associated conditions. The current smoking status of all the healthy controls were also recorded.

Study 3

34 children diagnosed to have ADHD by Dr. Frank Zaw (Consultant Child Psychiatrist) according to the DSM-IV criteria and attending Diana Princess of Wales Children's hospital in Birmingham for follow-up care were recruited in this study. Treatment details were taken from the clinical records and a history was taken for each child from the accompanying parent or guardian on associated conditions. The severity of ADHD was assessed by getting the parent or the guardian who lived with the child to fill a questionnaire designed by Dr. Frank Zaw, eliciting the degree to which the DSM-IV diagnostic criteria for ADHD were present (see Chapter 4 for details). Age, sex and ethnic group matched healthy controls were recruited from local schools.

8.2 Sample collection

Study 1

Plasma samples separated from the blood taken by venepuncture at the antecubital fossa from TS patients recruited for this study were brought by Dr. H.E.G. Rickards as 0.5ml aliquots. The plasma samples from age and sex matched child controls recruited for this study from Birmingham Children's hospital as mentioned above were supplied from the blood taken for routine investigations in the hospital. The adult controls were bled by Dr. H.E.G. Rickards at Aston University and plasma samples were separated as mentioned below in 8.3. All the plasma samples were stored at -70° C until assayed. These plasma samples were used by Mr. C.M. Gaynor for detecting KYN, tryptophan and neopterin and were used by me for the detection of plasma cortisol and antistreptolysin O titres (see chapters 3 and 4).

Study 2

Blood

7 ml of blood was collected from all the patients on the same day of recruitment to match the YGTSS determined on recruitment. All the bleedings were done into Li/ Heparin tubes by venepuncture at the antecubital fossa by the phlebotomists at the QEPH out patients department.

Dr. P. Karunasekera or myself bled the healthy controls the same way into Li/ Heparin tubes. On the same day as bled, plasma was separated from all the blood samples as described in 8.3 and 1ml aliquots of plasma were stored at -70° C in 2.5ml Li/ Heparin tubes until assayed for plasma INF-γ, neopterin, KYN and tryptophan.

Urine

A 1/ plastic bottle, 100ml measuring cylinder, pipette, 5ml universal tube, an instruction sheet on the collection procedure and a slip to fill in the name, total volume of urine collected and the date were given on recruitment or posted before the clinic attendance to patients for a 12 hour night-time urine collection. Patients collected night-time urine from 8 p.m. to 8 a.m. the following day and the total volume was measured at the end of the collection and 5ml of the total collection with the filled slip stating the total urine volume was handed over in the clinic or posted according to the instructions given. Similar sample of night-time urine was obtained from each matched control in the same month as the patient. These urine samples were used for the 6-sulphatoxymelatonin (aMT6S) assay. aMT6S is stable at room temperature for 5 days (Bojkowski *et al.*, 1987) and therefore all the urine samples were stored at -70° C within 5 days of collection until assayed. Any samples, which were received more than 5 days after collection, were discarded.

Study 3

Saliva

A 2ml sample of saliva was collected from all the ADHD children and their age and sex matched controls recruited in this study, by getting them to spit into a 5ml polypropylene tube. These samples were kept frozen at -70° C untill assayed for cortisol.

8.3 Separation of plasma from whole blood

Plasma was separated from whole blood for all the assays by centrifuging the blood contained in the Li/Heparin tubes at 3500 rpm for 10 min.

9. Detection of plasma antistreptolysin O titres (ASOT)

RapiTex® ASL test kit by Behringwerke AG (Germany) was used to detect ASOT. All reagents and plasma samples collected from study 1 were allowed to reach room temperature. 40µl from each sample of plasma to be tested were pipetted on to separate

fields on the test slide provided in the kit. 40µl of the control positive and negative serum provided in the kit were pipetted on to two separate areas on the same test slide. The contents of the RapiTex ASL reagent bottle were gently mixed and 40µl of this mixed suspension were placed on the test slide next to each of the plasma sample and the positive and negative serum samples. The two drops in each field were mixed thoroughly with a stirring rod and then the slide rotated through several planes to mix. After 2 min each field was examined for agglutination. Distinct agglutination demonstrates an ASOT, which exceeds 200IU/ml ± 20%. Further semi-quantitative analysis of all the plasma samples which showed distinct agglutination was also done by diluting the plasma samples with isotonic sodium chloride solution to detect the samples with a ASOT $\geq$ 300IU/ml and $\geq$ 400IU/ml.

10. Plasma cortisol assay

Coat-a-Count® commercial kit by Diagnostic Product Corporation (Los Angeles) was used to detect plasma cortisol levels by radioimmunoassay. Plasma samples collected in study 1 and stored at -70°C were defrosted and brought to room temperature. 25µl of the standards and plasma samples were dispensed into coated tubes provided in the kit. Four uncoated polypropylene tubes were used to define total and non-specific binding. 1ml of ^{125}I labelled cortisol was added to all the tubes. All tubes except the totals were incubated at 37°C for 45 min in a water bath and decanted at the end of the incubation. After decanting the tubes were drained on a rack and allowed to dry for 2-3 min. All the tubes were counted in a LKB Wallac Compugamma Universal gamma counter for 1 min. The protocol used in the gamma counter autocalculated the plasma cortisol levels in reference to the standard curve.

11. Salivary cortisol assay was done at the Department of Immunoassay of Withington Hospital in Manchester. I took the salivary samples collected in study 3, frozen on dry ice to Manchester.

12. Plasma Interferon-γ assay

INF-γ levels of the plasma samples collected in study 2 were determined using the Biotrak™ high sensitivity interferon-gamma human ELISA system by Amersham Life Science, Buckinghamshire, England. An antibody specific for human INF-γ has been coated on the microtitre plate provided in the kit. When plasma samples are pipetted into the wells the INF-γ if present is bound by this immobilized antibody. Plasma samples and all the reagents were allowed to reach room temperature prior to performing the assay. The wash buffer provided in the kit was diluted with distilled water (1:30) biotynylated antibody reagent provided in the kit was diluted (1:1) with the standard diluent supplied by the manufacturer,

Amdex amplification reagent provided in the kit was reconstituted with 11ml of distilled water and the INF-γ standard was reconstituted with 1.84ml of distilled water and mixed by gently inverting the vial to give a 2500pg/ml solution as instructed by the manufacturer. 400µl of standard diluent was pipetted into a polypropylene tube and 100µl of reconstituted INF-γ standard was added to it and mixed thoroughly making a 500pg/ml stock solution from which working standards (0.63, 1.25, 2.5, 5, 10 and 20 pg/ml) were prepared by serial dilutions. 100µl of the standard diluent were pipetted into 2 wells to detect non-specific binding. 100µl of the standards and 100µl of plasma samples were pipetted into appropriate wells in duplicate. The plate was covered with adhesive strip provided in the kit and incubated for 1 hour at room temperature (20-25°C) with continuous shaking of the plate on a plate shaker. Then the plate was decanted and washed with wash buffer. Four washes were done with filling the plate wells and decanting 3 times at each wash. After the last wash the plate was inverted and blotted against clean paper towelling. Following this washing 100µl of the diluted biotinylated antibody reagent was added into all the wells and covered with new adhesive strip and incubated for 2 hours at room temperature with continuous shaking. This biotinylated antibody was expected to bind to the INF-γ, which bound to the immobilised antibody, which coated the wells. The plate was decanted and washed 4 times as done after the first incubation to wash away any unbound biotinylated antibody. Then 100µl of Amdex amplification reagent was added to all the wells, to bind to the INF-γ, which was bound by both the immobilised and the biotinylated antibody. The plate was covered with a new adhesive strip and incubated at room temperature for 30min with continuous shaking. At the end of 30 min the plate was decanted and washed again as done before to remove any unbound amplification reagent. 100µl of TMB substrate solution was pipetted into all the wells and incubated for 1 hour at room temperature with continuous shaking without covering, for colour to develop in proportion to the amount of INF-γ bound in the initial step. Finally 100µl of the stop solution (0.18M sulphuric acid) was added to each well and the optical density of each well determined within 30 min using a spectrometer set up at 450 nm. The INF-γ levels in plasma samples were determined in reference to the standard curve constructed with the optical densities read for the known INF-γ standards.

13. Plasma tryptophan, kynurenine and neopterin were kindly assayed by Professor D. Fuchs at University of Innsbruck, Austria using high performance liquid chromatography (HPLC). The plasma samples collected in study 2 were sent frozen on dry ice by same day delivery courier service.

14. Urinary aMT6S was detected at Stockgrand Ltd., School of Biological Sciences, University of Surrey, UK by radioimmunoassay. aMT6S/ml was determined on all the urine samples collected in study 2 by the radioimmunoassay and the total night-time (8p.m.-8a.m.) urinary aMT6S excretion for all the patients and controls were determined by multiplyng the aMT6S/ml by the total night-time urine volume. The urine samples were taken frozen on dry ice by me to the Stockgrand Ltd. laboratory in Surrey.

Except for the plasma KYN, neopterin and tryptophan, urinary aMT6S and salivary cortisol assays done outside, all the other biochemical assays described here were done by me.

15. Statistical analysis of data

The data was analysed as described in the relevant results chapters using the Statistical Package for Social Sciences (SPSS).

The difference between test/patient and control groups were compared by Student's t-test (paired or independent samples as appropriate). Analysis of variance was used for the comparison of parametric data from more than two groups as mentioned in the relevant chapters. Comparison of non-parametric frequency data was done by Chi^2 test. Statistical significance was set at a probability level of 0.05. All the probabilities are values for two-tailed probability unless mentioned otherwise.

Spearman correlation coefficient was used for assessing correlations involving rating scales and the correlation of parametric data were assessed by the Pearson product-moment correlation coefficient.

All the arithmatic mean values are shown with one standard error (SEM).

16. Drug and reagent sources and vehicles used

Drugs

α-Methyl-5-hydroxytryptamine maleate	Tocris
Anthranilic acid	Sigma
DOI ((±)-1-(2,5-dimethoxy-4-iodopheny)-2-aminopropane)	RBI
Kynurenine	Sigma
Kynurenic acid	Sigma
Methysergide	RBI
myo-[^{3}H]Inositol	Amersham
Nicotinic acid	Sigma
Nicotinamide adenine dinucleotide	Sigma

Pargyline	Sigma
Picolinic acid	Sigma
Quinolinic acid	Sigma
3-Hydroxyanthranilic acid	Sigma
3-Hydroxykynurenine	Sigma
[^{3}H]Ketanserin	NEN
Xanthurenic acid	Sigma

For the animal experiments and the PI hydrolysis assays all the kynurenine pathway metabolites were dissolved in Krebs physiological solution. For the radioligand binding assays the drugs were dissolved in double distilled water or polyethylene glycol, depending on the solubility.

Reagents and other chemicals

Chloroform			BDH
Dowex anion exchanger			Sigma
Ethanol			Fisons
Formic acid (ammonium salt)			Sigma
Formic acid (approx. 99%)			Sigma
Krebs physiological solution:		g/l	
	Calcium chloride	2.5ml	BDH
	D-Glucose	2.1	Fisher
	Magnesium sulphate	0.29	BDH
	Potassium chloride	0.354	BDH
	Potassium dihydrogen orthophosphate	0.162	BDH
	Sodium bicarbonate	2.1	BDH
	Sodium chloride	6.92	BDH
Methanol			Fisher
Polyethylene glycol			Sigma
Sodium tetraborate			BDH
Sucrose			Fisons
Trizma pre-set crystals for Tris-HCl buffer			Sigma

CHAPTER 1

THE EFFECTS OF KYNURENINE PATHWAY METABOLITES ON DOI-INDUCED HEAD SHAKES IN MICE

CHAPTER 1

Introduction

Head-shakes and wet-dog shakes occur in rodents following administration of agents, which increase 5-HT activity, and have been shown to be due to activation of 5-HT_{2A} receptors (Kennett & Curzon, 1991). DOI ((±)-1-(2,5-dimethoxy-4-iodophenyl)-2-amino propane hydrochloride) is a direct 5-$HT_{2A/2C}$ agonist (Glennon *et al.,* 1986) and several studies have shown that systemic administration of DOI can induce head-shakes in mice (Heaton & Handley, 1989; Ogren and Fuxe, 1989). The head-shake response in rodents is a well-recognised behaviour response to 5-HT_{2A} receptor stimulation (Lucki *et al.,* 1984). Identical movements also occur spontaneously at a low rate in all furred and feathered species (Wei, 1981). These spontaneous movements appear to belong to the grooming repertoire (Wei, 1981), but their significance is otherwise unknown.

However in being sudden, rapid, recurrent, non-rhythmic and stereotyped, head-shakes fulfil the definition of motor tics as applied to humans (APA, 1995). Similar head-shakes and shoulder rotations occur frequently in TS (Robertson, 1989). Handley and Dursun in 1992 have suggested that tic-like movements such as head-shakes, which occur in rodents after a variety of pharmacological challenges may model the tics, observed in TS. In order to validate an animal model for any human condition one must provide face validity (similarities between the disorder and the model including pharmacological considerations), construct validity (a sound theoretical rationale) and predictive validity (which concerns primarily the prediction of drug actions in the clinic from the effects of drugs in the model) (Abrahamson & Seligman, 1977; McKinney & Bunney, 1969). Since the exact aetiology of TS is still not clear, one cannot assess the construct validity. Handley and Dursun (1992) have proposed the tic-like behaviours in rodents as a model for TS as they display face validity for the tics observed in TS.

Plasma kynurenine was shown to be elevated in TS patients (Dursun *et al.,* 1994; Gaynor *et al.,* 1997) and s.c. kynurenine (KYN) has caused potentiation of 5-HTP and DOI induced head-shakes in mice 2 hours after treatment (Handley & Miskin, 1977; McCreary & Handley, 1995; data presented here also confirms the same). These observations have raised the question whether this potentiation of DOI head shakes is by KYN itself or due to a further metabolite of it as the kynurenine pathway metabolism occurs in the liver and other extra hepatic tissues including the brain (see General Introduction).

Of the kynurenine pathway metabolites, KYN can cross the blood-brain barrier efficiently as a result of transport by the large neutral amino acid carrier (Fukui *et al.,* 1991). 3-OH

kynurenine (3-OHKYN) is taken actively into the brain by a sodium-dependent and a sodium-independent system (Eastman *et al.,* 1992). The other kynurenines enter the brain by passive diffusion and this process seems to be quite inefficient except for anthranilic acid (AA); due to their polar nature, neither kynurenic acid (KYNA) nor Quinolinic acid (QUINA) enters the brain efficiently under physiological conditions (Fukui *et al.,* 1991). But studies on rats by Luthman *et al.,* (1996) have shown that 3-OH anthranilic acid (3-OHAA) given s.c. was the most potent precursor of cerebral QUINA, while it was not as effective in raising peripheral QUINA. This observation indicates that an enhanced blood-brain barrier transport of 3-OHAA, and subsequent intracerebral metabolism occurred after systemic 3-OHAA loading. The contribution of peripheral 3-OHKYN to increasing cerebral QUINA was quite small in the above study. Luthman *et al.,* (1996) suggest major species difference in the kynurenine pathway and its regulation of cerebral QUINA after 3-OHKYN administration, based on their observations. AA given systemically or i.c.v. to rats did not appear to be a precursor of cerebral QUINA, suggesting that hydroxylation of AA to 3-OHAA does not occur *in vivo*, and this has been shown previously in a study done on gerbils (Luthman *et al.,* 1996; Saito *et al.,* 1993b). Studies done with radio labelled KYN have not shown any indication of KYN being converted to picolinic acid or anthranilic acid in the rat brain (Schwarcz *et al.,* 1996).
The aims of the experiments presented here were to explore whether the potentiation of DOI head shakes in mice by KYN is due to an action of its own or to a further metabolite of it in the kynurenine pathway and to determine whether other metabolites of the kynurenine pathway have any effect on DOI head shakes: which is a 5-HT_{2A} receptor mediated behaviour response.

Additional description of methods

Kynurenine pathway metabolites shown to enter the brain after systemic administration (see introduction) were administered s.c. to see their effects at different pre-treatment times and i.c.v. to see their immediate effects. The immediate effects were determined 10-15 min after i.c.v. administration of the metabolites.
Firstly the effect of s.c. kynurenine was determined at a dose of 0.5mg/kg (Handley & Miskin, 1977) at five different pre-treatment times: 20, 40, 60, 90 and 120 min. Then kynurenine was administered i.c.v. to see its direct immediate effect at a dose of 0.04μg/mouse: a dose which provides a relatively similar amount of kynurenine to the brain as that enters the brain after systemic administration of 0.5mg/kg (determined on the data from cerebral uptake studies done for kynurenine on rats by Gal and Sherman (1977)) and the determinations were done 15 min after treatment.

The effects of 3-OHKYN on DOI head shakes at a dose range of 0.5-5mg/kg (Handley & Miskin, 1977) were determined 2 hours after s.c. treatment and the immediate effects were determined 20 min after s.c treatment of 0.5mg/kg and 10min after i.c.v. treatment of 100μg/mouse.

The effect of AA was determined 2 hrs after s.c. treatment of a dose of 0.5mg/kg and 10 min after i.c.v. treatment of a dose of 0.5μg/mouse.

The effect of xanthurenic acid (XA) was also determined 10 min after i.c.v. treatment at a dose of 0.5μg/mouse.

The effect of Kynurenic acid was first tested with the highest safe dose free of neurological disturbances: 10μg/mouse (determined by preliminary experiments) administered i.c.v. Higher doses caused hypotonia and ataxia in animals. Four doses below the highest, were tested subsequently (5, 2.5, 0.5 and 0.25 μg/mouse). The effect was determined 10 min after i.c.v. treatment.

The effect of 3-OHAA was determined initially 2 hrs after s.c. administration of a 0.5mg/kg dose. Since 3-OHAA showed an inhibition of DOI head-shakes 2 hrs after s.c. treatment at this dose, its effect at different pre-treatment times (20, 40, 60, 90 and 120 min) was determined. The immediate effect of the metabolite was also determined 10 min after i.c.v. administration of 3-OHAA at a dose of 10μg/mouse.

The effect of QUINA was determined with the highest safe dose of 1μg/mouse, i.c.v.: free of convulsions (Lapin *et al.*, 1996) and four more doses below this: 0.5, 0.25, 0.125 μ g/mouse. The effect was determined for all the doses 10 min after i.c.v.treatment.

Since the percentage of KYN that reaches the brain after systemic administration is shown to be quite low (Gal & Sherman, 1977) and there is not much data on the production and concentrations of the metabolites of QUINA on its conversion to nicotinamide adenine dineucleotide (NAD) in the brain, the direct effect of these metabolites: picolinic acid, nicotinic acid and NAD were also determined 15 min after i.c.v. administration of each at a dose of 0.5μg/mouse.

All the experiments were done with paired controls.

Results

The number of DOI head shakes determined in 10 min for pairs of test and control mice of different treatment groups are shown in tables 1.1- 1.6. The statistical significance of the changes observed was tested by the paired t-test. These results are graphically presented as the mean percentage change ± SEM of DOI head-shakes frequency after treatment in figures 1.1-1.9. The mean percentage changes for each treatment group were

calculated from the percentage change determined for each test animal from its paired control.

Figure 1.1 shows the percentage changes of DOI head shakes frequency from parallel controls after different KYN treatments. These results show that KYN caused a statistically significant inhibition (paired t-test: $p<0.05$) of DOI induced head shakes 1 hour after s.c. treatment and a significant potentiation (paired t-test: $p<0.05$) 2 hours after s.c. treatment and 15 min after i.c.v. treatment.

3-OHKYN did not show a statistically significant change in DOI head shakes frequency at any pre-treatment time with any of the doses tested here after s.c. treatment and after i.c.v. treatment as shown in figures 1.2 and 1.3.

AA failed to show any significant change in DOI head shakes frequency after s.c. or i.c.v. treatment. XA also did not show any significant effect on DOI head shakes (figures 1.4 and 1.5).

KYNA did not show a significant effect on DOI head shakes frequency at any of the five concentrations tested (figure 1.6).

3-OHAA showed a significant inhibition (paired t-test: $p<0.05$) of DOI head shakes 60-120 min after treatment and did not show any effect 20-40 min after s.c. treatment and 10 min after i.c.v. treatment (figure 1.7).

QUINA showed a statistically significant inhibition (paired t-test: $p<0.005$) of DOI head shakes at all the concentrations (0.0625-1 μg/mouse) tested and the inhibition showed a dose dependent pattern (figure 1.8).

Picolinic acid, nicotinic acid and NAD also did not show a significant effect on DOI head shakes (figure 1.9).

Discussion

The results presented here show that out of the kynurenine pathway metabolites tested, KYN cause potentiation and inhibition of DOI head shakes at two different pre-treatment times following systemic treatment. Peak brain levels of KYN have been shown 2 hrs after systemic administration of KYN to rat brain (Gal and Sherman, 1977). The KYN which enters the brain has been shown to remain as KYN itself for up to 1 hr without significant metabolism to its further metabolites and therefore it is suggested as a storage form of neuroactive kynurenines in the brain (Speciale and Schwarcz, 1990).

In the presence of this data on KYN metabolism from previous studies, failure to potentiate DOI head shakes by any of the other metabolites of the kynurenine pathway and i.c.v. KYN showing potentiation of DOI head shakes as a direct immediate effect

suggests that the potentiation of DOI head shakes seen with KYN previously (McCreary and Handley, 1995) and with these experiments is due to an action of its own.

QUINA showed significant inhibition of DOI head shakes as a direct immediate effect at all the concentrations tested and the inhibition was dose dependent. The immediate and the most effective precursor of cerebral QUINA: 3-OHAA also caused inhibition of DOI head shakes 60-120 min after systemic treatment with no effect 20-40 min after systemic treatment and 10 min after i.c.v. treatment. These observations suggest that the effect seen with 3-OHAA is due to a further metabolite of it and it is likely to be QUINA. Speciale *et al.,* (1988) have shown a dose dependent increase of QUINA production in rat liver slices following incubation with KYN and peak serum QUINA levels occurring around 1 hr after intravenous KYN treatment to rats. Even though QUINA does not enter the brain efficiently under physiological conditions (Fukui *et al.,* 1991), Luthman *et al.,* (1996) have shown highest brain QUINA levels resulting in rats from peripheral administration of QUINA itself, compared with all its precursors. Therefore it is possible that the inhibition of DOI head shakes seen with KYN at 1 hr pre-treatment, may be due to the peripherally synthesised QUINA or its precursor, 3-OHAA from the systemically administered KYN entering the brain. The increasing levels of QUINA in the brain by this means may be masking the potentiation of DOI head shakes by KYN during the first part of the time-course experiments.

These experiments have clearly shown that out of the kynurenine pathway metabolites KYN causes potentiation and QUINA causes inhibition of DOI head shakes. The mechanism by which KYN and QUINA act to alter this 5-HT_{2A} mediated behaviour response remains to be explored.

Since QUINA is a NMDA agonist (Stone and Perkins, 1981) it may be acting via activation of these receptors. Infusion of NMDA into the caudate of anaesthetised cats has caused a decrease in extracellular 5-HT, and NMDA receptor antagonists stimulate 5-HT turnover in several brain regions (Becquet *et al.,* 1990; Whitton and Fowler, 1991; Whitton *et al.,* 1992; 1994). Kim *et al.,* (1998) have shown NMDA inhibiting and NMDA antagonists: AP-5, CPP, MK-801, ketamine, dextrophan and dextromethorphan potentiating 5-HT induced head shakes. Considering these results Kim *et al.,* (1998) have suggested that NMDA neurotransmission may modulate the 5-HT function at the 5-HT_2 site. It has also been reported that activation of NMDA receptors cause noradrenaline release in several cortical areas and in the cervical spinal cord (Wang and White, 1998; Pittaluga *et al.,* 1997; Nankai *et al.,* 1998). Since centrally injected noradrenaline has been shown to be highly potent in inhibiting the 5-HT-induced head shakes (Handley, 1970; Collier *et al.,* 1975), a possible mechanism for the antagonistic effect of QUINA on

DOI head shakes could be due to enhancement of central noradrenergic activity. With the above evidence, our results further suggest that NMDA receptor stimulation directly or indirectly modulates 5-HT_{2A} receptor functions.

Several other neurotransmitter systems also have been shown to influence the expression of 5-HT_2-receptor mediated head shakes. β_2 adrenoceptor agonists have shown potentiation of head shakes in mice (Ortmann *et al.,* 1981; Nimganokar *et al.,* 1983). Administration of apomorphine has shown inhibition of head shakes induced by 5-HTP (Corne *et al.,* 1963; Bedard and Pycock, 1977). Administration of benzodiazepines have caused potentiation of 5-MeODMT (serotonin receptor agonist (Glennon *et al.,* 1979)) induced head shakes in mice (Bedard and Pycock, 1977; Mathews and Smith, 1980). The NK-3 tachykinin receptor agonist senktide also has shown induction of head shake response in mice (Stoessl *et al.,* 1987; 1990). Different interactions of the noradrenergic system with head shakes behaviour induced by administration of 5-HTP and direct 5-HT_2 agonists have been shown (Heal *et al.,* 1986). The potentiation of DOI head shakes shown by KYN may be due to an action via one of the receptors shown to cause potentiation of head shakes following its activation or another receptor group which has not been explored.

3-OHKYN did not show a significant effect on DOI head shakes at any of the doses tested here. But previous studies have shown potentiation of 5-HTP induced head shakes in mice by lower doses of 3-OHKYN (0.5mg/kg.) and inhibition by higher doses (Handley and Miskin, 1978). This discrepancy in the effect of 3-OHKYN may be due to a different interaction of the neurotransmitter system via which 3-OHKYN exerts its effects, with head shake behaviour induced by 5-HTP and DOI.

A biologically active substance may alter a receptor-mediated response by acting as an exogenous biomolecular signal. The receptors are designed to receive a specific signal and initiate an enzymatic cascade that leads to a cellular response. The overall scheme of the signalling process involves five fundamental steps: ligand binding, receptor activation, signal transduction, effector activation and signal attenuation. Each of these steps corresponds to points at which signal variation can occur. Defects in the signalling process can lead to physiological abnormalities and possibly, human disease (Fuller and Shields, 1997).

Since results presented here show clearly that some of the kynurenines (KYN and QUINA) can effectively alter the head shake response which is a 5-HT_{2A} mediated central action, further experiments were done to determine whether they act by altering the stage of ligand binding and/or signal transduction. These results are presented in chapter 2.

Table 1.1. Raw data on DOI head shakes counted in test and control mice for 10 min to determine the effects of s.c. KYN at different pretreatment times and 10 min after i.c.v. treatment. The % changes were determined from this data: each test animal being expressed as % matched control.

0.04μg/mouse i.c.v. 10 min pre-treatment		**0.5mg/kg s.c. 20 min pre-treatment**		**0.5mg/kg s.c. 40 min pre-treatment**		**0.5mg/kg s.c. 60 min pre-treatment**		**0.5mg/kg s.c. 90 min pre-treatment**		**0.5mg/kg s.c. 120 min pre-treatment**	
test	**control**	**test**	**control**	**test**	**control**	**test**	**control**	**test**	**control**	**test**	**control**
28	22	24	22	21	21	16	21	14	14	30	21
17	13	21	17	21	25	16	22	19	20	27	18
21	16	20	19	18	21	11	21	15	15	30	18
25	20	20	20	21	20	11	17	14	14	24	18
25	21	24	22	25	24	14	19	18	21	24	22
25	21	20	20	26	26	12	18	18	20	22	13
		21	25	15	17			18	17		
				15	14						
paired t-test p<0.05		paired t-test p> 0.05		paired t-test p> 0.05		paired t-test p< 0.05		paired t-test p> 0.05		paired t-test p<0.05	

Table 1.2. Raw data on DOI head shakes counted in test and control mice for 10 min to determine the effects of different 3-OHKYN treatments. The % changes were determined from this data: each test animal being expressed as % matched control.

0.5mg/kg s.c. 2hrs pretreatment		**2.5 mg/kg s.c., 2hrs pretreatment**		**5 mg/kg s.c. 2hrs pretreatment**		**0.5mg/kg s.c., 20 min pretreatment**		**100μg/mouse i.c.v. 15 min pretreatment**	
test	**control**	**test**	**control**	**test**	**control**	**test**	**control**	**test**	**control**
25	24	25	27	23	21	21	24	17	15
33	33	25	29	24	23	17	19	13	11
24	26	25	29	20	22	18	22	23	19
13	14	25	27	20	19	19	23	15	14
18	14	22	25	23	23	19	23	17	15
17	18	21	24	26	24	18	16	11	10
29	30					16	15		
paired t-test p> 0.05		paired t-test p> 0.05		paired t-test p> 0.05		paired t-test p> 0.05		paired t-test p> 0.05	

Table 1.3. Raw data on DOI head shakes counted in test and control mice for 10 min to determine the effects of AA and XA treatments. The % changes were determined from this data: each test animal being expressed as % matched control.

AA: 0.5mg/kg s.c. 20 min pretreatment		AA: 0.5mg/kg s.c. 120 min pretreatment		AA: 0.5μg/mouse i.c.v. 15 min pretreatment		XA: 0.5μg/mouse i.c.v. 15 min pretreatment	
test	control	test	control	test	control	test	control
23	25	23	23	23	22	18	15
22	25	23	23	23	20	18	18
21	23	20	23	25	24	22	16
22	23	23	20	25	25	16	18
23	25	22	20	24	22	18	19
22	25	20	22	23	22	16	17
paired t-test p> 0.05		paired t-test p> 0.05		paired t-test p> 0.05		paired t-test p> 0.05	

Table 1.4. Raw data on DOI head shakes counted in test and control mice for 10 min to determine the effects of i.c.v. KYNA at a dose range of 0.25-10μg/mouse. The % changes were determined from this data: each test animal being expressed as % matched control.

0.25 μg/mouse		0.5 μg/mouse		2.5 μg/mouse		5 μg/mouse		10 μg/mouse	
test	control	test	control	test	control	test	control	test	control
14	22	18	21	29	22	26	24	17	20
22	22	20	25	28	26	28	26	11	12
12	11	17	11	27	26	28	17	8	8
15	11	20	21	24	17	25	24	14	12
22	21	18	21	28	26	27	26	5	7
22	22	20	21	26	26	28	25	7	7
paired t-test p>0.05		paired t-test p>0.05		paired t-test p>0.05		paired t-test p>0.05		paired t-test p>0.05	

Table 1.5. Raw data on DOI head shakes counted in test and control mice for 10 min to determine the effects of s.c. 3-OHAA at different pretreatment times and 10 min after i.c.v. treatment. The % changes were determined from this data: each test animal being expressed as % matched control.

0.5mg/kg s.c., 20 min pretreatment		**0.5mg/kg s.c., 40 min pretreatment**		**0.5mg/kg s.c., 60 min pretreatment**		**0.5mg/kg s.c., 90 min pretreatment**		**0.5mg/kg s.c., 120 min pretreatment**		**10μg/mouse i.c.v., 10 min pretreatment**	
test	**control**	**test**	**control**	**test**	**control**	**test**	**control**	**test**	**control**	**test**	**control**
16	17	16	15	8	15	10	17	15	21	15	14
14	16	16	17	14	18	13	19	18	28	15	17
22	20	17	16	18	22	12	18	15	24	16	16
23	22	14	17	15	18	15	23	26	34	22	20
21	23	22	20	13	17	14	22	23	32	14	13
23	22	22	23	16	18	17	21	16	26	21	19
								23	30		
paired t-test p>0.05		paired t-test p>0.05		paired t-test p<0.05		paired t-test p<0.05		paired t-test p<0.05		paired t-test p<0.05	

Table 1.6. Raw data on DOI head shakes counted in test and control mice for 10 min to determine the effects of i.c.v. QUINA at a dose range of 0.0625-1μg/mouse. The % changes were determined from this data: each test animal being expressed as % matched control.

0.0625μg/mouse i.c.v.		**0.125μg/mouse i.c.v.**		**0.25μg/mouse i.c.v.**		**0.5μg/mouse i.c.v.**		**1μg/mouse i.c.v.**	
test	**control**	**test**	**control**	**test**	**control**	**test**	**control**	**test**	**control**
18	21	15	19	14	22	10	15	13	22
17	21	15	19	17	22	7	16	17	23
17	20	14	19	18	23	13	22	16	23
18	20	14	18	14	24	14	21	16	24
20	22	14	18	18	24	12	20	11	23
21	23	14	18	15	23	11	17	17	24
				18	24	11	24		
						15	23		
paired t-test p<0.05		paired t-test p<0.05		paired t-test p<0.05		paired t-test p<0.05		paired t-test p<0.05	

Table 1.7. Raw data on DOI head shakes counted in test and control mice for 10 min to determine the effects of PA, NA and NAD treatments. The % changes were determined from this data: each test animal being expressed as % matched control.

PA: 0.5μg/mouse		**NA: 0.5μg/mouse**		**NAD: 0.5μg/mouse**	
test	**control**	**test**	**control**	**test**	**control**
19	20	19	16	26	21
13	15	17	16	22	27
26	24	24	22	26	23
20	18	21	19	22	26
19	19	17	20	28	28
20	19	20	20	24	25
paired t-test p>0.05		paired t-test p>0.05		paired t-test p>0.05	

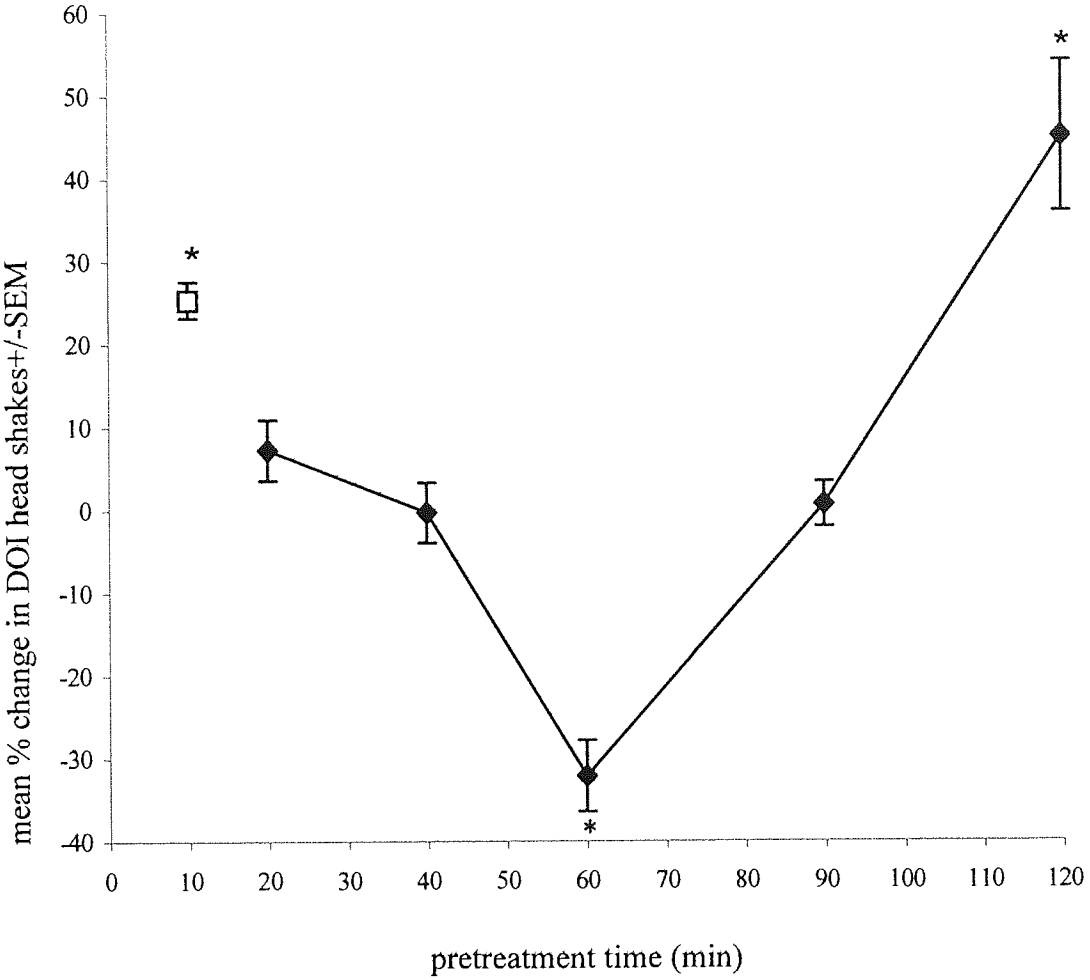

Figure 1.1. Effect of KYN on DOI head shakes frequency at different pretreatment times shown as % change from parallel controls. □ = drugs administered i.c.v., ♦ = drugs administered s.c. Each data point represents the mean % change from the paired control for each treatment group (N= 6/7/8 pairs per group). Vertical bars represent standard errors. * = head shake frequencies showed statistically significant change from the parallel controls (paired t-test, $p < 0.0001$).

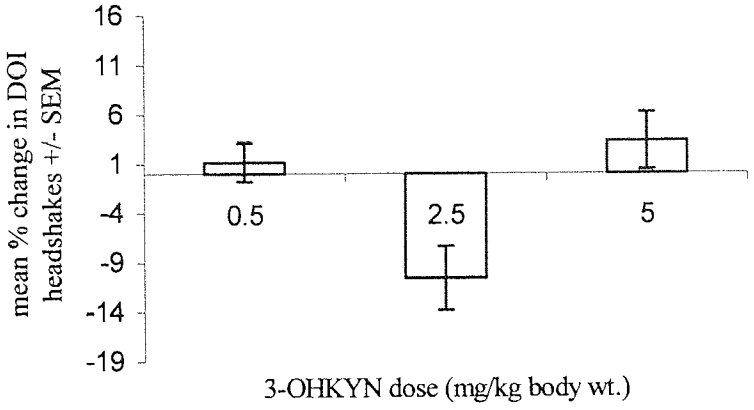

Figure 1.2. Effect of 3-OHKYN (0.5, 2.5 and 5mg/kg body weight) on DOI head shakes frequency 2 hours after s.c. treatment. Each data column represents the mean % change in DOI head shakes from the parallel control (N= 6/7 per group) and the vertical bars represent standard errors. The changes shown here were not statistically significant on comparison with parallel controls (paired t-test, $p > 0.05$).

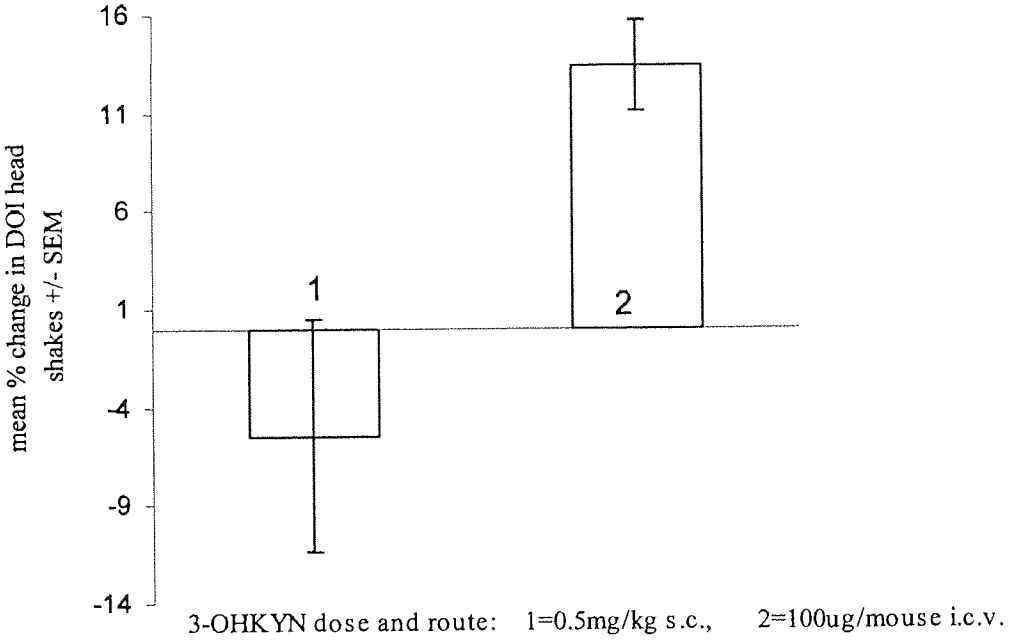

Figure 1.3. Effect of 3-OHKYN 20min after s.c. injection and 15min after i.c.v. injection. Each data column represents the mean % change in DOI head shakes from the parallel control (N= 6 per group) and the vertical bars represent standard errors. The changes shown here were not statistically significant on comparison with parallel controls. (paired t-test, $p > 0.05$).

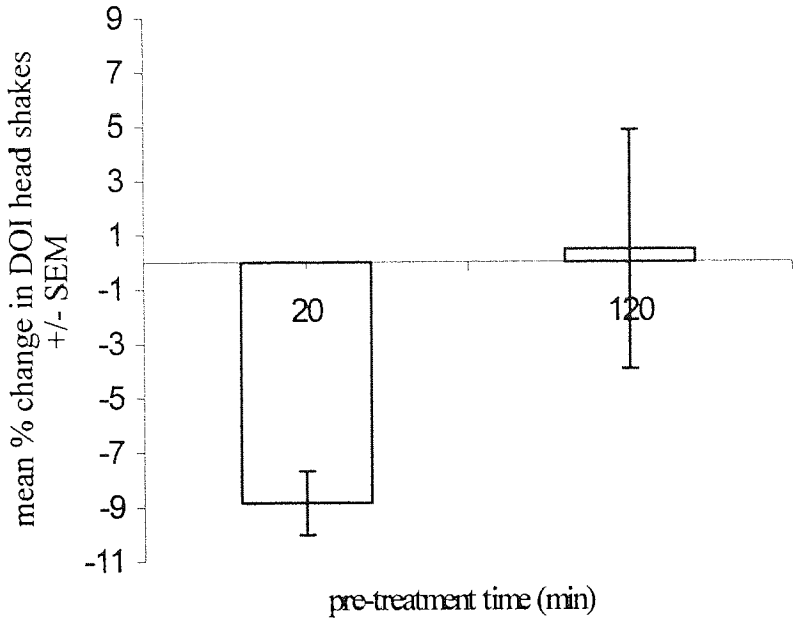

Figure 1.4. Effect of AA (0.5mg/kg) on DOI head shakes after s.c. treatment. Each data column represents the mean % change in DOI head shakes from the paired control and the vertical bars represent standard errors (N=6/group). These changes were not statistically significant on comparison with paired controls (paired t-test, $p > 0.05$).

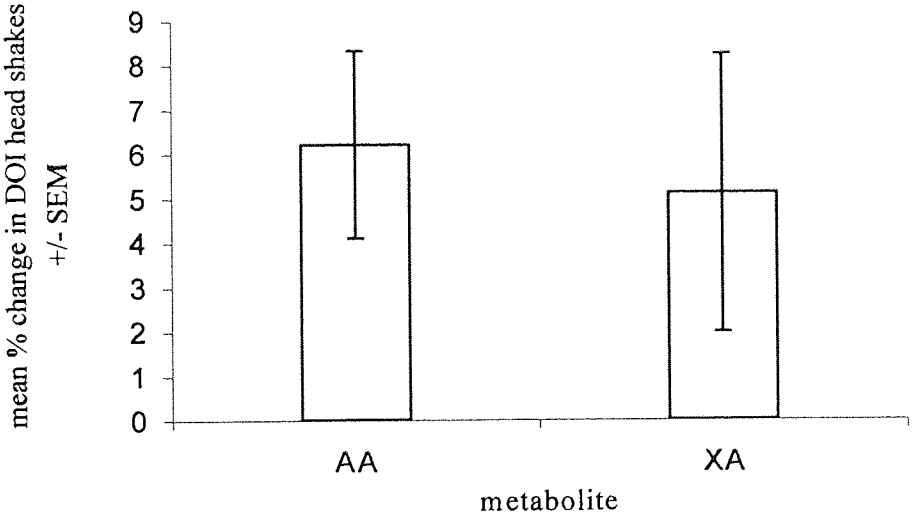

Figure 1.5. Effect of XA and AA on DOI head shakes after i.c.v. treatment. (Dose = XA: 5μg/mouse and AA: 0.5 μg/mouse). Each data column represents the mean % change in DOI head shakes from the paired control and the vertical bars represent standard errors (N=6/group). These changes were not statistically significant on comparison with paired controls (paired t-test, $p > 0.05$).

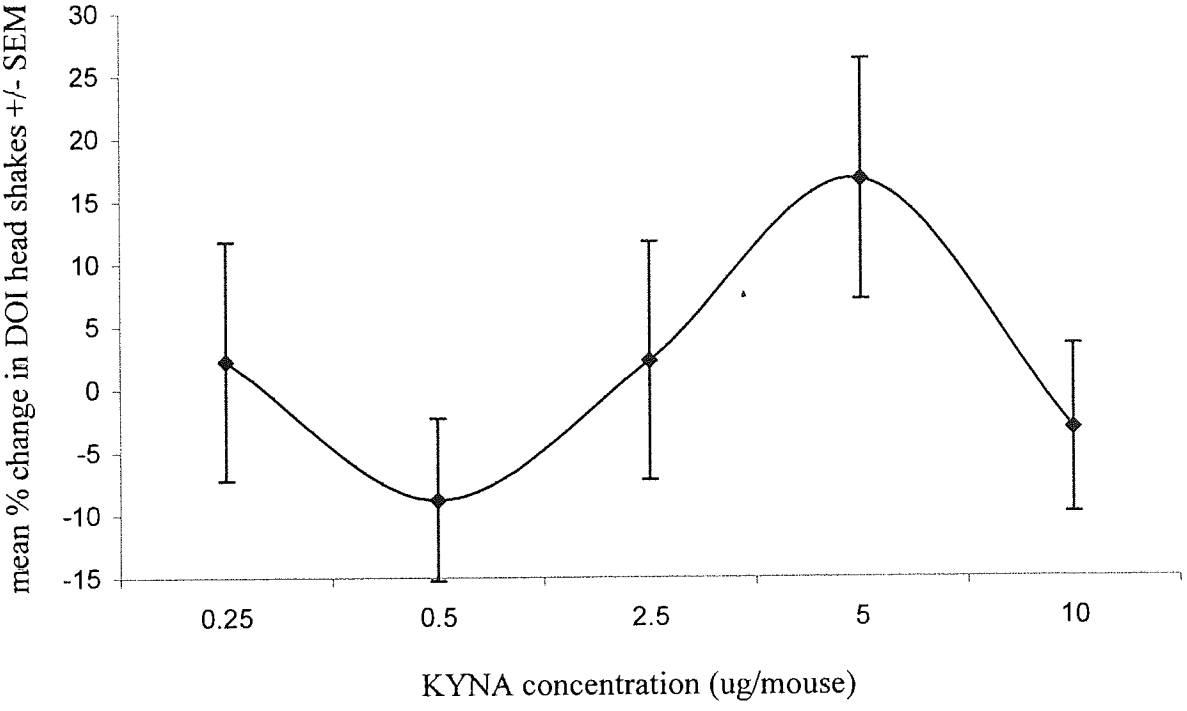

Figure 1.6. Effect of i.c.v. KYNA on DOI head shakes. Each data point represents mean % change in DOI head shakes from paired control for different treatment groups and vertical bars represent standard errors (N=6 per group). None of these changes were statistically different on comparison with paired controls (paired t-test, $p>0.05$).

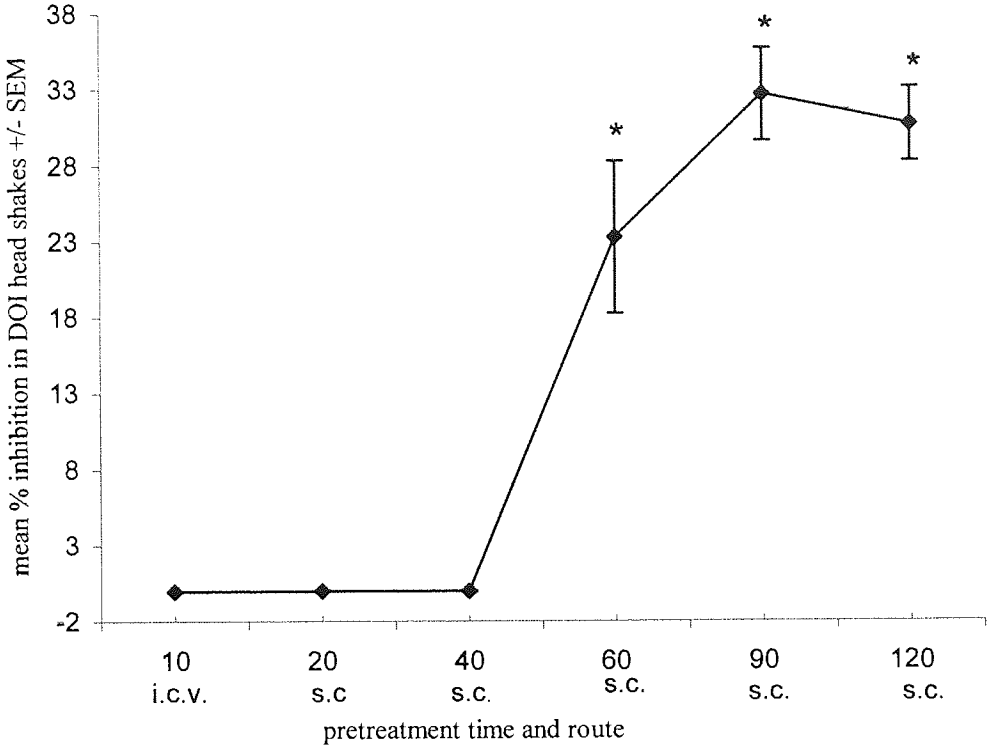

Figure 1.7. Effect of 3-OHAA on DOI head shakes at different pretreatment times. Data points represent mean % changes in DOI head shakes from paired controls and vertical bars represent standard errors, N=6 per group. * = statistically significant inhibition shown on comparison with parallel controls (paired t-test, $p < 0.0001$).

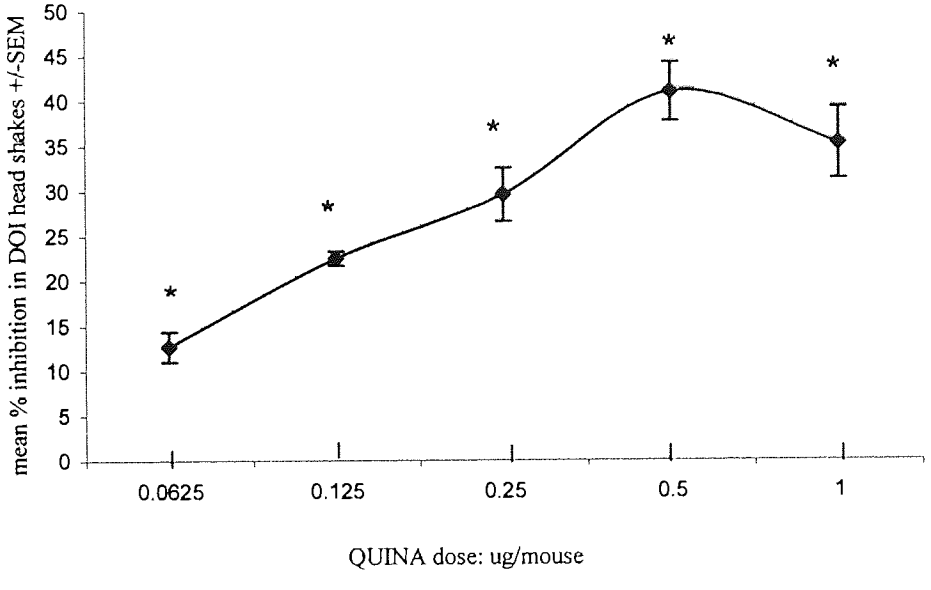

Figure 1.8. Effect of QUINA on DOI head shakes. Data points represent mean % changes in DOI head shakes from paired controls and vertical bars represent standard errors, N=6 per group. * = statistically significant inhibition shown on comparison with parallel controls (paired t-test, $p < 0.0001$).

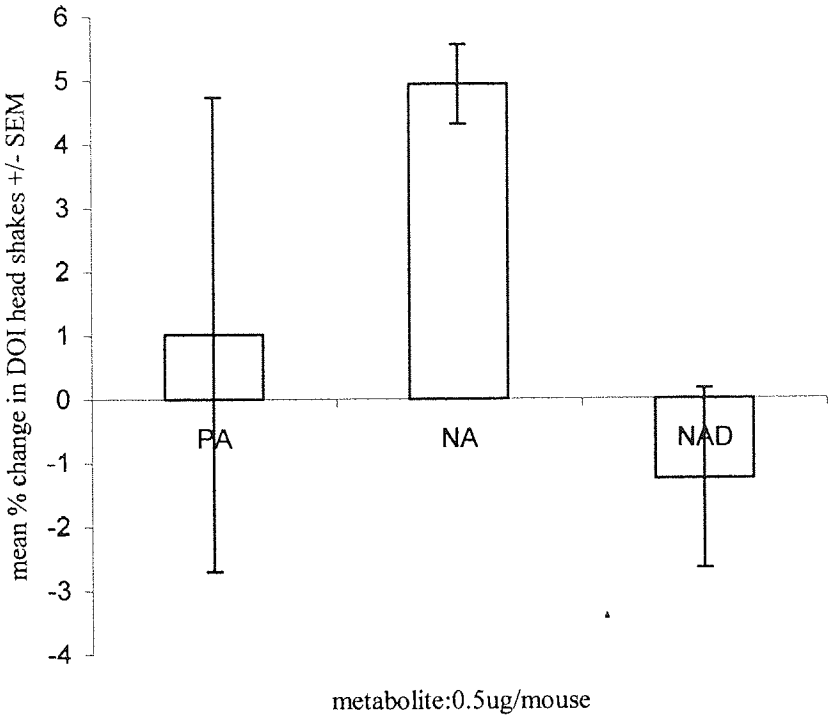

Figure 1.9. Effect of picolinic acid (PA), nicotinic acid (NA) and nicotinamide adenine dinucleotide (NAD) on DOI head shakes. Columns represent the mean % change in DOI head shakes from the paired control and the vertical bars represent standard errors (N=6 per group). These changes were not statistically significant on comparison with paired controls ($p>0.05$).

CHAPTER 2

THE EFFECTS OF KYNURENINE PATHWAY METABOLITES ON 5-HT_{2A} RECEPTOR BINDING AND 5-HT_{2A}-MEDIATED PHOSPHOINOSITIDE HYDROLYSIS.

CHAPTER 2

Introduction

The head shakes in rodents that have been characterised as a 5-HT_{2A} receptor mediated behaviour (Kennett & Curzon, 1991) and suggested to model the tics observed in Tourette syndrome (Handley & Dursun, 1992) occur spontaneously at a low rate (Wei, 1981) and are induced to occur at a high rate by agents increasing 5-HT_{2A} receptor activation (Green *et al.*, 1983). Previous studies have shown that the first stable metabolite of the kynurenine pathway, KYN, potentiates head shakes in mice induced by administration of the $5HT_{2A/2C}$ agonist DOI (McCreary & Handley, 1995) and results presented in Chapter 1 show that KYN potentiates DOI induced head shakes and QUINA inhibits them (see Chapter 1). Therefore it was worth detecting how other kynurenine pathway metabolites affect this 5-HT_{2A} receptor mediated function, in order to determine whether altered kynurenine pathway metabolites in TS could be a causative factor in the expression of tics, rather than an echophenomenon or a marker.

One way of studying interactions of substances with receptors is radioligand binding assay, by which the ability of a substance to act as an agonist/antagonist by direct binding to a receptor or the ability to alter binding of a known agonist/antagonist can be determined (Cooper *et al.*, 1998). Several neurotransmitters, hormones and other extracellular stimuli impart information to their target cells by stimulating cell-surface receptors that activate phospholipase C (phosphoinositidase C), which hydrolyses the bond between diacylglycerol and inositol 1,4,5-trisphosphate (IP3) portions of phosphatidylinositol 4,5-bisphosphate (PI), which is a quantitatively minor, but metabolically lively membrane lipid (Kirk *et al.*, 1981; Michell *et al.*, 1981). PI hydrolysis is a key event in signal transduction and is well recognised as the signal transduction pathway linked to the 5-HT_{2A} receptors located in the cerebral cortex (Conn & Sanders Bush, 1985). Both products of PI hydrolysis give rise to second messengers and thus represent a bifurcation in the signal pathway. The diacylglycerol functions by stimulating protein kinase C (Nishizuka, 1984; 1986) whereas IP3 mobilises Ca^{++}, which in turn activates several proteins resulting in a number of biological actions including gene expression (Berridge & Irvin, 1984; 1989; Fuller & Shields, 1998). The IP3 released to the cytosol is dephosphorylated back to free inositol. Two separate pathways have been shown for this dephosphorylation and in one pathway, IP3 is dephosphorylated sequentially to free inositol (Downes *et al.*, 1982; Storey *et al.*, 1984; Irvine *et al.*, 1986). The final dephosphorylation step in this sequential pathway is inhibited by Li^+ causing accumulation of inositol-4-monophosphate (I-1-P) (Hallcher & Sherman, 1980; Michell,

1986; Berridge, 1986). In addition to 5-HT, acetylcholine (muscarinic), noradrenaline (α_1), histamine (H_1), vasopressin (V_1), substance P, bradykinin, neurotensin and glutamate transmitters are also known to operate through this inositol lipid mechanism (see review by Berridge, 1986). Findings by Smith *et al.* (1990) have suggested that changes in receptor sensitivity as reflected in second messenger responses (5-HT mediated PI hydrolysis in cerebral cortical slices) correlate more closely with 5-HT_{2A}-mediated behaviour than with receptor binding.

The aim of the experiments presented here were to determine whether KYN and/or its further metabolites (3-hydroxy kynurenine (3-OHKYN), 3-hydroxy anthranilic acid (3-OHAA), quinolinic acid (QUINA), kynurenic acid (KYNA) and xanthurenic acid (XA)) could directly bind to 5-HT_{2A} receptors or whether some of the metabolites with important biological actions (KYN, KYNA and QUINA) might enhance 5-HT_{2A} receptor binding affinity by an indirect mechanism and to detect whether the kynurenine pathway metabolites which altered 5-HT_{2A} mediated head shakes (KYN and QUINA, see results presented in chapter 1), alter 5-HT_{2A} mediated PI hydrolysis and if they did whether the changes correlate with the alterations in 5-HT_{2A} mediated head-twitch response.

Additional description of experimental methods.

Mouse cortical tissue has been shown to be rich in 5-HT_{2A} receptors and 5-HT receptor binding sites are more stable after death (Van Wykim & Korf, 1981; Mann *et al.*, 1985). Therefore radioligand binding assays and PI hydrolysis assays were done on mouse cortical tissue as decribed under experimental methods.

Results

Radioligand binding assays

All the binding data was analysed using the EBDA iterative curve fitting program (Mc Pherson, 1983).

[^{3}H] Ketanserin binding was of high affinity and non-specific binding was low (0.12% of specific binding). Saturation binding analyses gave data, which fitted a single site-binding model with a Hill slope almost equal to 1 (0.98) and an *S* shaped semilogarithmic plot (figure. 2.1). The K_d calculated by non-linear regression analysis was 0.53nM and the B_{max} was 17300pmole/g wet weight of tissue. The figure 2.2 shows the graphical representation of the Scatchard analysis data from which K_d and the B_{max} was computed. On this graph the K_d is the negative reciprocal of the slope, and the B_{max} is the intercept on the x-axis.

The DPM (decays per min) counts obtained by scintillation counting from the first set of displacement experiments were used to determine the % displacement of [^{3}H] Ketanserin by the six kynurenine pathway metabolites tested. No detectable displacement was seen with the lower seven concentrations (0.1nM-0.5μM) of any of the six metabolites. The mean percentage displacements by the higher three concentrations (1, 10 and 100μM) of the six metabolites: KYN, 3-OHKYN, 3-OHAA, QUINA, KYNA and XA from six determinations are shown in fig.2.3-2.5 and these were low (< 50%) even at the highest concentration (100μM) tested, and did not show a concentration dependent pattern.
The second set of displacement experiments showed displacement of bound [^{3}H] Ketanserin by Methysergide dose dependently (Figure 2.6 shows the Methysergide displacement curve). The DPM counts from these experiments were used to determine the IC_{50} of methysergide with the EBDA iterative curve fitting program (McPherson, 1983). The IC_{50} values were converted to K_i values using the Cheng and Prusoff (1973) equation:

$$K_i = \frac{IC_{50}}{1+([\text{ligand}]/K_d)}$$

Table 2.1 shows the mean K_i values of methysergide: alone (control) and in the presence of three concentrations (1,10,100μM) of KYN, KYNA and QUINA from six determinations. The concentrations tested here are the highest three concentrations tested in the first set of displacement experiments. Statistical analysis by one-way ANOVA followed by Tukey-HSD showed that the K_i values of methysergide in the presence of kynurenines were not significantly different from control except for that obtained in the presence of KYNA at 100μM concentration which was significantly higher ($p<0.05$).

PI hydrolysis assays

[^{3}H] I-1-P was measured in the experiments presented here as a measure of PI hydrolysis. Results are expressed as a fold increase of sample I-1-P cpm over basal cpm (basal levels are calculated from tissue blank cpm subtracted from no drug cpm). Alpha methyl 5-HT caused dose dependent PI hydrolysis in mouse cortical tissue as expected in these experiments (see figures 2.7 and 2.8). The fold increase of 1-1-P at each concentration of alpha methyl 5-HT in the presence of 1μM concentration of KYN and QUINA were compared with the corresponding fold increase of 1-1-P by alpha methyl 5-HT alone (control) by paired t-test. Even though the alpha methyl 5-HT dose response curve was altered in the presence of KYN (figure2.7), the alteration of PI hydrolysis at any of the individual concentrations of alpha methyl 5-HT was not statistically significant.

Figure2.8 shows the alpha methyl 5-HT induced PI hydrolysis in the presence of QUINA and the PI hydrolysis seems to be inhibited by QUINA at all the concentrations of alpha methyl 5-HT, except at 10^{-6}M concentration while this inhibition is statistically significant at the higher three concentrations of alpha methyl 5-HT (10^{-3}-10^{-5}M) ($p< 0.05$, paired t-test).

Discussion

[^{3}H] Ketanserin is a high affinity selective ligand for 5-HT_{2A} receptors (Leysen *et al.*, 1981). The percentage displacements by the six kynurenine pathway metabolites tested here were low even at a concentration of 100μM. This suggests that none of these metabolites is likely to be a direct agonist at 5-HT2A receptors at physiologically relevant concentrations.

The K_i value is a measure of the affinity of the inhibitor (methysergide) for the receptor. The comparison of K_i values of methysergide was done to see whether there was an indirect effect of any of these three neuroactive kynurenines, KYN, KYNA or QUINA on 5-HT2A receptor binding, manifested as an alteration in the binding affinity of a known ligand (methysergide). But our data suggests that even an indirect effect of these metabolites is not present on 5-HT_{2A} receptor binding. On statistical analysis, kynurenic acid at a concentration of 100μM showed a significant increase in the K_i value of methysergide from that of the control, which may be associated with its relatively high displacement shown in the first set of displacement experiments. However, this indicated a decreased, rather than an increased affinity for methysergide in the presence of KYNA and this finding does not correspond with the head shake data for KYNA or KYN. These findings do not suggest that these kynurenines are likely to affect 5-HT_{2A} receptor binding affinity even indirectly.

Alpha methyl 5-HT is a 5-$HT_{2A/2C}$ agonist (Regunathan and Sourke, 1990). The alteration of the alpha methyl 5-HT PI hydrolysis dose response curve by KYN at 1μM concentration was not statistically significant at any point. Considering the dose at which KYN potentiated 5-HT_{2A}-mediated head twitch response, failure to affect PI hydrolysis at 1μM, suggests that the potentiation of 5-HT_{2A}-mediated head twitch response by KYN is unlikely to be due to an effect of it on 5-HT_{2A}-mediated PI hydrolysis. QUINA caused inhibition of 5-HT_{2A}-mediated PI hydrolysis significantly at higher concentrations of the 5-HT_{2A} agonist. The inhibition of PI hydrolysis by QUINA shown here is unlikely to be due to neurotoxicity as the dose used in these experiments (1μM) is less than the lowest QUINA dose detected to be neurotoxic on cultured neurones: ED50 for QUINA toxicity in cultured rat neurones following a 20 min exposure was 2mM (Kim and Choi, 1987).

The effect of QUINA on 5-HT_{2A}-mediated PI hydrolysis corresponded to the effect of QUINA on 5-HT_{2A}-mediated head twitch response, both being an inhibition (see Chapter 1). QUINA is a well recognised selective NMDA agonist (Stone and Perkins, 1981). There is conflicting evidence of NMDA as a stimulator of PI hydrolysis and it is less evident than that by the other excitatory amino acids, quisqualate and ibotenate (Recasens *et al.*, 1991). Besides a stimulating effect on PI hydrolysis, it has been reported that a number of EAAs, including NMDA inhibits PI hydrolysis induced by other neuroactive substances (Godfrey *et al.*, 1988; Jope and Li, 1989). The muscarinic-receptor-mediated PI hydrolysis (Noble *et al.*, 1989), 5-HT stimulated PI hydrolysis (Noble *et al.*, 1989) and adrenergic receptor-mediated PI hydrolysis (Gonzales and Moerschbaecher, 1989) in cortical slices have been shown to be inhibited by NMDA. Therefore the inhibition of PI hydrolysis detected with QUINA here may be secondary to its action on NMDA receptors. There is no clear evidence of the modes of EAA action to inhibit PI hydrolysis triggered by other neurotransmitters, but a number of speculative proposals on the modes are present and of them the following may be relevant to the inhibitory action of QUINA on 5-HT_{2A}-mediated PI hydrolysis: depolarization induced by EAA causing a substantial Na^+ influx, which in turn produces the inhibition observed (Baudry *et al.*, 1986) and releasing substances with inhibitory actions on PI hydrolysis (Linden & Delahunty, 1989).

None of the kynurenine pathway metabolites tested in these experiments including KYN and QUINA bound to 5-HT_{2A} receptors or indirectly altered 5-HT_{2A} binding. KYN also failed to show any significant effect on 5-HT_{2A}-mediated PI hydrolysis, leaving the mechanism by which KYN alters the 5-HT_{2A}-mediated head twitch response unresolved. Since there are many receptor interactions between several neurotransmitter systems and other substances, it is possible that KYN may be acting indirectly via another receptor system. There is a lot of evidence of other neurotransmitter systems modulating head shaking behaviour (Handley & Dursun, 1992). Even though QUINA did not directly bind or indirectly alter binding at the 5-HT_{2A} receptors, it significantly inhibited 5-HT_{2A}-mediated PI hydrolysis, and this may be the only mechanism or one of the mechanisms by which QUINA inhibits the 5-HT_{2A}-mediated head twitch response. Further research is required to elucidate the mechanisms by which KYN and QUINA interact with 5-HT_{2A} receptor activation. These findings together with the relevant background findings discussed in this chapter suggest investigations to detect whether KYN acts via some other unidentified receptor activation and whether the speculative proposals on the modes of inhibition of neurotransmitter mediated PI hydrolysis by EAA, occur in the case of inhibition of 5-HT_{2A}-mediated PI hydrolysis by QUINA.

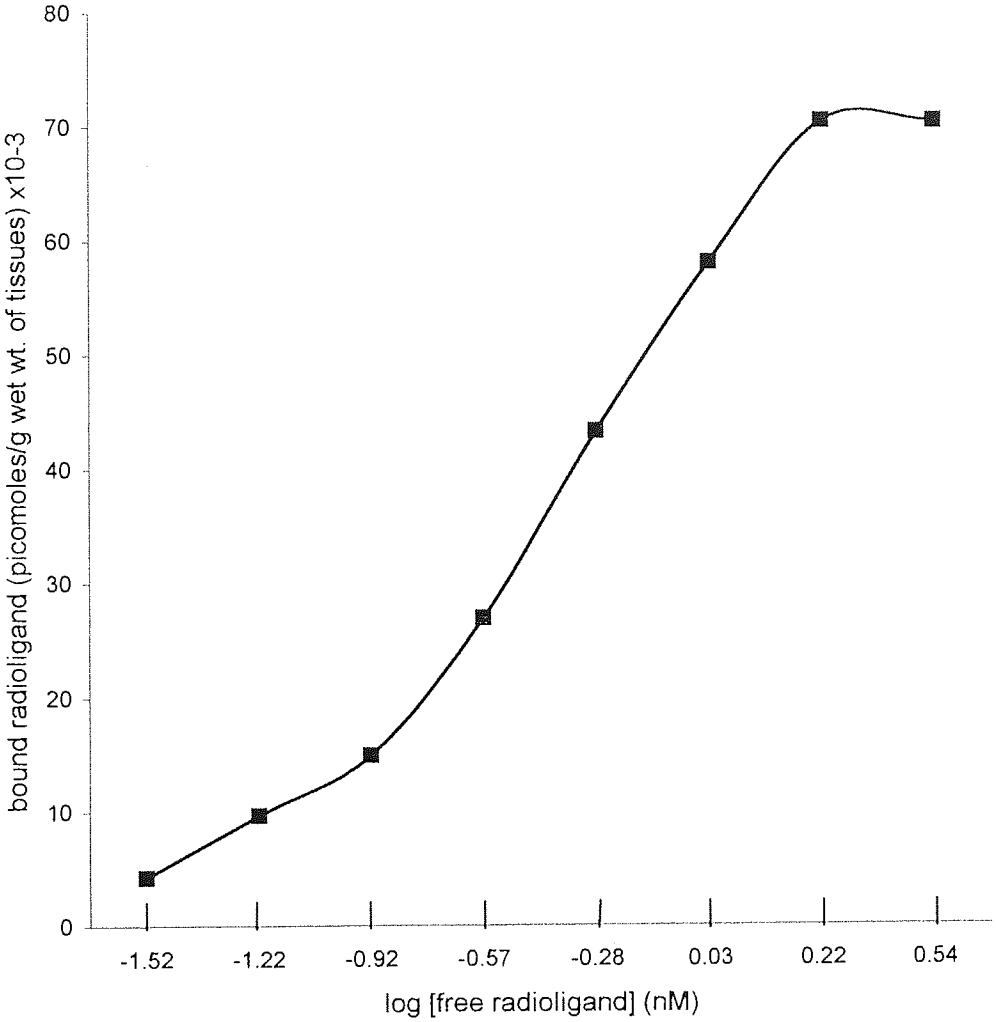

Figure 2.1. Semilog saturation binding plot for [^{3}H] Ketanserin. Specific binding is plotted as a function of the log of free radioligand concentration using the data from saturation binding experiments: each data point corresponds to the mean from 3 paired determinations.

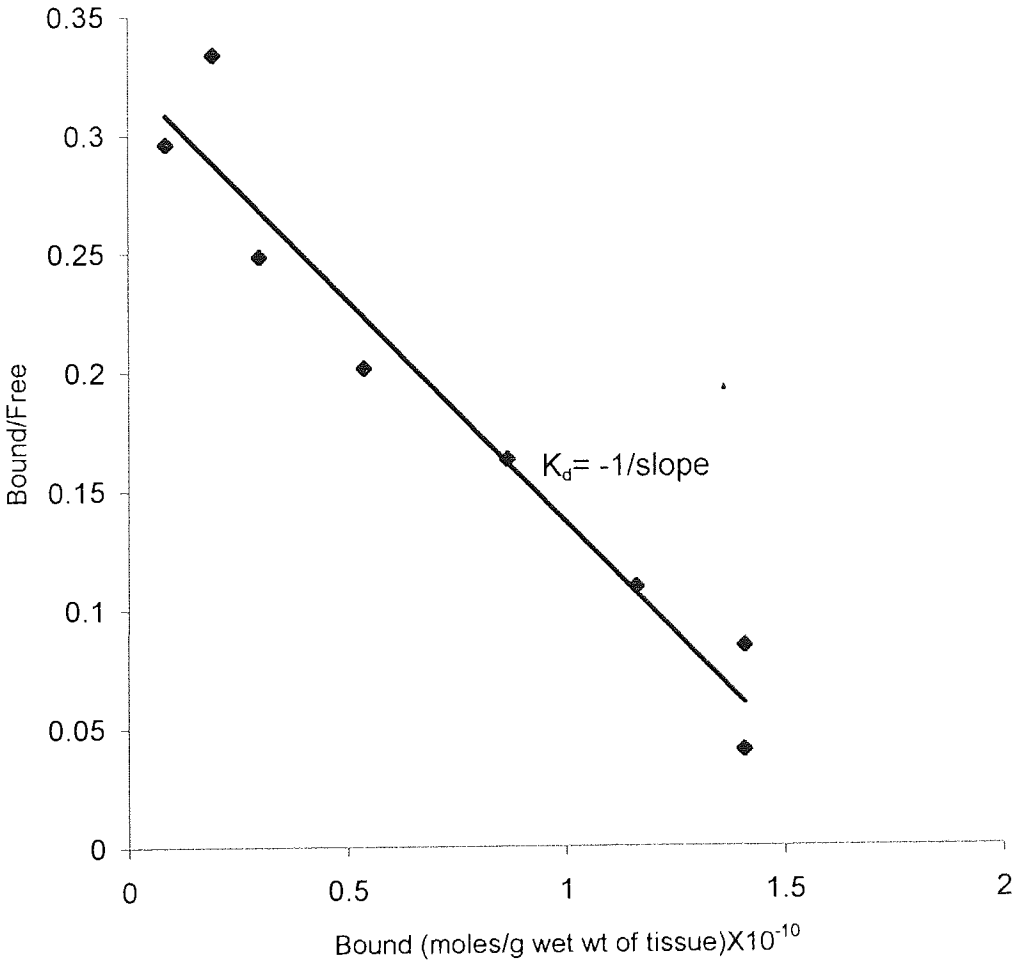

Figure 2.2. Scatchard plot for [3H] Ketanserin binding from saturation binding experiments (N=3 paired determinations). Intercept on the X axis =$1.7x10^{-10}$ moles/g wet wt. of tissue = B_{max}

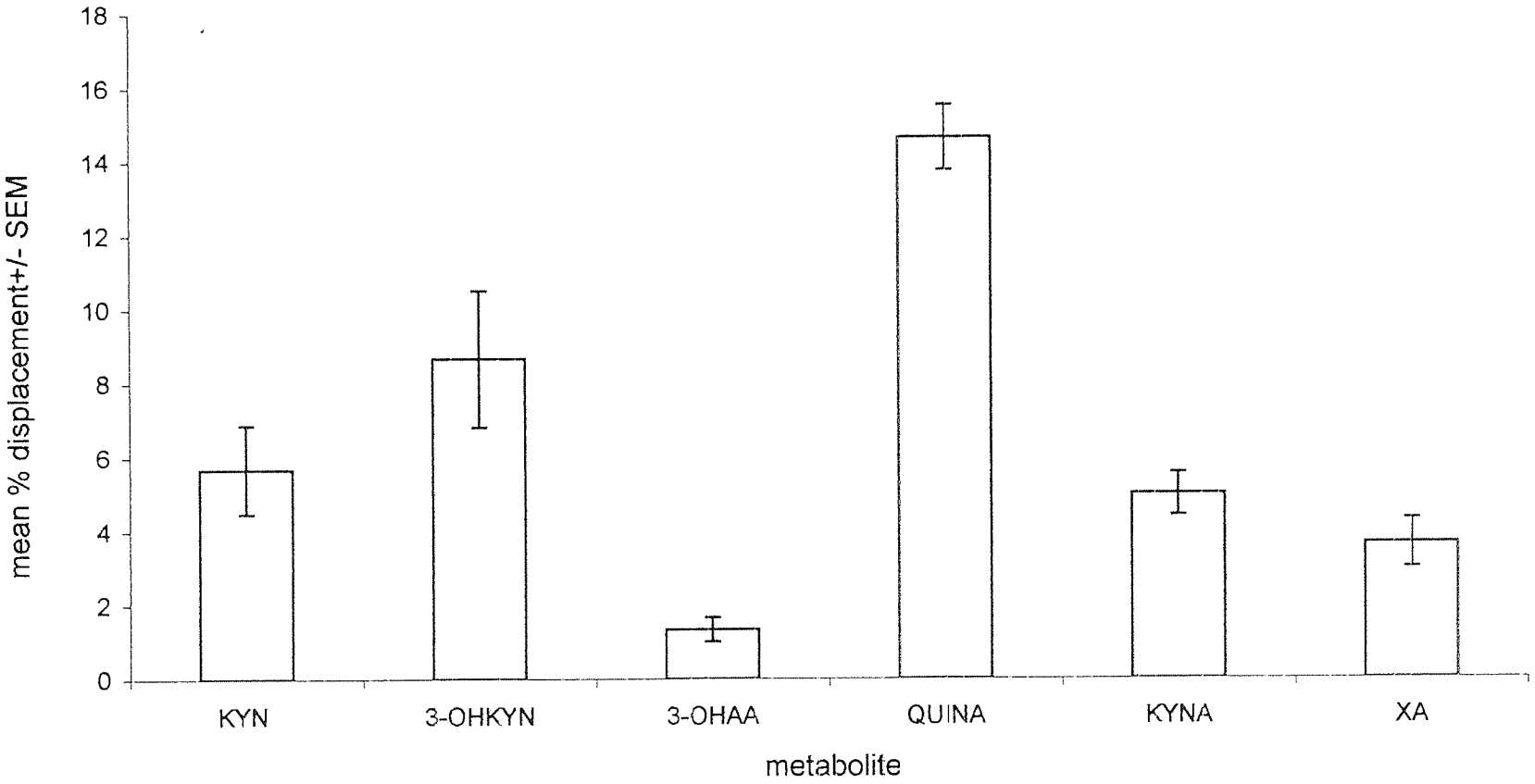

Figure 2.3. Percentage displacement of bound [^{3}H] Ketanserin at 5nM concentration by six kynurenine pathway metabolites at 1 micromolar concentration. Each data column represents mean from 5 paired determinations and vertical bars represent standard errors.

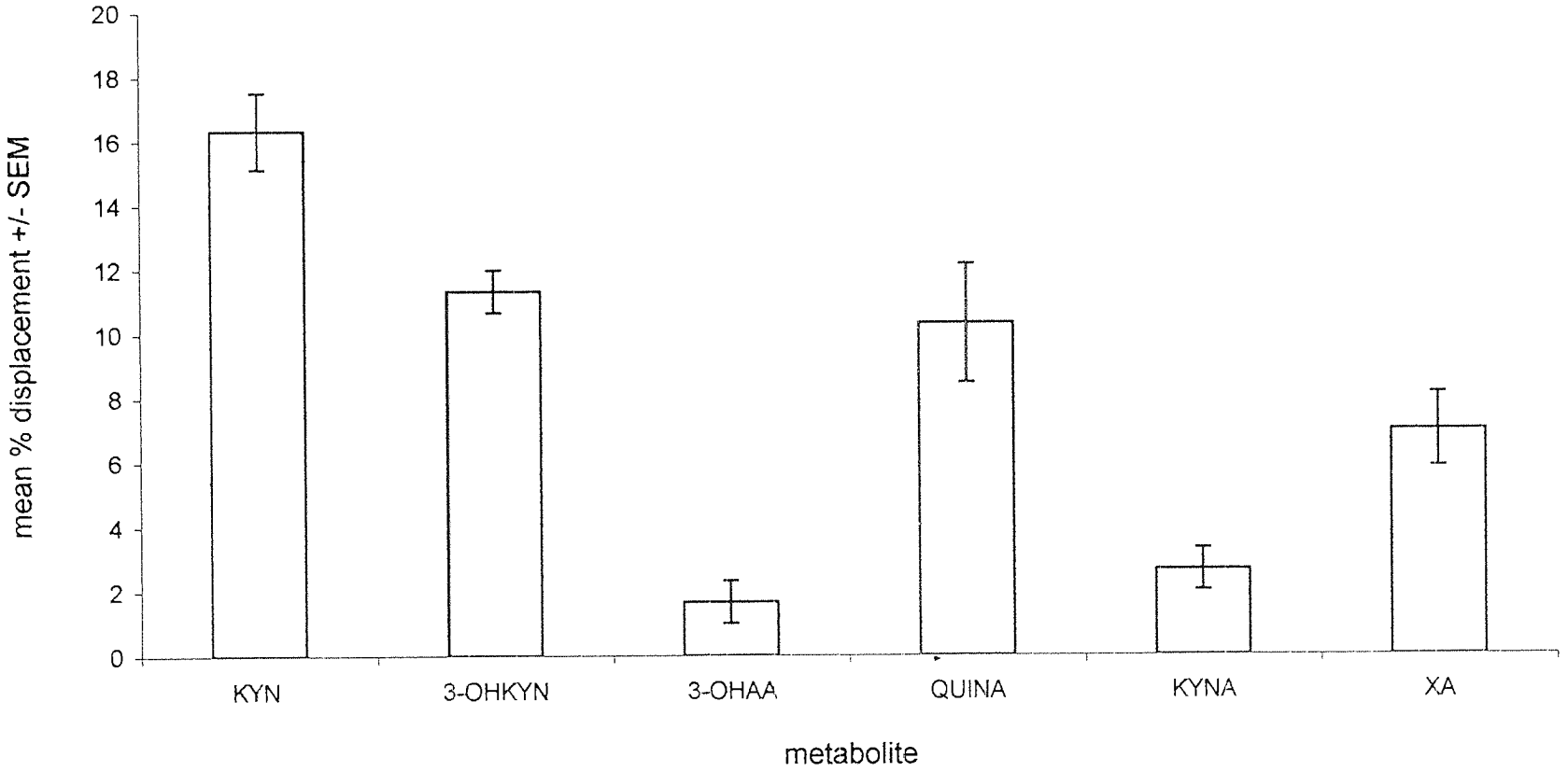

Figure 2.4. Percentage displacement of bound [3H] Ketanserin at 5nM concentration by six kynurenine pathway metabolites at 10 micromolar concentration. Each data column represents mean from 5 paired determinations and vertical bars represent standard errors.

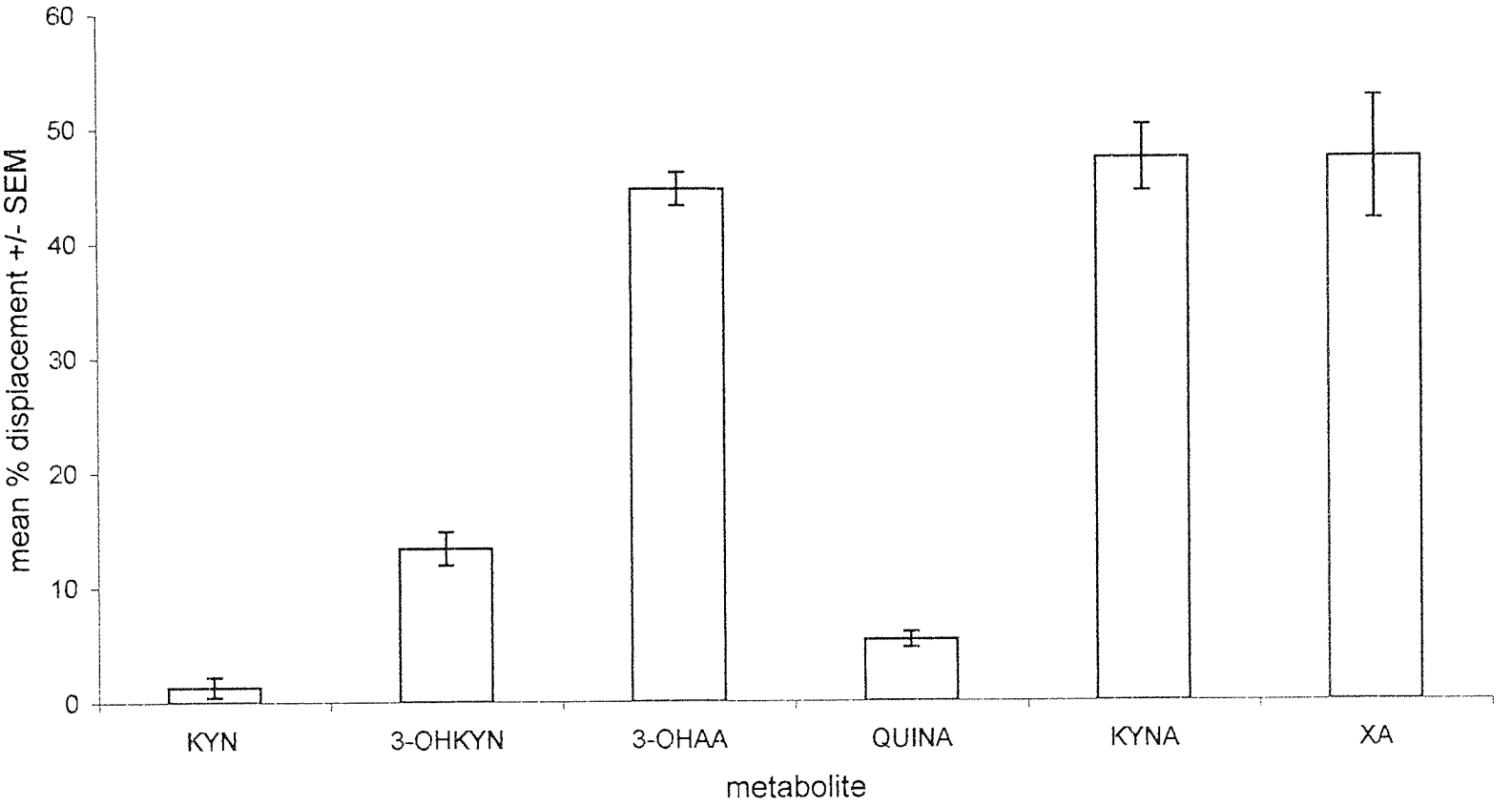

Figure 2.5. Percentage displacement of bound [3H] Ketanserin at 5nM concentration by six kynurenine pathway metabolites at 100 micromolar concentration. Each data column represents mean from 5 paired determinations and vertical bars represent standard errors.

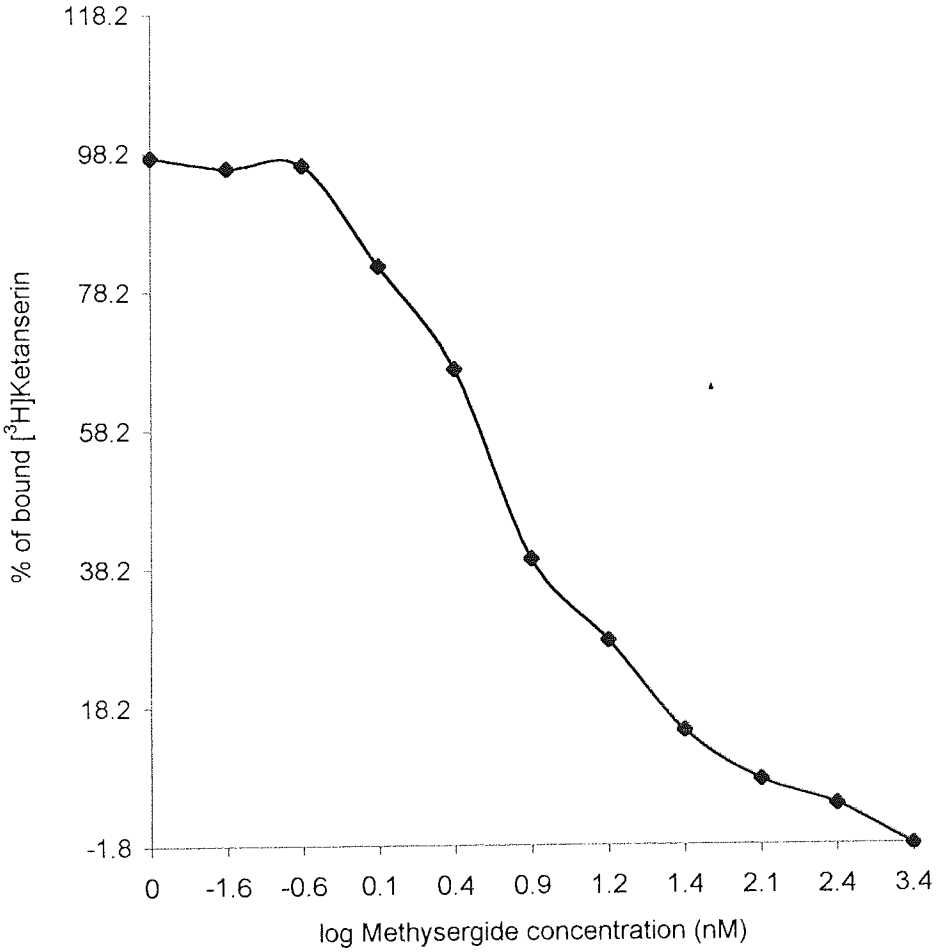

Figure 2.6. Methysergide displacement curve: displacement of bound [^{3}H]Ketanserin at 5nM concentration by Methysergide.

Metabolite	Concentration	K_i value (mean ± SEM) (nM)
none (control)		2.6 ± 0.1
kynurenine	1 μM	3.0 ± 0.5
	10μM	2.6 ± 0.7
	100μM	3.0 ± 0.5
kynurenic acid	1 μM	3.0 ± 0.5
	10μM	3.3 ± 0.4
	100μM	8.8 ± 1.1*
quinolinic acid	1 μM	3.2 ± 0.5
	10μM	3.4 ± 0.4
	100μM	3.1 ± 0.3

Table 2.1. Ki values of methysergide: alone (control) and in the presence of kynurenine, kynurenic acid and quinolinic acid. * = showed statistically significant difference from the control (independent samples t-test, $p<0.05$). The mean K_i values shown are from 5 determinations in duplicate.

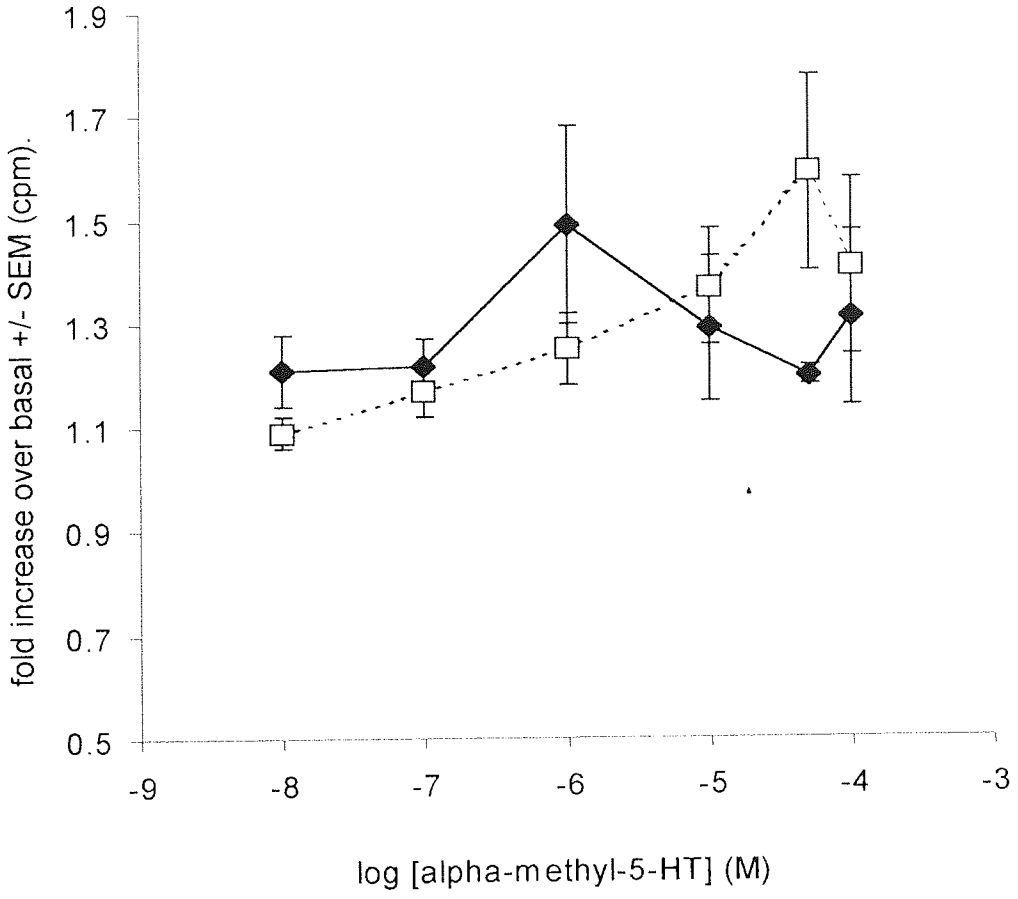

Figure 2.7. Effect of KYN on 5-HT_{2A} mediated PI hydrolysis. --□-- = alpha methyl 5-HT mediated PI hydrolysis (control dose response curve). —◆— = alpha methyl 5-HT dose response curve in the presence of 1μM KYN (test dose rsponse curve). Each point corresponds to the mean fold increase of cpm over basal from 8 paired determinations and the vertical bars represent standard errors. Basal PI hydrolysis activity = 805.± 18 cpm.

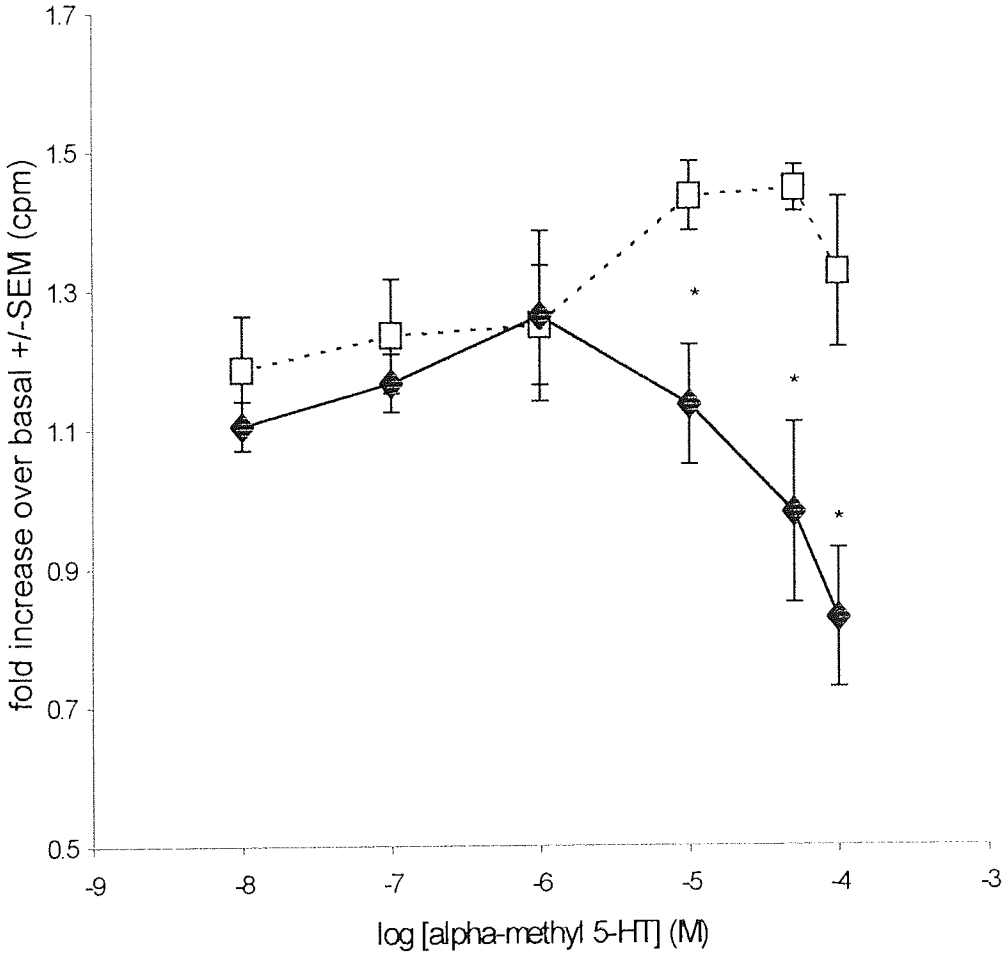

Figure 2.8. Effect of QUINA on 5-HT2A mediated PI hydrolysis. ---□--- = alpha methyl 5-HT mediated PI hydrolysis (control dose response curve). —◆— = alpha methyl 5-HT dose response curve in the presence of 1μMolar QUINA (test dose response curve). Each point corresponds to the mean fold increase of cpm over basal from 6 paired determinations and the vertical bars represent standard errors. Basal PI hydrolysis activity was 796cpm ± 14 cpm.
* = significantly lower than the control (paired t-test, $p < 0.05$).

CHAPTER 3

ANTISTREPTOLYSIN O TITRES IN TOURETTE SYNDROME AND THE EFFECT OF GROUP A β HAEMOLYTIC STREPTOCOCCAL IMMUNITY ON DOI-INDUCED HEAD SHAKES IN MICE.

CHAPTER 3

Introduction

Antibodies that attack parts of the brain have been suggested as a causative factor behind the symptoms of TS by a group of American researchers (see introduction). There is some evidence that an immune response to infections by bacteria such as group A beta haemolytic Streptococcus (GABHS) might be involved in TS. Antineuronal antibodies were first described by Husby and colleagues in 1976, to arise in response to GABHS infections and higher titres of antibodies against parts of basal ganglia were shown in the sera of children suffering from rheumatic chorea. This antibody reacting with neuronal cytoplasm was completely removed by absorption with GABHS membranes or with isolated human neurones from caudate nucleus and partially absorbed by GABHS cell wall preparations. Later studies by Swedo and Kiessling (1993;1994) showed a statistically significant increase in the incidence of antineuronal antibody positivity, and the levels of serum antineuronal antibodies against parts of basal ganglia, together with at least one marker (see below for serological markers) of streptococcal infection present, in children with TS, tic disorders or obsessive-compulsive symptoms (Keissling *et al.*, 1993,1994; Swedo *et al.*, 1997). Considering the spectrum of neuropsychiatric disorders suggested to be associated with GABHS and antineuronal antibodies, Swedo (1994) suggested the hypothesis that when genetically vulnerable children are exposed to a GABHS infection, antibodies are produced that mistakenly recognise cells within the basal ganglia and cause an inflammatory response. This inflammation is manifested as adventitious movements and psychiatric symptoms and these symptoms expression may depend on the epitope recognised by the antineuronal antibodies, extent of inflammation, chronicity of the insult, developmental stage of the child's immune system, inherited vulnerability or a combination of these factors. The serological markers used clinically to detect recent infection with GABHS are antistreptolysin O (ASO), antideoxyribonuclease B, antihyaluronidase and antistreptokinase. The cell bound antigens (M protein) and group A carbohydrate are other serological markers of GABHS infection and are used mainly for research purposes only (Gray, 1991; Greenwood, 1987). D8/17 B-cell alloantigen marker, a marker which had previously been recognised as positive in rheumatic fever has also been shown to be expressed more frequently in TS and obsessive compulsive disorder patients in the absence of rheumatic fever, when compared with healthy controls (Swedo *et al.*, 1997; Murphy *et al.*, 1997). All the evidence of increased incidence of GABHS immunity mentioned above have been reported in groups of children with recent onset TS or associated conditions. If GABHS immunity plays a

role in the causation of the symptoms of TS at least in some patients as hypothesised by Swedo (1994), evidence for GABHS immunity should be present in the presence of symptoms, showing increased levels of immune markers during exacerbations. The studies by which Swedo and Keissling have detected increased incidence of GABHS immunity in TS patients are those involving only children and adolescents. Therefore it is important to examine the same in a population including adult TS patients as well. With this data in the background the objectives of the experiments presented here were to:

1.determine the incidence of positive evidence for GABHS infection in a group of TS patients, including both children and adults having the disorder for a number of years (predominantly adults with the chronic disorder) and to compare this incidence with that of age and sex matched healthy controls.

2.determine the effect of GABHS immunity on DOI-induced head shakes in mice.

Additional description of methods

The evidence for recent GABHS infection was determined by detecting the plasma ASO titres (ASOT). The 72 TS patients and 46 age and sex-matched healthy controls included for this study were patients and controls recruited in study 1 (see Clinical study 1 under Experimental Methods). The ASOTs were detected by using RapiTex® ASL kit as described in Experimental Methods.

The GABHS prepared as described in Experimental Methods were used to immunise the mice to determine the effect of GABHS immunity on DOI-induced head shakes. The DOI-induced head shakes were determined as in Experimental Methods. Following the determination of DOI head shakes, all the mice were checked for the development of immunity against GABHS, as described under Experimental Methods.

Results

ASOT

Positive ASOT (>200IU/ml) were found in 21 out of the 72 patients and 15 out of the 46 controls tested. The percentage incidence of positive cases among the patient and the control groups are shown in figure 3.1 and they did not show a statistically significant difference. A significant difference was not detected even between the incidence of higher ASOTs (300 and 400IU/ml) detected in the two groups (Chi-square test: $P > 0.05$).

GABHS immunity on DOI head shakes

Figure 3.2 shows the GABHS grown on blood agar, from which the GABHS preparation for immunising the mice were prepared. ELISA done on the plasma from the mice

included in the experiments showed that all the test mice have developed immunity against the GABHS preparation and is shown as an antibody response. Figure 3.3 shows the absorbance corresponding to the plasma antibody titres in the test and control mice detected by a U.V. plate reader at 450nm setting. The mean absorbence at all six dilutions of the test mice plasmas showed a statistically significant increase when compared with that of the controls (paired t-test: $p< 0.05$).

Figure 3.4 shows the DOI induced head shakes frequency in mice injected with the GABHS and controls. The DOI head shakes frequencies of the test and the control groups did not show a statistically significant difference between the two even though the test mice showed a significant antibody response against GABHS. Immunised mice did not show any other abnormal movements or behaviours, when compared with the control mice. The immunised mice showed normal weight gain compared with the controls (mean body weight on day 30: test mice = 43.00 ± 1.9 g, control mice = 42.17 ± 1.3g).

Discussion

There was no significant difference in the incidence of positive evidence for recent GABHS infection in the patient and the control groups, when determined by ASOT. The detection method of ASOT has the disadvantage of giving false positive cases. False positive ASOT can be seen in people with hypercholesterolaemia and if serum is stored inadequately (Behringwerke A G, Limitations and interference, Rapitex ASL, 1995). Since the plasma used for this assay was stored at -70 °C prior to assay, false positive reactions due to inadequate storage is unlikely. But since a number of middle aged individuals were involved in the sample (patients and controls) presence of undetected and unknown cases of hypercholesteroleamia in some individuals may have contributed for some false positive cases, unequally in the patient and the control groups. If such false positive cases were present in the control group, it would have masked an increase in the incidence of ASOT positivity among the patients, on comparison between the patient and control groups. Even though ASOT is widely used as a marker of recent GABHS for several years, due to the possibility of having false positive cases the waning value of ASOT as a marker of recent GABHS has been suggested by Anyiwo *et al.*, (1989) on some of their findings. Therefore in the presence of shortcomings of the serological test used to detect evidence for GABHS infection, absence of a difference in positive evidence for GABHS infection among the patient and the control groups does not rule out a role of GABHS in the pathogenesis of TS. But if we detected a significantly higher incidence of evidence for GABHS infection, it would have contributed towards confirming a GABHS aetiology in TS. Therefore detection of evidence for recent

GABHS infection by a number of methods and considering the collective results may be worthwhile. Even though studies by Swedo and Kiessling showed at least one serological marker of GABHS infection positive (see Introduction above) a recent study by Singer et al., (1998) has failed to show increased incidence of ASOT or antideoxyribonuclease positivity in a group of children and adolescents with TS when compared with controls, despite them showing a significant increase in serum antineuronal antibodies against the putamen. Antibodies to several extracellular products of GABHS, such as ASO appear in the blood towards the end of the first week and disappear progressively during the succeeding months (Weatherall *et al.*, 1987). Since a majority of patients included in this study were adults, suffering from chronic symptoms of TS, this negative result does not rule out GABHS infection playing a part in the initial pathogenesis of TS in these patients. Therefore it may be worth detecting evidence for recent GABHS infection in a group of TS patients on initial presentation (at the time of onset of the disorder) or during exacerbation of symptoms. The researchers who contributed to the concept of PANDAS (see General Introduction) have also shown cases of OCD and TS following viral infections as well (Allen *et al.*, 1995). Studies by Fudje et al, (1996) and Murphy et al., (1997) have detected high incidence of B cell D8/17 expression in patients with childhood onset OCD and TS without a difference in the presence of antineuronal antibodies and antistreptococcal antibodies, suggesting that antistreptococcal antibodies may not be significant in TS, but the B-cell mediated immune mechanisms may still be important. These findings may also be relevant to the negative findings on ASOTs presented here.

Even though the mice developed a significant antibody response against the GABHS preparation they failed to show any alteration in DOI-induced head shake behaviour. Neither did they show any other abnormal movements or behaviours, when compared with control mice. Swedo (1994) has suggested the hypothesis that only genetically vulnerable children will develop adventitious movements and psychiatric symptoms following exposure to a GABHS infection. Genetic factors play a part in immunity and autoimmune conditions are shown to be dependent on genetic factors (see Introduction above). Therefore the failure of any of these experimental mice to show a change in the DOI head shakes frequency in response to GABHS immunisation may be due to a lack of genetic vulnerability. If any of the mice did show a change in head shake behaviour or any other behaviour that would mimic a neuropsychiatric disorder, our plan was to breed from such mice and to use as an animal model for further studies.

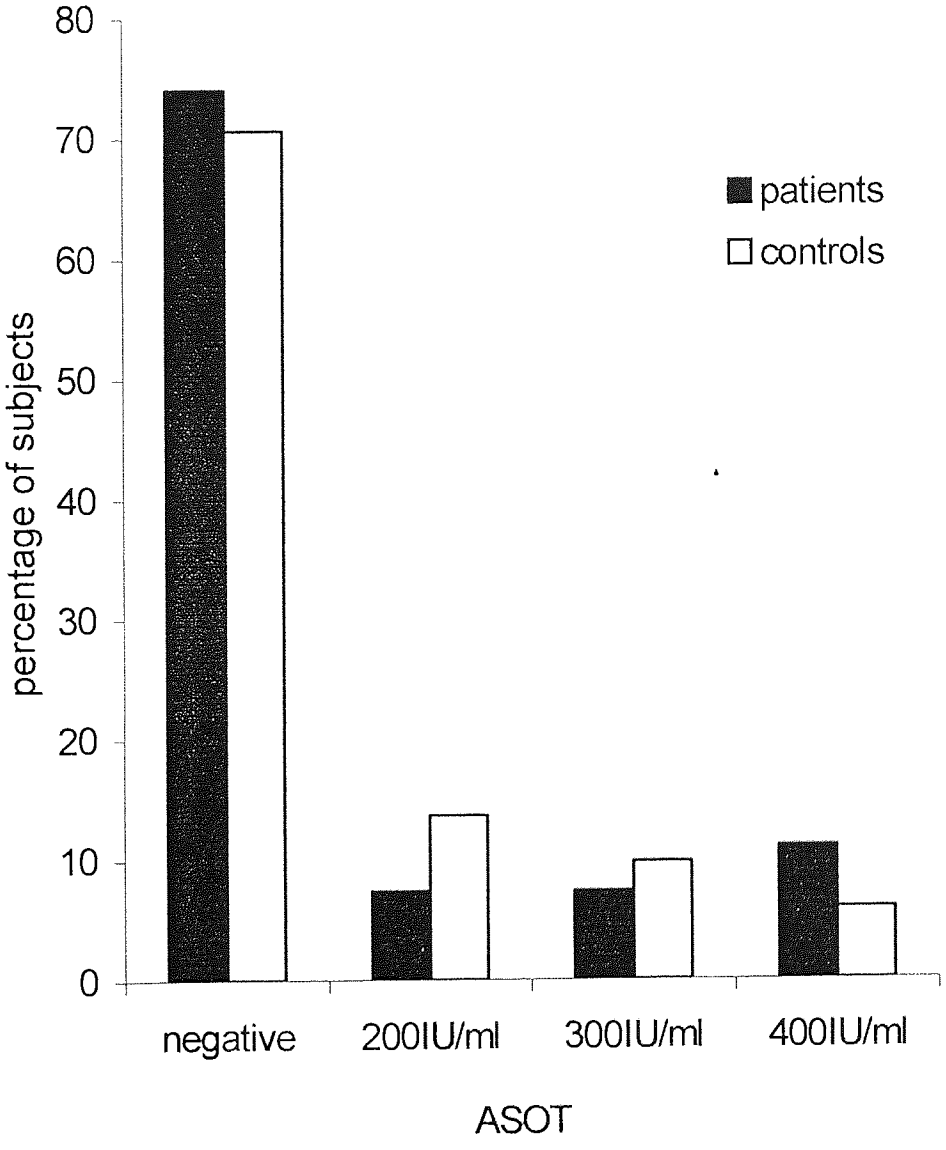

Figure 3.1. ASOTs in a group of TS patients and age and sex-matched controls. The percentage of subjects: patients and controls are shown in the Y axis and the X axis shows the ASOT. Positive ASOT = $\geq$ 200IU/ml. (Number of patients = 72, number of controls = 46).

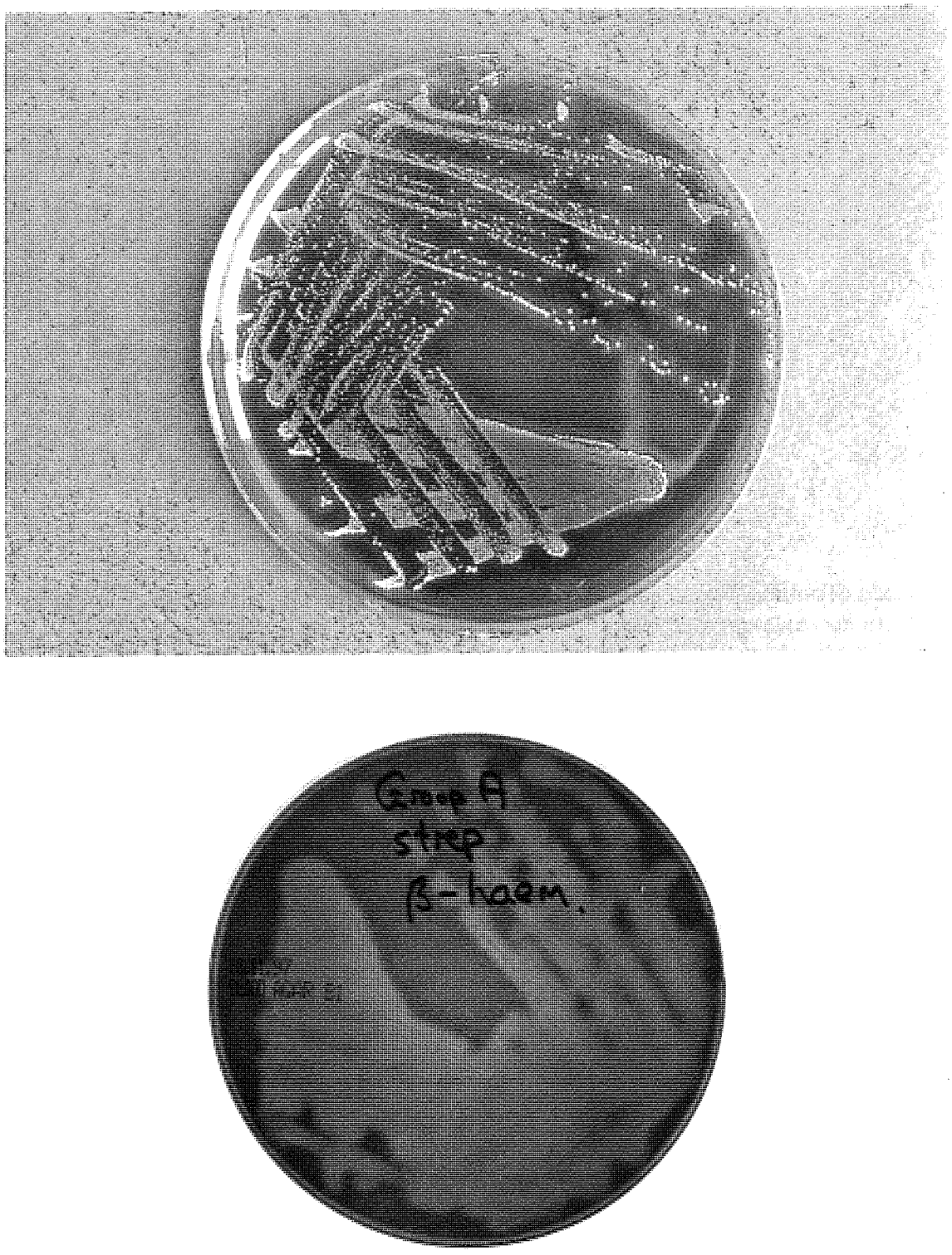

Figure 3.2. GABHS grown on blood agar from which the GABHS preparation for immunising the mice was prepared.

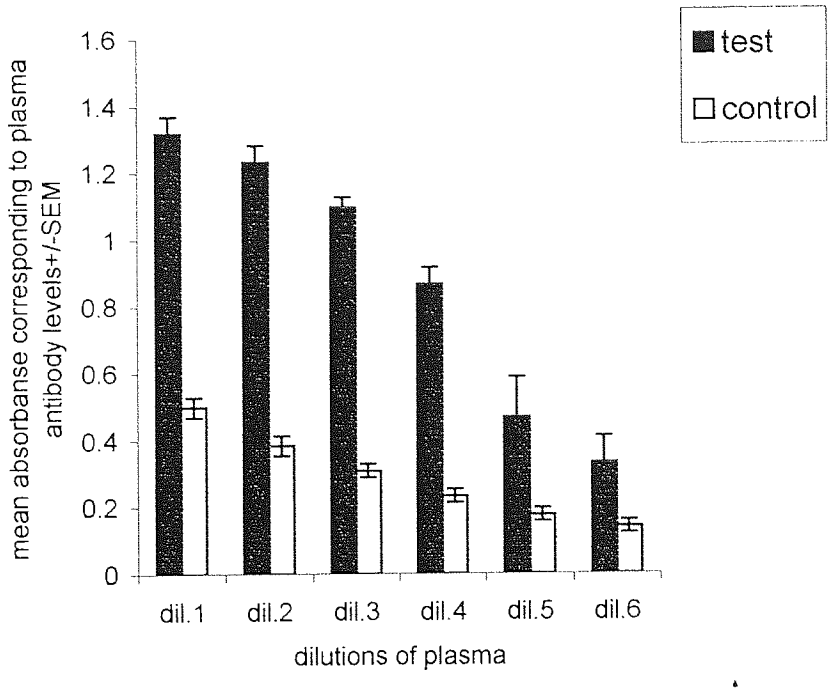

Figure 3.3. Mean plasma absorbance of test and control mice corresponding to plasma antibody titres against GABHS, detected by ELISA. Vertical bars represent standard errors (N=6/group). Mean absorbance at all 6 dilutions of the test plasmas showed statistically significant increase from that of control plasma (paired t-test, $p<0.05$).

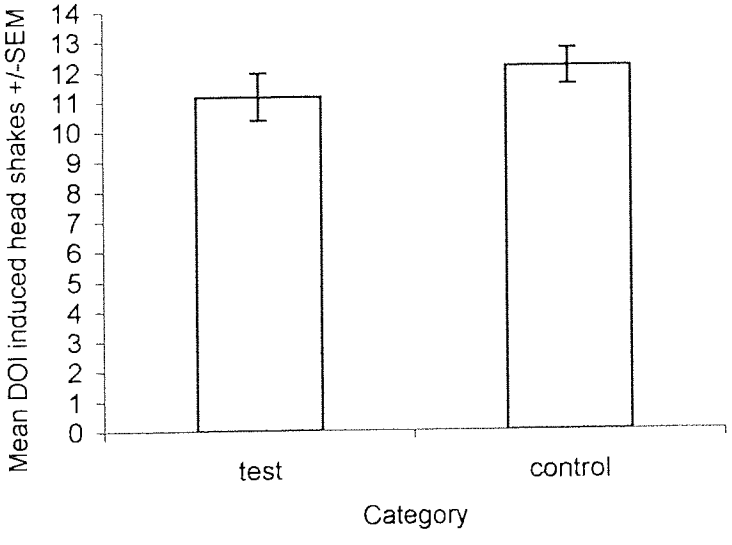

Figure 3.4. Effect of GABHS immunity on DOI induced head shakes. Mean DOI head shakes is shown by each column and vertical bars represent standard errors. N=6per group.

CHAPTER 4

CORTISOL SECRETION IN TOURETTE SYNDROME AND ATTENTION DEFICIT HYPERACTIVITY DISORDER.

CHAPTER 4

Introduction

The plasma concentrations of several important metabolites of the kynurenine pathway were measured by Mr. C. M. Gaynor in TS patients and age and sex matched controls, recruited in clinical study 1 described under Experimental Methods and KYN was found to be significantly elevated in TS patients when compared with age and sex matched healthy controls (Gaynor *et al.*, 1997). A more pronounced increase in the plasma KYN levels has been shown before in a small group of TS patients (7), when compared with age and sex matched healthy controls (Dursun, 1994). KYN is the first stable metabolite of the kynurenine metabolic pathway, which accounts for about 95% of tryptophan metabolism. The initial and the rate-limiting step of this pathway is catalysed by TDO in the liver and IDO in the extrahepatic tissues (refer to General Introduction). TDO activity is strongly induced by corticosteroids (Knox *et al.*, 1970). Cortisol is the main corticosteroid in humans synthesised and released by the adrenal cortex. Therefore it was decided to measure the plasma cortisol levels in the plasma samples from the group of TS patients on whom elevated plasma KYN levels were reported by Gaynor *et al.* (1997), and their age and sex matched controls to determine whether increased plasma cortisol levels in this group of TS patients played a role in causing increased plasma KYN levels (clinical study 1, refer to section on experimental methods for details).

The main physiological stimulus for the synthesis and release of cortisol by the adrenal cortex is adrenocorticotrophic hormone (ACTH), which is secreted by the anterior pituitary gland under the regulation of corticotrophin releasing factor derived from the hypothalamus. The circulating levels of cortisol also regulate the ACTH secretion. Therefore normal plasma cortisol levels are an index of the normal hypothalamic-pituitary-adrenal axis function. Cortisol has two main actions: those seen primarily in the resting state (facilitating the action of other hormones) and those which occur in response to a threatening/stressful environment. These latter actions are crucial for survival, and an individual deprived of his/her adrenal cortex is able to survive only in rigorously controlled conditions (Rang *et al.*, 1995). Therefore there is growing interest in the disturbances in the function of this axis playing a role in neuropsychiatric disorders, especially those affecting behaviour. King *et al.*, (1998) reported decreased salivary cortisol levels in response to a stressor in a group of subjects who were diagnosed to have Attention deficit hyperactivity disorder (ADHD) and continued to have all the features to satisfy the presence of ADHD a year after various psychosocial treatments, compared with the subjects of the same group who improved over the year of treatment and no

longer retained ADHD. On this observation King *et al.*, (1998) suggested that an impaired hypothalamic-pituitary-adrenal axis response to stress may be a marker for the more developmentally persistent form of ADHD. Lower salivary cortisol levels have also been shown before and after an anticipated stressor in prepubertal boys who were assessed to have impulsivity and aggressive delinquency, on significantly higher scores achieved on rating scales adapted to measure behaviour, when compared with age matched boys who were assessed to have normal age appropriate behaviour (Moss *et al.*, 1995). The behaviourally disturbed boys included in this study were children whose fathers were psychoactive substance abusers and a diminished cortisol response has been detected in these adults as well, compared with normal individuals (Moss *et al.*, 1995). With this evidence on diminished cortisol response to stressors in individuals with behaviour disturbances and since an interesting significant relationship between ADHD symptom severity and plasma cortisol levels were observed in the TS patients of study 1, who were diagnosed to have associated ADHD (see results below), we further decided to measure cortisol secretion (by measuring salivary cortisol levels) in a group of subjects whose primary diagnosis was ADHD, to compare with age and sex matched healthy controls and to examine the relationship with their ADHD severity (methodology described under clinical study 3 in the Methods section).

In the absence of a standard rating scale for the determination of the ADHD severity alone, separating it from other behaviour disorders, we used a questionnaire prepared by Dr. F. Zaw based on DSM-IV diagnostic criteria to determine the ADHD severity of the patients included in study 3. Since this questionnaire was not validated as a rating scale before, one of our aims was also to begin to validate this questionnaire doing the same study (see additional description of methods).

ADHD has been diagnosed in 20-90% of children with TS attending clinics (Robertson, 1994) and Comings and Comings (1987) have suggested a genetic link between TS and ADHD (refer to General Introduction for details). Brain imaging studies have shown abnormalities in the caudate nucleus both in TS (Wolf *et al.*, 1996) and ADHD (Mataro *et al.*, 1997). The DSM-IV criteria for the diagnosis of ADHD include:

* the persistence of 6 or more of the symptoms of inattention or hyperactivity-impulsivity to a degree that is maladaptive and inconsistent with developmental level for at least 6 months.
* some inattentive or hyperactive-impulsive symptoms that caused impairment were present before age 7 years.
* some impairment from the symptoms is present in two or more settings.

* clear evidence of clinically significant impairment in social, academic or occupational function is present.
* the symptoms do not occur exclusively during the course of a pervasive developmental disorder, schizophrenia or other psychotic disorder and are not better accounted for by another mental disorder (mood, anxiety, dissociative or a personality disorder).

The symptoms of inattention and hyperactivity-impulsivity listed in DSM-IV are shown in the questionnaire used in clinical study 3 to assess ADHD severity (refer to questionnaire in pages 109-111). In most individuals the symptoms of ADHD attenuate during late adolescence and adulthood, although a minority experience the full complement of symptoms into mid-adulthood. Other adults may retain only some of the symptoms, in which case the diagnosis of ADHD, in partial remission is used (APA, 1995). Therefore ADHD is a condition predominantly seen in children and adolescents and hence we recruited our sample of patients with a primary diagnosis of ADHD from a Psychiatry clinic of a children's hospital.
In this study (study 3) we decided to measure salivary cortisol, instead of plasma cortisol as it was not practical to obtain blood samples from healthy children recruited as controls solely for research purposes, and since it is well established that salivary cortisol levels reflect cortisol secretion and correlate well with the plasma cortisol levels (Aardal-Eriksson et al., 1998;Schmidt, 1998).

Additional description of methods
Since cortisol shows a diurnal variation in its secretion with higher levels in the morning and lower more stable levels in the afternoon (Rang *et al.*, 1995), we chose to determine the afternoon (12.00-17.00 hrs) cortisol levels in both the studies (study 1 and 3) and therefore all the blood samples from both the patients and controls in study 1 and salivary samples from the patients and controls in study 3 were taken between 12.00-17.00 hrs.
Since a seasonal variation in cortisol secretion has been shown (Handley *et al.*, 1980), the salivary samples from the controls in study 3, were taken in the same month as the patient to whom he or she was matched to enable comparison between the 2 groups without the interference of a monthly variation. This study was done over 2 months (from February to March 1999). 34 children diagnosed by Dr. F. Zaw (Consultant Child & Adolescent Psychiatrist), according to DSM-IV criteria, between 5 -15 years of age, and 25 healthy controls were recruited over these two months to give 25 ADHD subject/age, sex and month matched healthy control pairs for comparison of salivary cortisol levels between patients and controls. No controls were found for 9 patients according to the

matching criteria and therefore their salivary cortisol levels were not used for the comparison between patients and controls. The relationship with different categories of symptoms of ADHD were examined in the whole sample of ADHD patients recruited in the study (N=34). All the patients included in this study happened to be white children and therefore to avoid any influence of ethnic difference if present on cortisol secretion, all the healthy controls were also chosen from white children.

The following scoring system was used in deciding a severity score from the questionnaire prepared by Dr. F. Zaw for the determination of ADHD severity: never = 0, sometimes = 1, often = 2 and always = 3. Since this questionnaire has not been validated and used before, in addition to getting a parent who lived with the child to fill the questionnaire in the clinic, on his/her observations of the child's behaviour over the past 6 months, we also sent the same questionnaire to every child's school requesting the child's class teacher to fill it on his/her observations of the child's behaviour in the class. By doing this we expected to compare the scores achieved by the two means in order to check the validity of the questionnaire.

Oppositional Defiant Disorder (ODD) and ADHD have been identified as predictors of Conduct Disorder (CD) while ODD and CD come in the differential diagnosis of ADHD (Spender and Scott, 1996; APA, 1995). Because of these associations between ADHD, ODD and CD, in order to reconfirm that our sample of ADHD subjects were those primarily suffering from ADHD and not other related disorders, and in order to determine the incidence of related disorders among these children, all the subjects included in study 3 were also assessed for the symptoms of ODD and CD according to the DSM-IV criteria by including the relevant questions in the same questionnaire used to assess the ADHD severity. An information sheet was filled for each subject giving the details of the presence and the severity of each of these related disorders (see questionnaire in pages 108-110 and the information sheet in page 112). A relevant history was also taken from each parent who filled the questionnaire on his/her ADHD child to identify the presence of other psychopathologies in this group of children that were not assessed by the questionnaire.

The presence of other neuropsychiatric conditions were diagnosed according to DSM-IV criteria by using the observations recorded in the questionnaire and from the history and the examination of the child by me under the guidance of Dr. F. Zaw.

Unfortunately in clinical study 1, plasma samples from TS patients have been collected gradually throughout the course of the year (July, 1995-June 1996), while the majority of controls have been bled in March 1996. Therefore to avoid the discrepancy in the season of investigation, between patients and controls we only compared the plasma cortisol

levels of TS patients from study 1 bled between March – June, 1996 (N=27) with the controls bled during the same period (N=44). The increase in plasma KYN levels reported by Gaynor *et al.* (1997) in this group of TS patients was also by similar comparison. Another unintended factor made the examination of the relationship of the ADHD severity of the TS patients of study 1 with plasma cortisol levels complicated: the assessment of patients recruited earlier in the study has been based on the DSMIII-R (APA, 1987) criteria, while DSM-IV criteria has been used to score patients recruited in the latter part of the study. Prof. M. Robertson did these assessments and we received the scores with the DSM edition used for each patient. Therefore we examined the relationship of the ADHD severity with plasma cortisol levels considering the patients assessed on the two different DSM editions as two separate groups.

Patients included in both the studies (study 1 and study 3) were on various medications for their neuropsychiatric problems.

All the statistical analyses of the results were done using the Statistical Package for Social Sciences (SPSS). The comparisons of cortisol levels between the patients and controls to determine the significance of difference were done by t-tests and the relationship between cortisol levels and the severity assessments of ADHD were examined by Spearman correlation coefficients. The significance of the monthly variations in cortisol levels was tested by analysis of variance.

Results

Study 1

The monthly distribution of the blood sample collection from patients and controls is shown in table 4.1 and the plasma cortisol levels of patients and controls by the Month is shown in figure 4.1. The analysis of variance showed significant variation in plasma cortisol level depending on the month in the patient and the control groups ($F_{1,131}$ 634, $p<0.05$).

On comparison of plasma cortisol levels between TS patients and healthy controls as described above in additional methods, there was no significant difference between the two groups (independent samples t-test, $p> 0.05$, see figure 4.2).

56 out of the 72 TS patients (77%) included in this study had associated ADHD diagnosed according to DSMIIIR or DSM-IV criteria. On examining the relationship between plasma cortisol levels and ADHD severities of the TS patients with associated ADHD from the whole group of TS patients (N=72) included in study 1, the group of patients assessed for ADHD severity according to the presence of DSMIIIR criteria

(N=32), showed no significant relationship between plasma cortisol levels and ADHD severities (correlation coefficient, $p > 0.05$), but there was a significant inverse relationship between the plasma cortisol levels and the ADHD severities in the group of patients assessed according to the presence of DSM-IV criteria (N=23, correlation coefficient, $r = -0.61$ and $p < 0.05$, see figure 4.3).

There was no significant correlation of plasma cortisol levels with the tic severity (YGTSS) of the TS patients (correlation coefficient, $p > 0.05$).

The table 4.2 shows the medications taken by the TS patients included in study 1 at the time of taking the blood sample.

Study 3

Table 4.3 shows the number of patients and controls recruited and their mean salivary cortisol levels in each month. There was no significant variation in the salivary cortisol levels by month, within the 2 months (February and March 1999) of the study (analysis of variance, $F_{1,56}$ 0.082; $p > 0.05$).

There were 31 males and 3 females in the sample of ADHD children, showing a 10.3:1 male:female ratio.

The comparison of salivary cortisol levels in patients and controls showed significantly lower salivary cortisol levels in ADHD patients (paired t-test $p<0.005$ and independent samples t-test, $p<0.05$, see figure 4.4).

The assessment of ADHD severity showed a mean severity score of 37.12 ± 2.07 out of a total score of 54, according to our assessment from the questionnaire filled in the clinic by a parent living with the child. Even though we sent the same questionnaire to the schools of all the ADHD children included in the study, to be filled by the class teacher on the child's behaviour in school, only 17 questionnaires were returned. These 17 assessments done on teachers' observations in schools showed a mean score of 27.56 ± 2.96 out of the total score (54) and significantly differed from the score determined on parents' observations of the same children (paired t-test $p<0.05$), always achieving a lower severity score on teacher's observations, than the severity score determined on parent's observations, on examining the 2 different scores for each individual child, except in the case of the 3 unmedicated patients included in this group who scored almost the same by both means (see table 4.4 for details). Even though the ADHD severity scores determined on the parents' observations and the teachers' observations were significantly different, they positively correlated with each other (correlation coefficient, $r = 0.70$ and $p<0.005$).

The relationship between the salivary cortisol level and the ADHD severity determined on the parents' observations for the 34 children with ADHD were examined and no significant relationship was found between these parameters (correlation coefficient, $p>0.05$). Neither was a significant relationship detected between the salivary cortisol levels and the ADHD severity determined on teachers' observations for the 17 children who were also assessed by the teachers' observations (correlation coefficient, $p>0.05$).
Even when the scores of attention deficit (AD) and hyperactivity-impulsivity (HD) were taken separately (see questionnaire and information sheet) and examined the relationship with the salivary cortisol levels, no significant relationship was seen between these symptom severity and salivary cortisol level.
Table 4.5 shows the different medications taken by the 34 ADHD children during the study. Since these children were on a number of different medications and some of them were on multiple medications it was not possible to examine the effect of one particular medication on cortisol secretion.
Table 4.6 shows the frequency of neuropsychiatric conditions other than ADHD, that were diagnosed according to the DSM-IV criteria in the group of 34 children primarily affected with ADHD (based on the questionnaire filled by the parent in the clinic, history given by the parent and clinical assessment of the child). All 32 children satisfied the criteria for the diagnosis of ODD and the ODD severity showed a strong significant positive relationship with the ADHD severity (correlation coefficient, $r = 0.69$ and $p<0.001$). 7 of the children included in this group suffered from epilepsy and were on antiepileptic medications. The number of patients having different associated conditions was too small (except for ODD and TS) to compare with the patients not having those conditions with respect to salivary cortisol. ODD was present in 100% of this group of ADHD patients. On comparison of ADHD patients having TS and not, there was no significant difference between the 2 groups in salivary cortisol levels (t-test, $p>0.05$).

Discussion

There was no significant difference between the plasma cortisol levels in TS patients and their controls in study 1. This suggests that the difference in plasma KYN reported between this same group of TS patients and their controls (Gaynor *et al.*, 1997) is unlikely to be due to an alteration in the cortisol secretion in the TS patients. As expected the plasma cortisol levels showed a significant monthly variation. Even though the ADHD severity scores determined according to the presence of DSM criteria in study 1 was not a validated severity analysis, a significant inverse relationship between the plasma cortisol levels and the ADHD severity determined according to the presence of

DSM-IV criteria was seen where increased ADHD severities corresponded to lower plasma cortisol levels and *vice versa*. No relationship between plasma cortisol and ADHD severity determined according to the presence of the DSMIIIR criteria were seen. On inquiring from the clinicians who assessed the TS patients in study 1, we found out that the ADHD severity score given for the TS patients who had associated ADHD was the number of DSM criteria of ADHD present in these patients at the time of the study. Therefore the ADHD severity score referred to in study 1 is on a checklist based on the DSM criteria, rather than on a severity rating scale. On these results we may suggest that the plasma cortisol shows an inverse relationship with the number of DSM-IV criteria of ADHD present in a patient and therefore lower cortisol secretion is a possibility in more established ADHD.

In study 3 significantly lower salivary cortisol levels were detected in the ADHD subjects, when compared with age, sex, ethnic group and month matched controls. This observation together with the inverse relationship between plasma cortisol and ADHD symptoms in study 1, further support the suggestion of impaired cortisol response and the presence of a disturbance in the hypothalamic-pituitary-adrenal axis in the individuals affected with ADHD, made by a few researchers (see Introduction above).

Relatively high salivary cortisol has been detected in children reported as contemporaneously shy by their mothers and who were behaviourally inhibited, compared with the children having age appropriate behaviours (Schmidt *et al.*, 1997). This observation taken together with the finding of low salivary cortisol in children with ADHD, compared with children with normal behaviour, further supports the hypothesis of disturbed hypothalamic-pituitary-adrenal axis playing a role in neuropsychiatric disorders affecting behaviour. These observations suggest that both lower and higher cortisol responses can lead to behavioural problems. Altered cortisol responses may also be a consequence of the altered experiencing of stress by these children due to some other factors, since cortisol is a stress hormone, the shy children may be experiencing a greater degree of stress in a given situation leading to higher cortisol secretion, while ADHD children experience less stress leading to lower cortisol secretion.

In study 3, neither ADHD severity nor the AD or HD severity when taken separately showed a significant relationship with the salivary cortisol levels. On going through the medication profiles of these children it was seen that the medication dosing had been done in such a way as to provide for positive effects during the period that the child was in school, in order to help with the children's continuation of schooling. Therefore the significant difference seen between the ADHD severity scores determined on parent's

observations and the teacher's observations in school in the same medicated child may well be due to the effects of medication. The more structured environment provided in any school compared with home might also be influencing the behaviour, in giving lower indices of the ADHD severity in school. Under these circumstances we could not prove the validity of the scoring system used in this study based on the DSM-IV diagnostic criteria. Therefore further studies are suggested on unmedicated ADHD children to validate this scoring system in order to have a robust severity scale to be used in clinical research and for determining the efficacy of treatment.

All 32 ADHD children included in study 3 showed associated ODD according to DSM-IV criteria. The ODD severity assessed as described in additional description of methods, also did not correlate with the salivary cortisol levels, but positively correlated with the ADHD severity. The symptoms of ODD are associated with aggression and therefore the aggressive behaviours contributing towards the scores of ADHD cannot be ruled out in this group of children. Aggressive behaviour in boys with ADHD has failed to show any significant relationship with the salivary cortisol levels and on this observation together with the large body of literature available on the complexity of the biological substrate of aggression it is suggested that the biological factors do not correlate with the behaviours associated with aggression (Kaneko *et al.*, 1993). These may explain the absence of a significant relationship between the salivary cortisol and the ADHD severity in this group of children.

Due to the wide variety of medications taken by the patients in both studies 1 and 3 we could not analyse the effects of medications on cortisol secretion. Most patients included in study 3 were on more than 1 medication making the task more difficult. The majority of patients included in study 1 and a fair proportion of patients in study 3 were on neuroleptics and it has been shown that the 24-hour plasma cortisol profile is preserved in schizophrenic patients on neuroleptics (Jiang & Wang, 1998). In the absence of evidence from studies on patients with different medications and unmedicated patients we cannot totally rule out the medications taken by the subjects included in the two studies affecting plasma and salivary cortisol levels and these influences contributing to the differences in cortisol levels between patient and control groups. There were 7 (21.88%) children suffering from epilepsy included in the group of ADHD subjects in study 3 and they were on different antiepileptic medications. It is reported that Sodium valproate and Ethosuximide cause attention deficit and hyperactivity and carbamazepine causes aggressive behaviour as side effects (BMA & RPSGB, 1998). Therefore the possibility of these drugs influencing the severity of ADHD and playing a role in the causation of ADHD in the epileptic children cannot be disregarded.

In agreement with the higher incidence of TS seen in ADHD and *vice versa*, a 56.25% incidence of TS were detected in the group of children with ADHD in study 3 and 77% of TS patients had associated ADHD in study 1.

This study gives strong evidence of an impaired cortisol response in ADHD and shows the different array of psychopathologies associated with ADHD and TS. It is worth carrying out studies on larger groups of TS and ADHD subjects with different associated conditions to examine the type of associations between them and the biochemical disturbances prevalent in them in order to find more effective therapies with less adverse effects.

ASSESSMENT FORM 1.0
ADAPTED FROM DSM IV APA 1994

PATIENTS NAME:	DOB:
COMPLETED BY:	DESIGNATION: [Parent / Teacher]
DATE:	

[In the last 6 months]

INATTENTION		NEVER	SOMETIMES	OFTEN	ALWAYS
1.	Fails to give close attention to details or makes careless mistakes in schoolwork, work, or other activities.	☐	☐	☐	☐
2.	Has difficulty sustaining attention in tasks or play activities.	☐	☐	☐	☐
3.	Does not seem to listen when spoken to directly.	☐	☐	☐	☐
4.	Does not follow through on instructions and fails to finish schoolwork, chores, or duties in the workplace (not due to oppositional behaviour or failure to understand instructions).	☐	☐	☐	☐
5.	Has difficulty organising tasks and activities.	☐	☐	☐	☐
6.	Avoids, dislikes, or is reluctant to engage in tasks that require sustained mental effort (such as schoolwork or homework).	☐	☐	☐	☐
7.	Loses things necessary for tasks or activities (e.g., toys, school assignments, pencils, books or tools)	☐	☐	☐	☐
8.	Is easily distracted by extraneous stimuli.	☐	☐	☐	☐
9.	Is forgetful in daily activities.	☐	☐	☐	☐

HYPERACTIVITY					
1.	Fidgets with hands or feet or squirms in seat	☐	☐	☐	☐
2.	Leaves seat in classroom or in other situations in which remaining seated is expected.	☐	☐	☐	☐
3.	Runs about or climbs excessively in situations in which it is inappropriate (in adolescents or adults, May be limited to subjective feelings of restlessness)	☐	☐	☐	☐
4.	Has difficulty playing or engaging in leisure activities quietly.	☐	☐	☐	☐
5.	Is "on the go" or acts as if "driven by a motor".	☐	☐	☐	☐
6.	Talks excessively.	☐	☐	☐	☐

		NEVER	SOMETIMES	OFTEN	ALWAYS
IMPULSIVITY					
1.	Blurts out answers before questions have been completed.	☐	☐	☐	☐
2.	Has difficulty waiting turn.	☐	☐	☐	☐
3.	Interrupts or intrudes on others (e.g., butts into conversations or games).	☐	☐	☐	☐
OPPOSITIONAL DEFIANCE					
1.	Loses temper	☐	☐	☐	☐
2.	Argues with adults	☐	☐	☐	☐
3.	Actively defies or refuses to comply with adults' requests or rules	☐	☐	☐	☐
4.	Deliberately annoys people	☐	☐	☐	☐
5.	Blames others for his or her mistakes or misbehaviour	☐	☐	☐	☐
6.	Is touchy or easily annoyed by others	☐	☐	☐	☐
7.	Is angry and resentful	☐	☐	☐	☐
8.	Is spiteful or vindictive	☐	☐	☐	☐

	CONDUCT DISORDER	YES	NO
	[In the last 6-12 months] **AGRESSION TO PEOPLE AND ANIMALS**		
1.	Bullies, threatens or intimidates others	☐	☐
2.	Initiates physical fights	☐	☐
3.	Has used a weapon that can cause serious physical harm to others (e.g., a bat, brick, broken bottle, knife, gun)	☐	☐
4.	Has been physically cruel to people		
5.	Has been physically cruel to animals	☐	☐
6.	Has stolen while confronting a victim (e.g., mugging, purse snatching, extortion, armed robbery)	☐	☐
7.	Has forced someone into sexual activity	☐	☐

		YES	NO
	[In the last 6-12 months] **DESTRUCTION OF PROPERTY**		
1.	Has deliberately engaged in fire setting with the intention of causing serious damage	☐	☐
2.	Has deliberately destroyed others' property (other than by fire setting)	☐	☐
	DECEITFULNESS OR THEFT		
1.	Has broken into someone Elses house, building or car	☐	☐
2.	Lies to obtain goods or favours or to avoid obligations (i.e., "cons" others).	☐	☐

SERIOUS VIOLATIONS OF RULES

1.	Stays out at night despite parental prohibitions, beginning before age 13 years	☐	☐
2.	Has run away from home overnight at least twice while living in parental or parental surrogate home (or once without returning for a lengthy period)	☐	☐
3.	Truants from school, beginning before age 13 years	☐	☐

FOR USE BY DEPARTMENT OF PSYCHIATRY ONLY:

PATIENT NAME:

ADHD SCORE

INATTENTION ☐☐

HYPERACTIVITY ☐☐

IMPULSIVITY ☐☐

ADHD SEVERITY SCORE ☐☐

AD / HD Combined Type
AD / HD Predominantly Inattentive Type
AD / HD Predominantly Hyperactive Impulsive Type

ODD ☐☐

CONDUCT DISORDER RATING

MILD

MODERATE

SEVERE

CRITERIA FOR ADHD

*Six or more on inattention or Hyperactivity - Impulsivity for at least 6 months
*symptoms causing impairment were present before 7 years
*Impairment from symptoms is present in 2 or more settings
*Evidence of clinically significant impairment in social/academic /occupational functioning
*Symptoms do not occur exclusively during the course of

PDD
Schizophrenia
Other Psychotic Disorders
Not accounted for by other mental disorder

CRITERIA FOR ODD

*Four or more features on Oppositional Defiance lasting for atleast 6 months
*Clear evidence of clinically significant impairment in social/academic /occupational functioning
*Symptoms do not occur exclusively during the course of a psychotic or mood disorder
*Criteria for conduct disorder not met

CRITERIA FOR CONDUCT DISORDER

*3/more symptoms in the past 12 months with at least 1 in the past 6 months
*The disturbance causes clinically significant impairment in social/academic /occupational functioning

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QUESTIONNAIRE COMPLETED BY: TEACHER / PARENTS (DELETE AS APPROP.)

Table 4.1. Number of blood samples collected each month from TS patients and healthy controls, over the whole duration of the study (study 1:July, 1995-June, 1996)

Month	number of controls	number of TS patients
July, 1995	1	3
August, 1995	0	2
September, 1995	0	2
October, 1995	0	10
November, 1995	0	2
December, 1995	0	17
January, 1996	0	4
February, 1996	0	5
March, 1996	30	9
April, 1996	5	7
May, 1996	7	5
June, 1996	2	6
Total	45	72

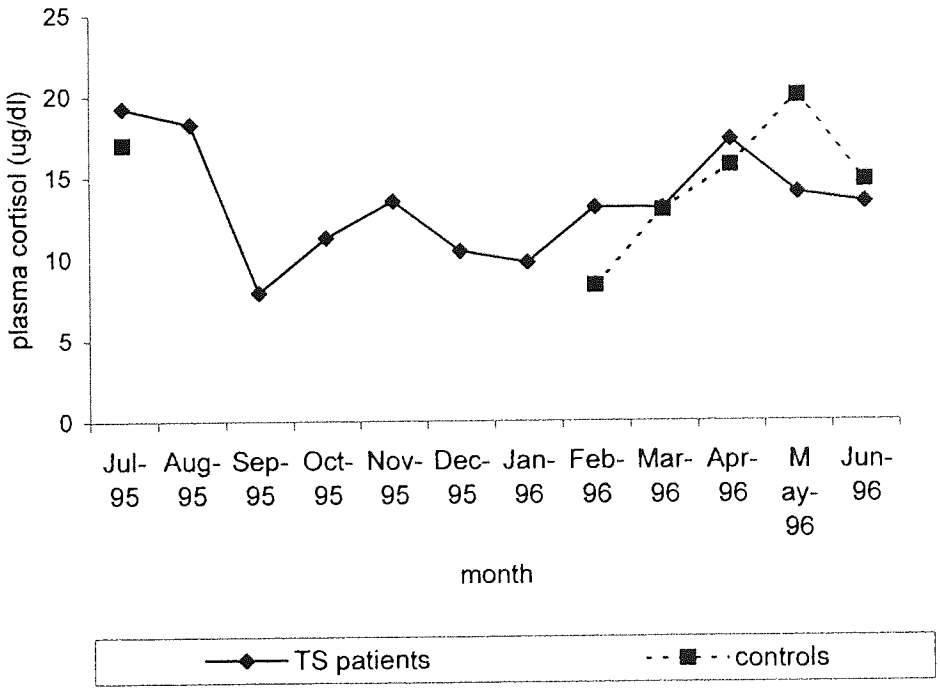


Figure 4.1. Monthly variation of plasma cortisol in TS patients and controls.

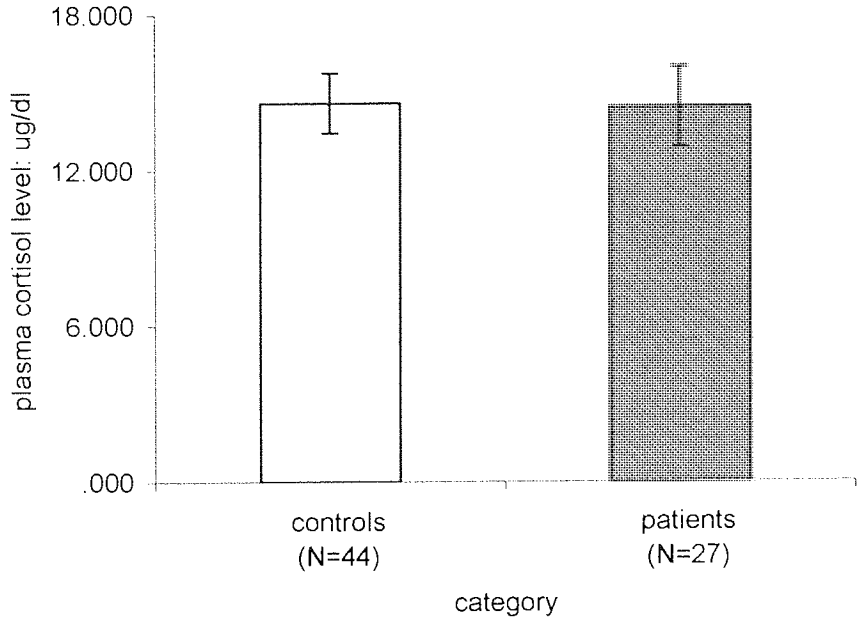


Figure 4.2. Plasma cortisol levels in TS patients and healthy controls bled between March-June 1996. The columns represent mean plasma cortisol level for each group and vertical bars represent standard errors.

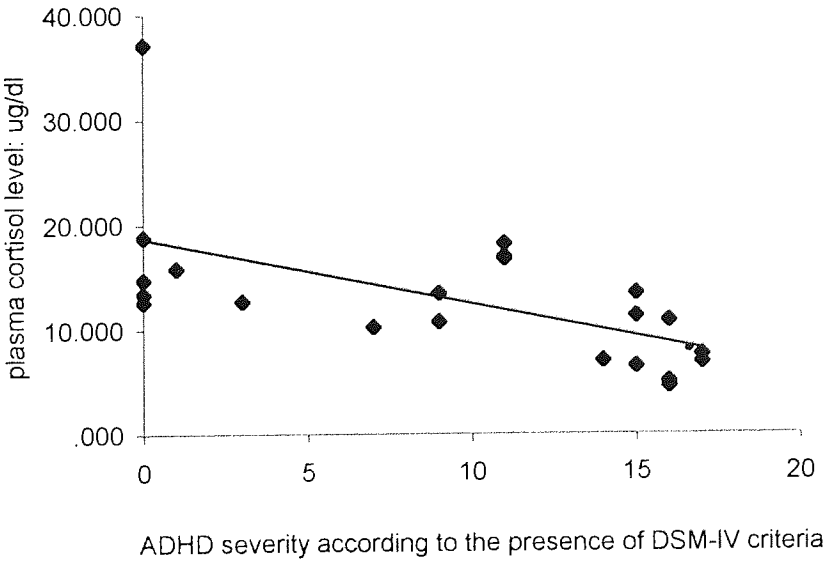


Figure 4.3. Correlation between plasma cortisol and ADHD severity rating using DSM-IV in TS patients with associated ADHD (Pearson Product moment correlation coefficient, $r = -0.61$, $p<0.05$).

Table 4.2. Different medications taken by the TS patients included in study 1, at the time of taking the blood samples for plasma cortisol detection.

Medications	**Number of patients**
Neuroleptics: Haloperidol, Pimozide and Sulpiride	29
Nicotine	2
α_2-adrenergic agonists: Clonidine	7
Antidepressants	9
Hypnotics	2
Anticonvulsants	3

Table 4.3. Number of ADHD children and healthy controls recruited in each month over the 2 months of the study 3 and the salivary cortisol detected.

Month	Patients		Controls	
	number	salivary cortisol (nM/l) mean ± SEM	number	salivary cortisol (nM/l) mean ± SEM
February '99	16	2.59 ± 0.28	16	3.32 ± 0.35
March '99	16	2.67 ± 0.25	9	3.72 ± 0.42

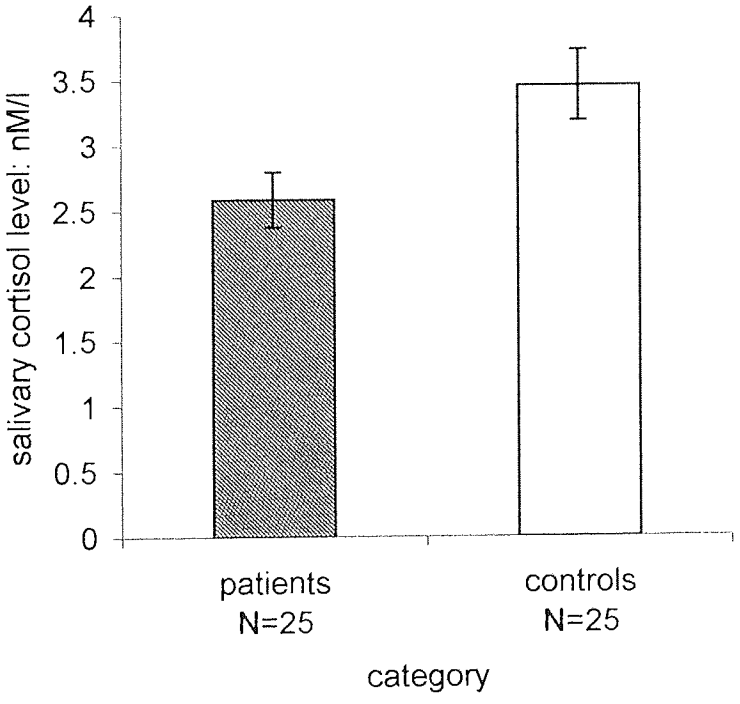


Figure 4.4. Salivary cortisol levels in ADHD patients and healthy controls of study 3. The columns represent the mean salivary cortisol for each group and vertical bars represent standard errors.

Table 4.4. Comparison of the ADHD severity scores determined by parents' observations and teachers' observations and the medication state in the 17 children assessed by the school.

Medication status + = on medication/s - = not on any medication	**ADHD severity score on parent's observations**	**ADHD severity score on teacher's observation**
-	30	30
-	20	23
-	44	44
+	49	38
+	50	46
+	44	25
+	22	11
+	51	28
+	40	34
+	53	44
+	33	29
+	36	24
+	46	23
+	36	15
+	42	19
+	45	35
+	24	4

Table 4.5. Medications taken by the ADHD children at the time of severity assessments and salivary sample collection.

Medications	**Number of patients on:**
Central nervous system stimulants:	
Methyl phenidate	7
Dexamphitamine	10
Antidepressants: SSRIs	6
TCAs	2
Neuroleptics: conventional: Haloperidol, Sulpiride	2
atypical: Olanzapine, Amisulpiride	5
α_2-adrenergic agonist: Clonidine	1
Melatonin	3
Antiepileptics: Carbamazepine, Sodium valproate, Ethosuximide	7

Table 4.6. The neuropsychiatric conditions other than ADHD, that were present in the group of 32 ADHD children included in study 3.

Neuropsychiatric condition	**number of children affected (N= 32)**	**percentage from the total**
Oppositional defiant disorder	32	100
Conduct disorder	6	18.75
Chronic motor tics	4	12.5
Tourette syndrome	18	56.25
Obsessive compulsive disorder/behaviours	4	12.5
Trichotillomania	3	9.38
Learning disorder	4	12.5
Nocturnal enuresis	2	6.25

CHAPTER 5

A STUDY OF PLASMA INF-γ, NEOPTERIN, KYNURENINE, TRYPTOPHAN AND NIGHT-TIME URINARY 6-SULPHATOXYMELATONIN EXCRETION IN TOURETTE SYNDROME.

CHAPTER 5

Introduction

Two previous studies have shown increased plasma KYN levels in TS patients. The first study carried out in a small sample (7 patients and 10 age and sex matched controls), showed a marked increase in plasma KYN levels in TS patients and this was not associated with a change in plasma tryptophan levels or an increase in the plasma neopterin levels (Dursun, 1992). However reduced plasma tryptophan in TS patients was reported by Comings (1990a,b) and on this observation he has suggested increased metabolism of tryptophan via the kynurenine pathway in TS. The second large cross sectional study (Clinical Study 1 described under Clinical Methods: 72 patients and 46 controls) showed a smaller, but a statistically significant increase in plasma KYN levels associated with increased plasma neopterin levels in TS patients (Gaynor *et al.*, 1997). This study also did not show a difference in the plasma tryptophan levels among patients and controls. The two studies have used different methods for measuring plasma KYN and neopterin levels. These different findings in TS patients, together with the finding of KYN's potentiating effect on DOI induced head shakes in mice, which models TS in humans (McCreary & Handley, 1995; results presented in Chapter 1) warranted further examination of plasma tryptophan, KYN and neopterin in a group of TS patients.

Since cortisol induces TDO, which is the first and the rate-limiting enzyme of the hepatic kynurenine pathway (Knox *et al.*, 1970), the plasma cortisol levels were measured in the group of TS patients who showed increased plasma KYN levels and in their matched controls from the large cross sectional study mentioned above. The plasma cortisol levels did not show any change between the patient and control groups (see results in Chapter 4) and did not correlate with the plasma KYN levels (Gaynor *et al.*, 1997). Since both neopterin and KYN synthesis is induced by interferons and INF-γ is shown to be the most potent interferon in inducing the rate limiting enzymes catalyzing the synthesis of both neopterin, and KYN in extrahepatic tissues (Takikawa *et al.*, 1986; Bitterlich *et al.*, 1988;

Goldstein *et al.*, 1989), it was also decided to detect the plasma INF-γ levels in the patients and controls included in this study (Clinical study 2 described under Experimental methods).

Neopterin is reported to be low in healthy current smokers (Diamondstone *et al.*, 1994) and significantly higher plasma KYN levels have been detected in non smoking TS patients when compared with smokers (Gaynor *et al.*, 1997). Therefore the current smoking status of all the individuals included in this study was recorded to determine the effects of smoking on the different biochemicals detected here.

Walsh *et al.* (1994) have shown melatonin's ability to inhibit hepatic TDO activity, resulting in decreased production of KYN. This observation suggests that an alteration in melatonin secretion may alter the plasma KYN levels by affecting the hepatic tryptophan metabolism via the kynurenine pathway. Melatonin has also shown inhibitory effects on the 5-HT_{2A} mediated head twitch response in rats and 5-HT_{2A} mediated PI hydrolysis in the rat cortex (Eison *et al.*, 1995), further making it interesting to investigate melatonin in TS. Symptoms of TS show a seasonal pattern with a tendency to wane during the summer months (Goetz, 1986), a period in which melatonin secretion is elevated compared to the winter months (Arendt *et al.*, 1979). The symptoms of TS worsen during puberty and early adolescence (Bruun, 1984), and the onset of puberty is associated with changes in pineal melatonin secretion, (Kitay, 1954; Silman *et al.*, 1979; Cohen *et al.*, 1982; Waldhauser *et al.*, 1984;1986). Considering these findings on TS and melatonin secretion, together with the reported modulatory effects of melatonin on dopaminergic functions (Zisapel *et al.*, 1982; Dubocovich, 1983; Cremer-Bartel *et al.*, 1983a;b; Bradbury *et al.*, 1985) and the observation of TS present in association with a tumour in the pineal gland (Lakke & Wilmink, 1985), Sandyk & Kay (1990) suggested that the development of TS may be linked to alterations in pineal melatonin function and that studies on melatonin secretion in TS patients may lead to the development of new therapeutic strategies. Considering this background data on melatonin with regard to TS, and since neuroactive melatonin has not been assessed in TS so far, we decided to detect the melatonin secretion in

TS patients and the matched controls included in this study. Since melatonin secretion is maximal during night-time, being almost undetectable during day light (Arendt, 1985) and for practical reasons such as compliance of the individuals included in the study, night-time melatonin secretion in individuals was determined in this study. 6-Sulphatoxy-melatonin (aMT6S), is the major excretory metabolite of melatonin (Jones *et al.*, 1969; Fellenberg *et al.*, 1981), and it closely correlates, both quantitatively and qualitatively with plasma melatonin in humans over 24 hrs of the day, with small shifts in the timing of melatonin secretion in the course of the year well reflected in urinary aMT6S (Bojkowski *et al.*, 1987a; Nowak *et al.*, 1987; Bojkowski and Arendt, 1988). aMT6S measurement in urine provides a robust, simple and a reliable assessment of melatonin secretion (Arendt *et al.*, 1985; Bojkowski *et al.*, 1987b). Therefore night-time melatonin secretion in the TS patients and controls included in this study was determined as aMT6S excreted in the night-time urine (8p.m -8a.m.).
A high incidence of obsessive compulsive behaviours (OCB) in TS patients have been confirmed by several studies (Montgomery *et al.*, 1982: Pitman *et al.*, 1987; Robertson *et al.*, 1988). According to the APA (1995) obsessive compulsive disorder (OCD) is found in 35-50% of TS sufferers, while tics or a history of tics is present in 20-30% of those suffering from OCD. OCB are characterized by the presence of recurrent, stereotyped behaviours and/or thoughts that intrude into consciousness or actions. When symptoms result in significant distress or impairment, it is classified as a 'disorder' (Singer & Walkup, 1991). Since the biochemicals assayed in this study have not been investigated in people having OCB, it was also decided to compare the TS patients who had associated OCD/B with other TS patients and healthy controls.

The aims of this study were to:

1. detect whether increased plasma KYN is present in this group of TS patients as previously reported and to detect whether there are differences in plasma tryptophan, KYN/tryptophan ratio, neopterin, INF-γ and melatonin secretion in this group of TS patients, when compared with matched controls.

2. determine the relationship of plasma INF-γ, neopterin, KYN, tryptophan levels, KYN/tryptophan ratio and night-time urinary aMT6S with the severity of tics in TS.

3. determine the relationship between plasma INF-γ, neopterin, KYN, tryptophan, and urinary aMT6S in TS patients and healthy individuals.

4. determine the effects of medications commonly taken by the patients included in this study, on plasma INF-γ, neopterin, KYN, tryptophan levels, KYN/tryptophan ratio and melatonin secretion.

5. detect whether there is any difference in the expression of plasma INF-γ, neopterin, KYN, tryptophan, KYN/tryptophan ratio and night-time melatonin secretion in TS patients who suffered with associated obsessive compulsive disorder/behaviours (OCD/B).

6. determine whether there is any effect of the current smoking status on plasma INF-γ, neopterin, KYN, tryptophan levels, KYN/tryptophan ratio or on melatonin secretion in TS patients and healthy individuals.

Additional description of methods (Clinical study 2)

The TS patients included in this study were those diagnosed to have TS according to the DSM-1V criteria, by Dr. H. E. G. Rickards (Consultant Neuropsychiatrist, QEPH, Birmingham) and their tic severity was assessed using the Yale Global Tic Severity Score (YGTSS) (Leckman *et al.*, 1989), by me under the guidance of Dr. H. E. G. Rickards in parallel with the collection of blood and urine samples, to determine the relationship of different biochemicals assayed with the tic severity. Patients who did the night-time urine collection more than two weeks apart from the collection of the blood sample were assessed a second time for the tic severity at the time the urine samples were received. Recruitment of patients and collection of blood and urine samples for this study were done between August 1998 and March 1999. Most patients and controls included in the study gave both blood and urine and a few gave only blood or urine samples. Blood samples were collected from 27 TS patients and 24 age, sex and ethnic group matched controls for plasma INF-γ, neopterin, KYN and tryptophan assays. Since plasma neopterin

levels have shown difference according to the ethnic group, with higher levels among whites than in blacks (Diamondstone *et al.*, 1994) all the controls were also matched for ethnic group as well. To nullify the effect of a diurnal or a seasonal variation, if present in the expression of the biochemicals detected in plasma, all the blood samples from both the patients and controls were taken between 12.00–17.00 hrs, and control blood samples were taken in the same month as from the patient to whom he/she was matched. Urine samples were received from 31 TS patients and 22 age, sex and ethnic group matched controls. Since melatonin shows a diurnal and a seasonal variation all the urine collections were done from 8 p.m. to 8 a.m. and the controls also provided urine samples in the same month as the patient to whom he/she was matched. All together 32 TS patients and 27 healthy controls were included in this study.

Most of the patients were on one or more medication for TS and/or for conditions associated with TS (table 5.3 shows the medications taken by the patients included in the study). None of these patients were on any other medication at the time of blood and urine samples collection and for 2 weeks prior.

The patients who were identified as having associated OCD/B were those diagnosed according to DSM-IV criteria and scored on the Leyton obsessional inventory (Cooper, 1970).

All the statistical analyses were done using the SPSS statistical package and the significance of differences between different groups were compared by independent samples t-test. Further analysis with 1-way Anova, followed by Tukey's Post-hoc test was done where appropriate. Presence of a monthly variation in the expression of different biochemicals was tested by analysis of variance and relationships between different parameters were tested by Pearson product-moment or Spearman correlation coefficient. Statistical significance was set at a probability level (p) of 0.05.

Results

Figure 5.1 shows the age distribution and gender of TS patients and healthy controls included in this study. There were 2 patients of Pakistani and 1 patient of

Chinese ethnic origin. All the other patients recruited in this study were British whites. Control sample included 1 Chinese, 2 Pakistani origin and 25 British white healthy individuals.

Figures 5.2 and 5.5 show the monthly distribution of the collection of blood and urine samples from patients and controls for plasma INF-γ, neopterin, KYN, tryptophan and night-time urinary aMT6S assays: figure 5.2 shows the number of patients and controls from whom blood samples were obtained and figure 5.5 shows the number of patients and controls from whom urine samples were received each month.

Of the biochemicals measured in plasma, KYN and tryptophan showed a significant monthly variation (analysis of variance, KYN: $F_{3,28}$ 2.936; $p<0.05$, tryptophan: $F_{3,28}$ 3.042; $p<0.05$, see figures 5.3 and 5.4). The night-time urinary aMT6S also showed a significant monthly variation (analysis of variance, $F_{6,51}$ 3.626; $p<0.05$) as expected. Lower night-time urinary aMT6S excretion is seen in the Winter months for both the patients and the controls (see figure 5.6).

On statistical analysis of the results, none of the biochemicals measured here showed a significant difference between the TS patients and controls (see tables 5.1 and 5.2).

Table 5.1. Mean plasma INF-γ, neopterin, KYN, tryptophan and KYN/tryptophanx1000, detected in TS patients and matched healthy controls.

Biochemical	**Patients** mean ± SEM (N=27)	**Controls** mean ± SEM (N=24)	**t-test analysis between groups**
INF-γ (pg/ml)	0.54 ± 0.14	0.68 ± 0.24	p>0.05
neopterin (nM)	6.93 ± 0.60	7.59 ± 0.80	p>0.05
KYN (μM)	1.88 ± 0.10	2.10 ± 0.12	p>0.05
tryptophan (μM)	60.41 ± 3.4	58.87 ± 2.86	p>0.05
KYN/tryptophan x 1000	31.75 ± 1.21	37.87 ± 3.32	p>0.05

Table 5.2. Mean night-time urinary aMT6S excretion detected in TS patients and matched controls.

	night-time urinary aMT6S (ng/collection)	
	Patients (N= 31)	**Controls (N=22)**
mean ± SEM	14722.88 ± 1949.38	13129 ± 2232.54
t-test analysis	$p > 0.05$	

The tic severity detected by the YGTSS in this group of 32 TS patients ranged between a minimum score of 12 and a maximum of 42 with a mean of 26.88 ± 1.52, out of a total score of 55. These tic severity scores did not show any significant relationship to the substances (plasma INF-γ, neopterin, KYN, tryptophan, KYN/ tryptophan x 1000 or night-time urinary aMT6S) measured in this group of patients (correlation coefficient, $p>0.05$ for all). They also did not show any significant relationship to the age of the patient or the month in which the assessment was done.

On examining the relationship between different biochemicals in patients and healthy controls together as one sample, night-time urinary aMT6S excretion showed a significant negative correlation with plasma KYN ($r = -0.41$ and $p< 0.05$) and KYN/tryptophan x 1000 ($r = -0.34$ and $p<0.05$), while plasma INF-γ showed a positive correlation with plasma neopterin ($r = 0.39$ and $p< 0.05$). KYN, tryptophan and KYN/ tryptophan x 1000 positively correlated with each other as expected. The relationships between different biochemicals were examined separately within the patients and healthy controls. In the healthy control group, the same significant relationships between substances as seen in the whole sample (negative correlation between night-time urinary aMT6S excretion and plasma KYN ($r = -0.52$ and $p< 0.05$), positive correlation between plasma INF-γ and plasma neopterin ($r = 0.61$ and $p< 0.05$) and positive correlation between plasma KYN, tryptophan and KYN/ tryptophan x 1000) were seen, and in addition INF-γ positively correlated with plasma KYN ($r = 0.45$ and $p< 0.05$, see figure 5.7) and even more strongly with the KYN/tryptophan ratio ($r = 0.61$ and $p< 0.005$). There was no such significant correlation between INF-γ and KYN in TS patients (see

figure 5.8). None of the correlations were significant ($p>0.05$) other than the positive correlations between plasma KYN, tryptophan and KYN/ tryptophan x 1000 ($p< 0.05$), in the TS patients group, although the correlations showed the same trends as in healthy controls.

Out of the 32 TS patients included in this study only 5 were unmedicated. The table 5.3 shows the different medications taken by the patients at the time of the study.

Table 5.3. The medications taken by the patients included in the study.

Medications	**Number of patients**
Neuroleptics: Haloperidol, Pimozide and Sulpiride	21
Antidepressants: Serotonin reuptake inhibitors (SSRIs): Paroxetine and Fluoxetine Tricyclic antidepressants (TCAs): Dothiepin and Clomipramine,	8 4
Methylphenidate	2
Benzodiazepines: Diazepam and Clonazepam	3
Propranolol	1
Zopiclone	1
Antimuscarinic drugs: Procyclidine	1
α2-adrenergic agonists: Clonidine	1

Effects of neuroleptics and antidepressants (SSRIs and TCAs) on the different biochemicals were examined by comparing the levels of the different biochemicals in, patients on neuroleptics/antidepressants, patients who were not on those medications and matched healthy controls.

As shown in table 5.5 and figure 5.9, night-time urinary aMT6S excretion showed a statistically significant difference between the TS patients, who were on neuroleptics (N=18) and those who were not on neuroleptics (N=13), with higher levels in the TS patients who were not on neuroleptics (independent samples t-test, $p< 0.05$). None of the other substances showed a significant difference

between these two groups (see table 5.5). On comparison of night-time urinary aMT6S excretion of these two groups of TS patients and healthy controls, the differences were not statistically significant (1-way Anova followed by Tukey's post hoc test, $p>0.05$), casting doubt on the reliability of the significant difference seen between the patients on neuroleptics and patients not on neuroleptics by t-test analysis.

Patients on antidepressants: SSRIs or TCAs (N=11) showed significantly lower plasma KYN levels (table 5.5 and figure 5.10) when compared with patients who were not on antidepressants (N=16) (independent samples t-tests, $p<0.05$). Further statistical analysis by 1-way Anova followed by Tukey's post-hoc test showed significantly lower plasma KYN levels in TS patients on antidepressants when compared with both healthy controls (N=24) and TS patients not on antidepressants ($F_{2,49}$ 3.5313; $p<0.05$). None of the other substances showed a significant difference between these groups (see table 5.5).

Table 5.4 shows all the associated conditions suffered by the TS patients included in the study.

Table 5.4. Associated conditions suffered by the TS patients included in the study.

Associated conditions	**number of patients**
Attention deficit disorder/Hyperactivity disorder/Attention deficit hyperactivity disorder	7
Depressive disorder	6
Learning disorder	1
Obsessive compulsive disorder/behaviours (OCD/B)	15

Out of the 27 TS patients whose plasma INF-γ, neopterin, KYN and tryptophan were assayed 14 had associated OCD/B and out of the 31 patients whose night-time urinary aMT6S was assayed, 15 had associated OCD/B. On comparison of the different biochemical levels between TS patients having associated OCD/B with healthy controls, plasma KYN levels were found to be significantly lower in

TS patients having associated OCD/B (independent samples t-test, $p<0.05$, see figure 5.11). Further statistical analysis by 1-way Anova did not show a significant difference in the plasma KYN levels between different groups of subjects (TS patients with OCD/B, TS patients without OCD/B, healthy controls), doubting the reliability of the significance of the low plasma KYN seen in TS patients with OCD/B, with reference to healthy controls by t-test analysis. Other biochemicals in TS patients with OCD/B did not show a significant difference on comparison with healthy controls or TS patients without OCD/B (t-test and 1-way Anova, $p>0.05$) (see table 5.5 below).

Table 5.5. Mean ± SEM detected for the different biochemicals, separating patients into subgroups, according to their medications and presence or absence of associated OCD/B.

substance	**controls**	**patients**					
		on neuroleptic	not on neuroleptic	on anti-depressants	not on anti-depressant	OCD/B +	OCD/B -
aMT6s (ng/collection)	13129.36 ± 2232.54 (N=22)	12038.06 ± 1780.2 (N=18)	20763.75 ± 4388.71 (N=13)	19675.33 ± 4305.19 (N=11)	12101.00 ± 1712.78 (N=20)	12846.5 ± 2413.70 (N=15)	16912.0 ± 413.70 (N= 16)
INF-γ (pg/ml)	0.68 ± 0.24 (N=24)	0.45 ± 0.10 (N=17)	0.69 ± 0.33 (N=10)	0.66 ± 0.19 (N=10)	0.48 ± 0.18 (N=17)	0.37 ± 0.10 (N=14)	0.69 ± 0.25 (N=13)
neopterin (nM/l)	7.41 ± 0.81	7.48 ± 0.88	6.01 ± 0.56	7.53 ± 1.05	6.68 ± 0.74	6.78 ± 0.70	7.07 ± 0.98
KYN (μM/l)	2.1 ± 0.12	1.88 ± 0.13	1.87 ± 0.14	*1.6 ± 0.2	2.0 ± 0.1	1.75 ± 0.10	2.00 ± 0.16
tryptophan (μM/l)	58.43 ± 2.95	58.24 ± 3.52	64.10 ± 7.17	51.02 ± 1.02	62.64 ± 6.53	60.04 ± 5.49	60.75 ± 4.40
KYN x 1000 / tryptophan	37.87 ± 3.32	32.54 ± 1.30	30.44 ± 2.43	31.36 ± 1.96	31.93 ± 1.53	30.30 ± 1.58	33.11 ± 1.79

* = significantly lower than that of patients not on antidepressants and controls (1-way Anova, $F_{2,49}$ 3.5313; $p<0.05$)

On comparison of the different biochemicals between smokers and non-smokers, statistically significant lowering of the plasma INF-γ levels were seen in the

smokers (table 5.6 and figure 5.12), both among patients and controls (independent samples t-test, $p<0.05$). None of the other substances were significantly different between smokers and non-smokers ($p>0.05$) (see table 5.6).

Table 5.6. Mean ± SEM detected for different substances in smoking and non-smoking patients and healthy controls.

Substance	Patients		Controls	
smoking status	**smoker**	**non-smoker**	**smoker**	**non-smoker**
aMT6s (ng/collection)	16309.00 ± 4175.56 (N=13)	13883.18 ± 2082.91 (N=18)	7094.40 ± 3270.40 (N=5)	14904.35 ± 2610.18 (N=17)
INF-γ (pg/ml)	0.31 ± 0.12* (N=10)	0.67 ± 0.2 (N=17)	0.43 ± 0.23* (N=7)	0.78 ± 0.33 (N=17)
neopterin (Nm/l)	7.45 ± 1.33	6.63 ± 0.57	6.84 ± 1.77	7.92 ± 0.88
KYN (μM/l)	1.81 ± 0.20	1.92 ± 0.10	1.93 ± 0.15	2.16 ± 0.15
tryptophan (μM/l)	58.29 ± 4.76	61.65 ± 4.74	60.77 ± 5.13	58.20 ± 3.49
KYN x 1000 / tryptophan	30.69 ± 1.74	32.27 ± 1.65	39.56 4.22	33.07 4.18
* = significantly lower than the levels for non-smokers when compared within the group (patients and healthy controls)				

Discussion

This group of TS patients did not show an increase in plasma KYN levels as reported in the earlier studies, instead TS patients who were on antidepressants (SSRI or TCA), as treatment for associated conditions showed significantly lower KYN levels when compared with TS patients who were not on antidepressants and with healthy controls. TS patients who had associated OCD/B showed significantly lower KYN levels when compared with healthy controls, but on further statistical analysis using 1-way Anova taking the patients who did not have associated OCD/B also into account, no significant difference in the plasma KYN levels were shown between groups. Since these subgroups of TS patients were small, further studies on larger samples of TS patients with and without associated OCD/B is required to discard or accept the significantly lower KYN

seen by t-test analysis in TS patients having associated OCD/B in this study. Low plasma KYN levels that return to normal in remissions have been shown in endogenous depression (Orlikov *et al.*, 1994). Therefore it is possible that the lower plasma KYN levels in TS patients suffering from depression contributed towards the low plasma KYN levels seen in TS patients on antidepressant medication. Inhibitory effects of TCAs on hepatic TDO has also been shown by animal studies where animals were treated with Amitriptyline, Imipramine and Tranylcypromine (Mangoni, 1974). The lower plasma KYN seen in TS patients on antidepressants may be due to a similar inhibitory effect on the hepatic TDO by the SSRIs (Paroxetine/Fluoxetine) and TCAs (Clomipramine and Dothiepin) taken by them. The SSRIs inhibit the liver cytochrome P450 isoenzymes and it is shown that Fluoxetine is highly potent and Paroxetine is weakly potent in this inhibition (Stahl, 1997), but inhibition of hepatic TDO activity by SSRIs has not been shown. The failure to see increased plasma KYN levels in this group of TS patients, as previously reported may be due to the lower plasma KYN levels in TS patients on antidepressants, who formed a major part (21 out of the 27 patients) of the TS patient group included in this study. These results suggest studies to investigate the effects of SSRIs on tryptophan metabolism.

The results of this study showed a significant monthly variation in plasma KYN. Therefore another possibility for the discrepancy of findings on KYN in these different studies is the difference in the monthly distribution of the different studies. Out of the 27 patients and the 24 controls on whom plasma KYN were assayed in this study were those bled from August to February, except for the 2 patients and 1 control bled in March 1999, while in the large cross-sectional study mentioned above out of the two previous studies, the plasma KYN levels have been compared in TS patients and controls bled from March-June 1996. A variation in the pattern of KYN synthesis occurring in different months in the TS patients may have contributed to the discrepancies of findings in different studies, that investigated plasma KYN in TS.

Plasma neopterin levels were also not increased when compared with healthy controls, in this group of TS patients nor did they correlate with plasma KYN

levels as show by one of the two studies, which investigated plasma neopterin in TS together with KYN (Gaynor *et al.*, 1997). But plasma INF-γ positively correlated with the plasma neopterin, KYN and more strongly with KYN/tryptophan ratio in healthy controls, showing that plasma neopterin and KYN production is regulated by INF-γ in healthy individuals, in agreement with the clinical, *in vitro* and *in vivo* studies showing the regulatory effect of INF-γ on neopterin and KYN production (refer General Introduction). Neither KYN nor neopterin significantly correlated with INF-γ in TS patients suggesting a disturbance in the normal KYN and pteridine metabolism in TS, even though a significant difference in the plasma levels of the individual substances were not found between TS patients and healthy controls in this study.

The absence of a significant relationship of any of the substances detected here with the tic severity in this mainly medicated group of TS patients, does not rule out them playing a part in the pathogenesis of tics in TS. Similar studies are required in unmedicated patients to make conclusions.

TS patients who were not on neuroleptics showed higher urinary aMT6S excretion when compared with TS patients who were on neuroleptics and healthy controls. Statistical significance with t-test was seen on comparison of TS patients on neuroleptic medication and not. In order to take the control group into account, 1-way Anova followed by Tukey's Post-hoc test was done on controls, patients on neuroleptics and patients not on neuroleptics. Neither 1-way Anova or Tukey's test showed significant difference in the urinary aMT6S excretion between these groups. This casts doubt on the reliability of the significant difference seen by t-test analysis, between the urinary aMT6S excretion in TS patients on neuroleptics and not on neuroleptics. Urinary aMT6S excretion shows great inter-individual variability (Bojkowski and Arendt, 1988) making it more difficult to make conclusions on small groups like this. Therefore further work is required on a large sample having more cases within subgroups to get conclusive evidence for the presence of a disturbance in the melatonin secretion or the excretion of aMT6S in TS.

Urinary aMT6S and plasma KYN showed significant negative correlation and this can be explained on the findings by Walsh *et al.* (1994), where melatonin inhibited hepatic TDO activity, resulting in decreased production of KYN. This significant correlation was seen only when the patients and the healthy controls were taken as one sample and when healthy controls were taken as a separate group. There was no significant correlation between urinary aMT6S and plasma KYN when the patients were taken as a separate group. This further suggests the presence of altered melatonin secretion and KYN production in TS, leading to a disturbance in the normal correlation seen between the two substances among healthy individuals.

On comparison of different substances among smokers and non-smokers in the whole sample and within the patient and the control groups, INF-γ was significantly lower in the smokers. This finding suggests that smoking has a significant inhibitory effect on systemic INF-γ expression. Cigarette smoking has been shown to alter the production of a number of cytokines by several cells and a number of *in vitro* and *in vivo* experiments have shown modulatory effects of nicotine on cytokine production (McCrea *et al.*, 1994; Tappia *et al.*, 1995; Dandrea *et al.*, 1997; Sugano *et al.*, 1998), but the effect of cigarette smoking on systemic INF-γ expression has not been clearly shown (Satoh *et al.*, 1995).
The findings of this clinical study lead to a number of speculations as mentioned above, that suggest further experiments in larger samples of TS patients and healthy controls to determine the exact biochemical disturbances in TS.

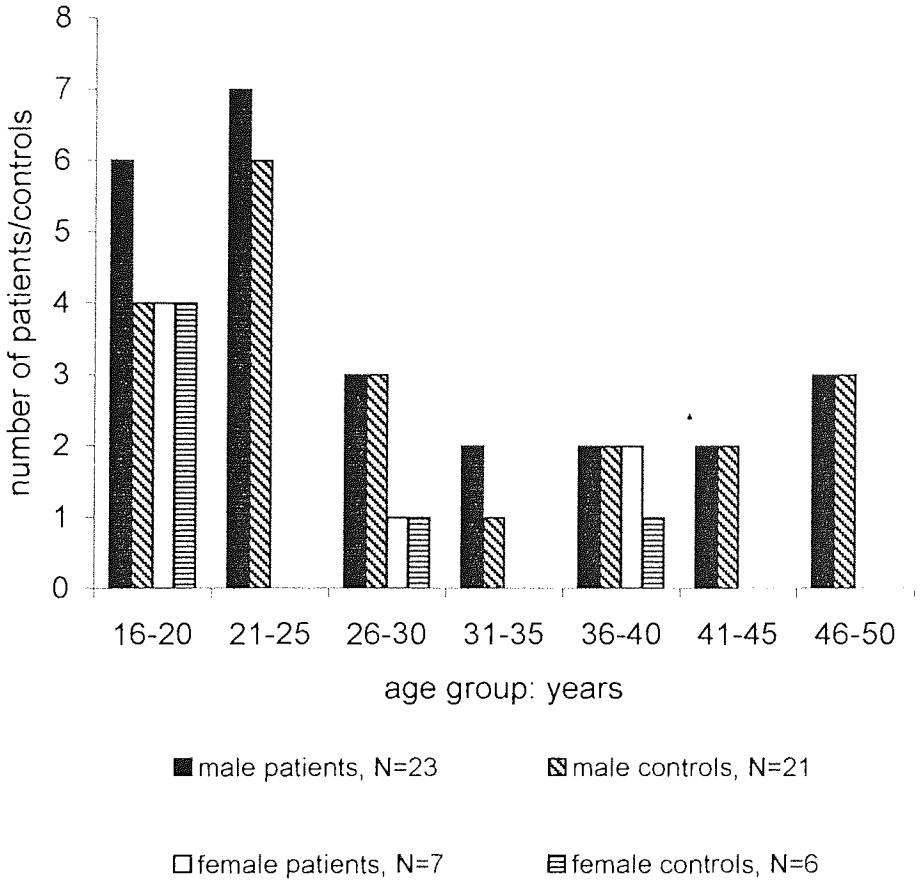


Figure 5.1. Age distribution and gender of the patients and controls included in the study.

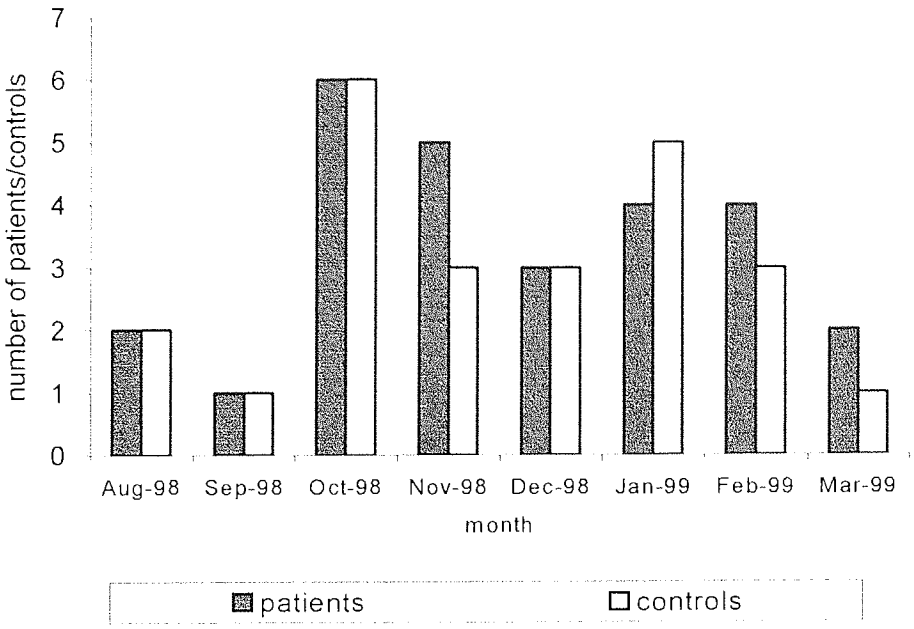


Figure 5.2. Number of patients and controls bled each month over the study period for plasma INF-γ, neopterin, KYN and tryptophan assays. patients, N= 27 and controls, N= 24.

Figure 5.3

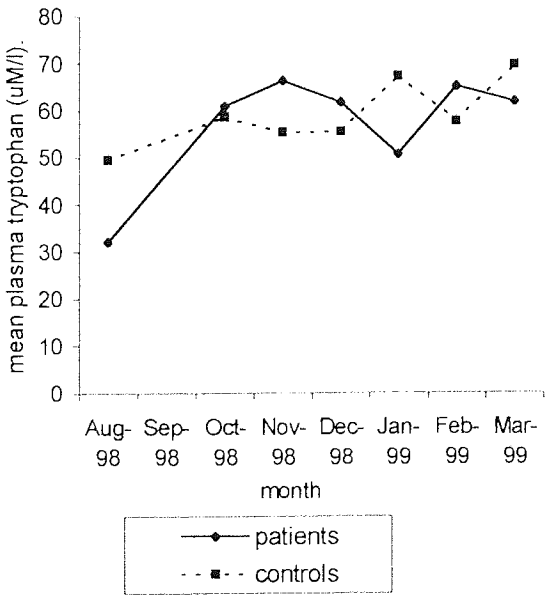
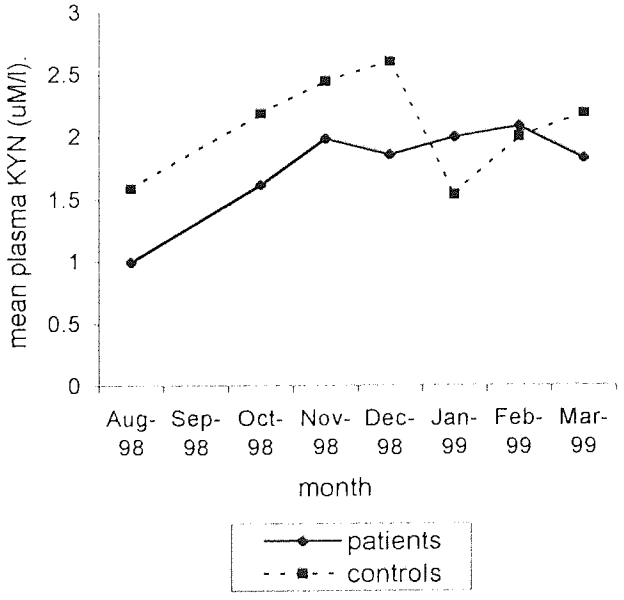


Figure 5.4



Figures 5.3 and 5.4: Variation of plasma tryptophan and KYN seen in patients and controls by month (analysis of variance: $p < 0.05$ for both tryptophan and KYN in patients and controls).

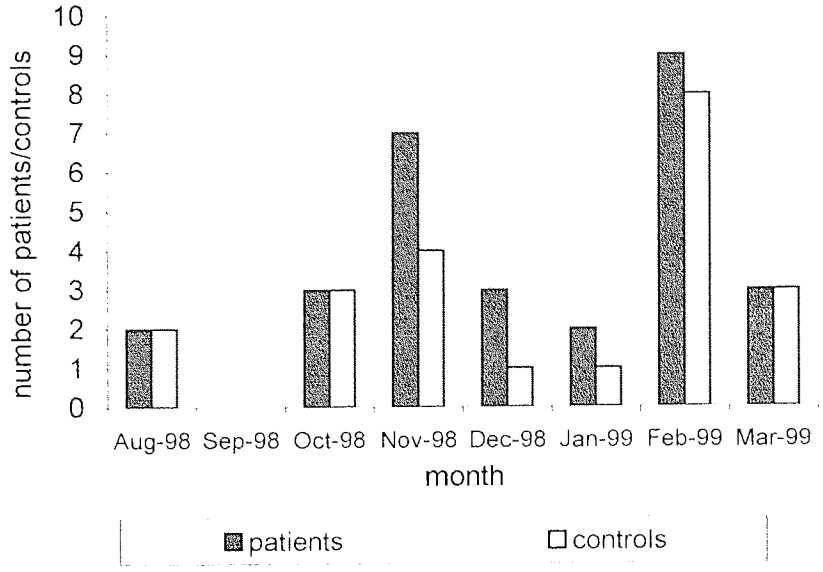


Figure 5.5. The number of patients and controls who gave urine samples in each month over the study period for aMT6S assay. patients, N=29 and controls, N= 22.

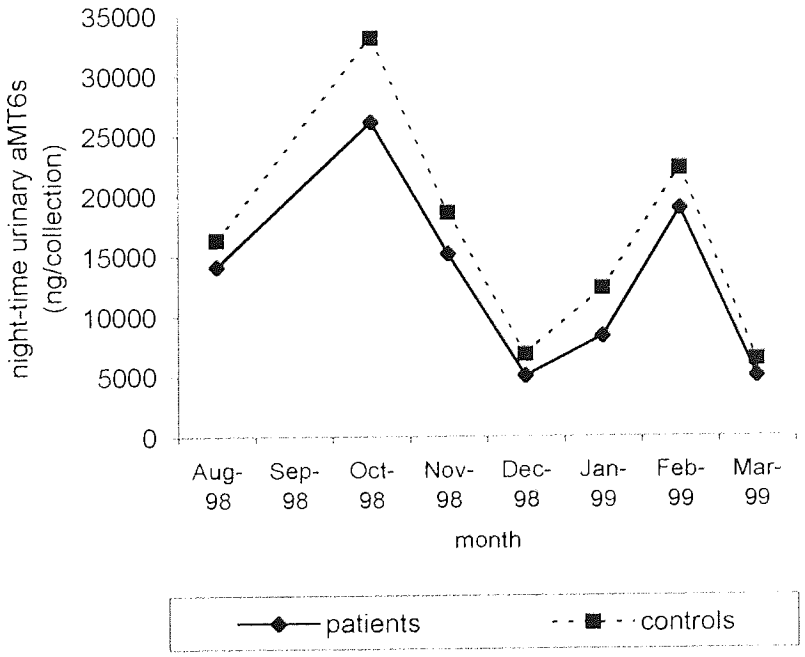


Figure 5.6. Variation of night-time urinary aMT6S excretion in patients and controls according to the month of collection.

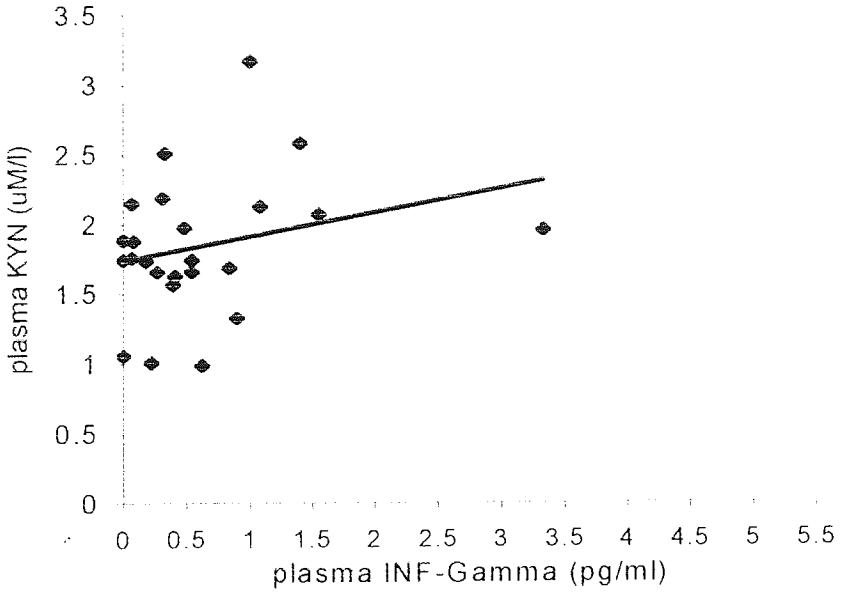


Figure 5.7. Correlation between plasma INF-γ and KYN in healthy controls (Pearson Product moment correlation coefficient, $r = 0.45$ and $p<0.05$).

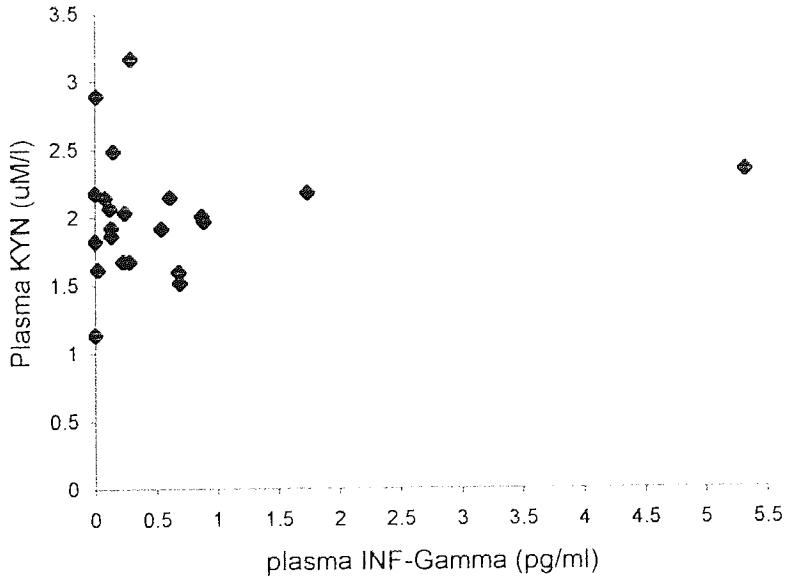


Figure 5.8. Plasma INF-γ and KYN in TS patients. There was no significant correlation between plasma INF-γ and KYN (Pearson Product moment correlation coefficient, $p>0.05$).

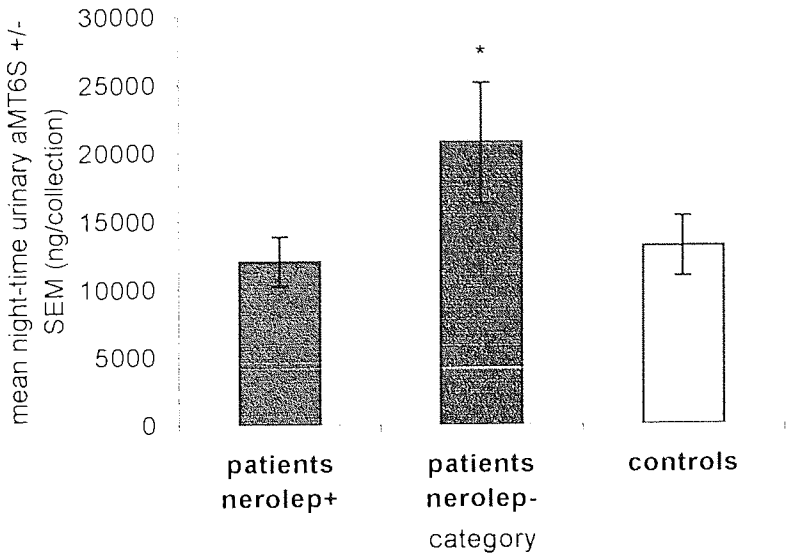


Figure 5.9. Night-time urinary aMT6s excretion in TS patients on neuroleptics (patients neurolep+, N= 18), TS patients not on neuroleptics (patients neurolep-, N= 13) and matched controls (controls, N= 22). Columns represent mean aMT6S excretion for each group and vertical bars represent standard errors.
* = significantly higher than in patients neurolep+ (t-test, $p<0.05$).

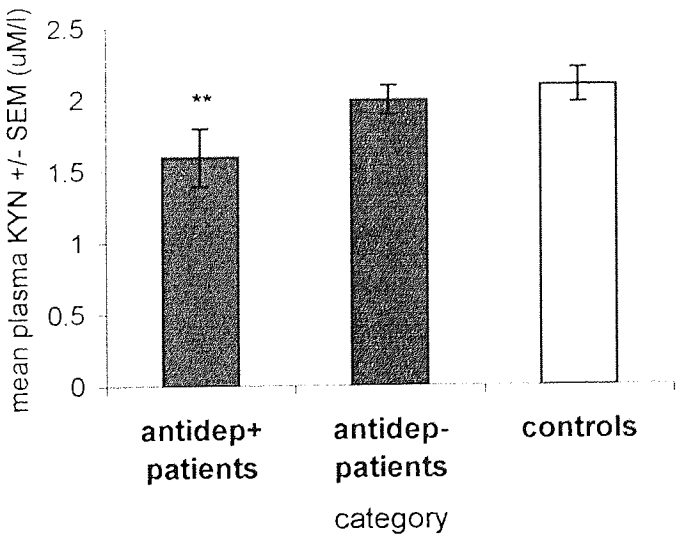


Figure 5.10. Plasma KYN levels in TS patients on SSRI and TC antidepressants (antidep+ patients, N= 11), TS patients not on antidepressants (antidep- patients, N= 16) and matched healthy controls (controls N=24). Columns represent mean plasma KYN for each group and vertical bars represent standard errors.
** = significantly lower than in antidep- patients and controls (analysis of variance, $F_{2,49}$ 3.5313; $p<0.05$ and t-tests, $p<0.05$).

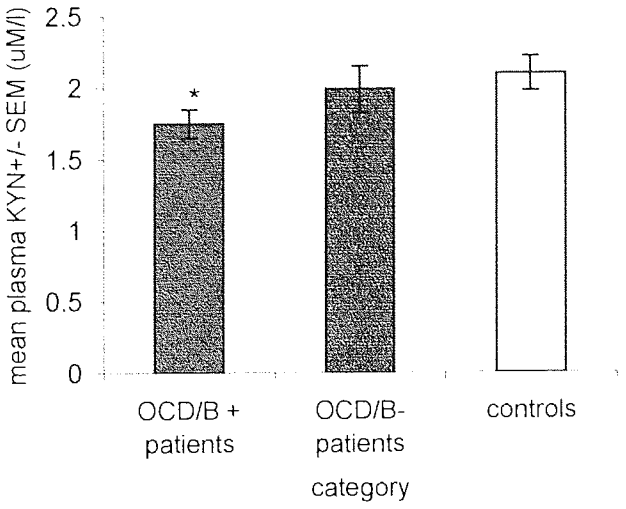


Figure 5.11. Plasma KYN levels in TS patients having associated OCD/B (OCD/B+ patients, N= 14), TS patients not having associated OCD/B (OCD/B- patients, N= 13) and matched healthy controls (controls, N= 24) Columns represent mean plasma KYN for each group and vertical bars represent standard errors. * = significantly lower than in controls only (t-test, $p<0.05$).

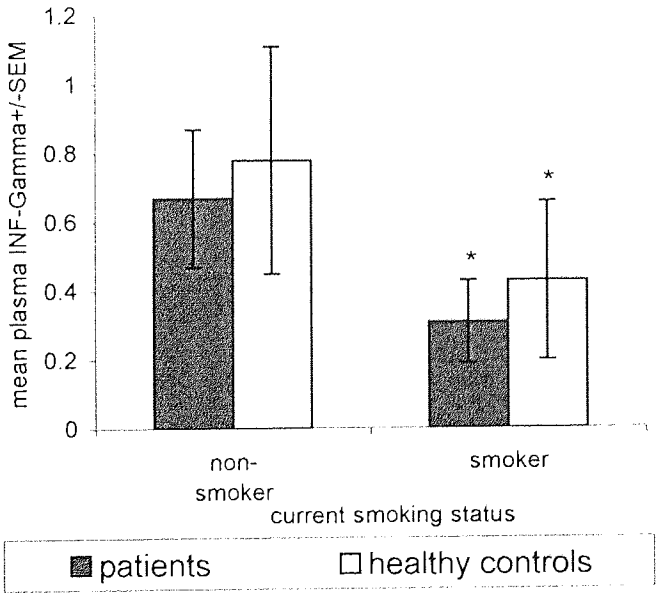


Figure 5.12. Plasma INF-γ levels in smoking and non-smoking patients and healthy controls. The columns show the mean plasma INF-γ levels for each group and the vertical bars represent standard errors. Patients, N=27 (10 smokers and 17 non-smokers), healthy controls, N=24 (7 smokers and 17 non smokers) * = significantly lower than the levels for non-smokers within the same group (t-test analysis, $p<0.05$).

GENERAL DISCUSSION

GENERAL DISCUSSION

The purpose of the pre-clinical studies done in this project was to investigate the mechanisms of action of kynurenine pathway metabolites in influencing tics-like behaviours in mice. The animal studies clearly showed that KYN, the first stable metabolite of the pathway potentiates 5-HT_{2A}-mediated head shakes in mice which have been proposed to model tics in TS (Handley and Dursun, 1992). QUINA which is a NMDA agonist (Stone & Perkin, 1981) produced along the kynurenine pathway, showed dose dependent inhibition of the 5-HT_{2A}-mediated head shake response. 3-OHAA which is the immediate precursor of QUINA in the kynurenine pathway also showed inhibition of the 5-HT_{2A}-mediated head shake response only at higher pre-treatment times suggesting that the effect was due to a further metabolite, which was likely to be QUINA.

The radioligand binding studies presented in Chapter 2 showed that neither KYN nor QUINA directly bind or indirectly modify the binding of a known ligand (methysergide) to 5-HT_{2A} receptors. Therefore it is evident that the effects of KYN and QUINA detected on the 5-HT_{2A}-mediated head shake response is not due to a direct agonist or an antagonist effect on the 5-HT_{2A} receptors.

The finding that 5-HT_{2A}-mediated head shake response is inhibited by QUINA is in agreement with the findings by Kim *et al.* (1998), which showed inhibition of 5-HT-induced head shakes by NMDA, and potentiation by NMDA receptor antagonists. This raises the question how NMDA receptor stimulation affects a 5-HT_{2A}-mediated response. The PI hydrolysis studies presented in Chapter 2 showed that QUINA inhibited 5-HT_{2A}-mediated PI hydrolysis, and previous studies on PI hydrolysis have shown that NMDA receptor stimulation inhibits PI hydrolysis mediated by several other neurotransmitter systems, including 5-HT (refer to the discussion of Chapter 2). Therefore it is possible that the inhibition of the 5-HT_{2A}-mediated head shake response seen with QUINA may be due to an inhibition of the second messenger pathway linked with 5-HT_{2A} receptor stimulation (PI hydrolysis), following NMDA receptor stimulation by QUINA. However

it has been reported that NMDA receptor stimulation also modifies several other neurotransmitter systems by means of increasing or inhibiting neurotransmitter release (Keita *et al.*, 1997; Nankai *et al.*, 1998). Increased release of noradrenaline in the CNS following NMDA stimulation is well documented (Pittaluga *et al.*, 1997; Wang & White, 1998) and noradrenaline has been shown to be highly potent in inhibiting 5-HT_{2A}-mediated head shakes (Handley, 1970; Collier *et al.*, 1975). Therefore this inhibition of 5-HT_{2A}-mediated head shakes by the noradrenaline released after NMDA receptor stimulation is another possibility for the inhibitory effect of QUINA detected on 5-HT_{2A}-mediated head shakes. Even though Kim *et al.* (1998) showed potentiation of 5-HT induced head shakes in mice by several NMDA antagonists (AP-5, CPP, MK-801, ketamine, dextrophan and dextromethorphan), we did not detect a significant potentiation of 5-HT_{2A}-mediated head shakes in mice by KYNA, which is a broad spectrum NMDA antagonist produced along the kynurenine pathway metabolism of tryptophan (Perkin & Stone, 1982). Since the NMDA receptor has a very complex structure with a number of subunits, the different exogenous antagonists show marked variability in specificity and potency in binding to these sites of the NMDA receptor (Seeburg, 1993; Hollmann & Heinemann, 1994; Danysz et al., 1995; Rogawski, 1993; Evans *et al.*, 1982). KYNA interacts with the glycineB site of the NMDA receptor and is a very weak and non-selective, competitive antagonist of the NMDA receptor, as it is also a competitive antagonist at AMPA/Kainate receptors (Birch *et al.*, 1988; Stone, 1991). Of the NMDA antagonists with which Kim *et al.* (1998) showed potentiation of 5-HT_{2A}-mediated head shakes, AP-5 and CPP are potent and selective antagonists, (Evans *et al.*, 1982; Davies *et al.*, 1986), while MK-801, ketamine, dextrophan and dextromethorphan are potent, selective and non-competitive antagonists (Wong *et al.*, 1986; MacDonald *et al.*, 1991; Netzer *et al.*, 1993) of the of the NMDA receptor. Therefore the failure to see a significant potentiation of the 5-HT_{2A}-mediated head shakes by KYNA may be due to its low potency of action at the NMDA receptor, compared with the NMDA antagonists with which Kim *et al.* (1995) reported potentiation of 5-HT_{2A}-mediated head shakes.

The mechanism by which KYN potentiates 5-HT_{2A}-mediated head shakes is not clear. It is possible that KYN may be acting via an unrecognized receptor group that influences 5-HT2A-mediated head shakes. Stone (1991) implied a physiological role of KYN in the modulation of NMDA receptor sensitivity, by an observation of KYN activity on hippocampal neurons similar to that of glycine. The convulsant effect of KYN is well established (Lapin, 1981a) and this suggests a stimulatory effect of KYN on NMDA receptors rather than an inhibitory effect. But it is possible that that KYN may be having an inhibitory effect on another site of the NMDA receptor and its potentiation of 5-HT_{2A}-mediated head shakes may be by a mechanism similar to that of NMDA antagonists potentiating 5-HT_{2A}-mediated head shakes demonstrated by Kim *et al.* (1998). This is an area worth researching further. All our findings of animal and *in vitro* studies taken together with the related findings by other researchers, suggest that it will be useful to further explore the mechanism of the actions of these metabolites, KYN and QUINA on different neurotransmitter systems, in order to decide how they modify this 5-HT_{2A}-mediated central action.

Two previous clinical studies reported increased plasma KYN in TS patients (Dursun, 1992; Gaynor *et al.*, 1997), when compared with age and sex matched healthy controls. The earlier study (Dursun, 1992) out of the two showed a much higher magnitude of difference in plasma KYN levels between patients and controls when compared with the later study (Gaynor *et al.*, 1997). Neither of these studies showed lower plasma tryptophan levels in TS patients, when compared with healthy controls, as reported by Comings (1990a,b). The study by Gaynor *et al* (1997) also showed increased plasma neopterin levels in the TS patients, while the study by Dursun (1992) did not detect such a change. Under physiological conditions the metabolism of tryptophan via the kynurenine pathway, leading to the production of KYN, predominantly occurs in the liver (Knowles *et al.*, 1989). TDO is the first and the rate limiting enzyme of the kynurenine pathway in the liver, which catalyses the production of formylkynurenine, which in turn is converted to KYN. Cortisol induces the liver IDO activity (refer to General Introduction). However an associated increase in the plasma cortisol levels was not found (refer to results in Chapter 4) in the group of TS patients on whom elevated plasma KYN

levels were reported by Gaynor *et al.* (1997). In the absence of increased plasma cortisol levels to account for the increased plasma KYN levels, and in the presence of associated increased plasma neopterin levels in the same group of TS patients, it was speculated that the increased plasma KYN detected in TS patients may be due to increased IDO activity, induced by cytokines (Gaynor *et al.*, 1997). Considering these clinical findings of plasma KYN in TS, we measured plasma tryptophan, KYN, neopterin and INF-γ in the TS patients and the matched healthy controls in the clinical study presented in Chapter 5. This study did not reveal elevated plasma tryptophan, KYN or neopterin levels in TS patients as reported before, but showed a disturbance in the positive correlation between plasma KYN, neopterin and INF-γ compared with the age, sex and ethnic group matched healthy controls. The healthy controls showed a significant positive correlation between plasma INF-γ, neopterin and KYN as expected, in agreement with the induction of IDO and GTPC-1, by INF-γ (refer to General Introduction). In addition plasma KYN was found to be lower in TS patients having associated OCD/B, when compared with the healthy controls, but statistical analysis of this lower KYN levels was not convincing (see results in Chapter 5). TNFα is a cytokine clearly shown to potentiate the inducing effects of interferons on IDO (Werner-Felmayer *et al.*, 1989) and significantly lower plasma TNFα levels have been detected by Monteleone *et al.* (1998) in subjects with OCD, in comparison to healthy controls. Higher turnover of serotonin has also been reported in OCD, leading to increased metabolism of tryptophan via the serotonin pathway (Bornstein & Baker, 1992). In the presence of these findings showing a possibility of lower KYN production in subjects with OCD/B the finding of lower plasma KYN levels in TS with associated OCD/B cannot be totally disregarded, even though the statistical significance was not convincing. We also found significantly lower plasma KYN levels in TS patients on antidepressants, when compared with TS patients who were not on antidepressants and healthy controls. This finding could be explained by the observation of the inhibitory effect of TCAs on liver TDO activity in animals, but such an action has not been shown with SSRIs (refer to discussion of Chapter 5). Our findings suggest that future animal experiments should be carried out to detect the effects of SSRIs on hepatic tryptophan metabolism. These different factors influencing plasma KYN levels in the TS patients may have contributed to the failure to detect increased plasma KYN levels in this

group of TS patients, in contrast to the previous studies. Our study also showed a significant monthly variation in plasma KYN and tryptophan levels in both the patient and the control groups. The two previous studies which examined the plasma KYN levels for comparison with healthy controls have been performed in different months of the year compared with ours (Dursun, 1992; Gaynor *et al.*, 1997). It is also well accepted that the symptoms of TS show a seasonal variation (Goetz, 1986). As shown in figure 5.4 in Chapter 5, during the months of our study the monthly variation of plasma KYN levels in patients and controls were mostly parallel to each other, suggesting that the monthly variation did not differ between the two groups. However this pattern may be different in other months, when there may be a different pattern of seasonal variation of plasma KYN production occurring in TS patients, compared with healthy individuals. This difference of KYN synthesis may be restricted to a particular period of the year and the two previous studies would have fellen during such a period. The difference in the magnitude of increase in patient plasma KYN levels from controls in the two previous studies also may be due to this monthly variation between them. It is worth carrying out larger studies spread over one whole calendar year examining the same patients monthly to test this hypothesis. Absence of increased plasma levels of KYN does not rule out increased levels of KYN in the brain and it may be that both increased brain KYN and plasma KYN levels are found in more severely affected TS patients or during the periods of increased severity of the disorder. Therefore it would be useful to determine the KYN levels in the CNS by means of CSF analysis in TS patients and healthy controls. Such studies will have a lot of practical and ethical implications as obtaining CSF is considered an invasive procedure.

Even though our study did not detect significantly higher plasma levels of KYN, neopterin or INF-γ in TS patients with reference to healthy controls, a disturbance in the IDO mediated KYN production in TS patients was made evident when compared with healthy individuals. A significant positive relationship between plasma INF-γ, neopterin and KYN was present as expected in the control group and was absent in the TS patients (refer to results in Chapter 5). The night-time urinary aMT6s detected as an index of melatonin secretion in this study showed a significant negative correlation with plasma

KYN only in the healthy control group, in agreement with the animal experiments showing inhibition of liver TDO activity by melatonin (Walsh *et al.*, 1994). This disturbed relationship also suggests a disturbance of the KYN synthesis in TS patients. Comparing the TS patients on the basis of taking neuroleptic medication, a significantly higher urinary aMT6s was detected in TS patients not on neuroleptics, while the urinary aMT6s excretion in TS patients taking neuroleptics was closer to that of the controls. The significance of this difference was not statistically conclusive (see results in Chapter 5). With these doubtful results from statistical analysis on small subgroups we cannot draw any conclusions, but considering this observation with the disturbed relationship between plasma KYN and urinary aMT6s excretion in TS patients, when compared with healthy controls, we suggest further experiments to detect melatonin secretion in larger groups of TS patients both unmedicated and on different medications.

Another interesting finding of our clinical studies was the significantly lower plasma INF-γ levels detected in the smokers of both TS patient and healthy control groups. There are several reports of modified cytokine responses in smokers and *in vivo* and *in vitro* experiments showing the influence of nicotine on cytokine production (see discussion of Chapter 5). In the light of these results it is interesting to explore whether the lower INF-γ produced in smokers is due to the process of smoking or due to an effect of nicotine itself. Since nicotine is used as a therapy on its own and in combination with neuroleptics in the treatment of TS (refer to General introduction), it is important to find out the effects of nicotine on immune mechanisms in order to identify its efficacy as a immune-modulatory agent and possible side effects. Although lower plasma neopterin levels were found in healthy individuals who smoke, when compared with healthy non-smokers (Diamondstone *et al.*, 1994), in our sample of healthy individuals the plasma neopterin levels which positively correlated with INF-γ was lower in the smokers, but this difference from the non-smokers was not statistically significant. The positive correlation between plasma INF-γ and neopterin was disturbed in the TS patients and there was no significant difference in the plasma neopterin levels between smoking and non-smoking patients.

Considering the findings of animal, *in vitro* and clinical studies presented in this thesis with regard to the kynurenine pathway in TS, it is suggested that investigating for an imbalance in the systemic and/or central KYN and QUINA production in TS may be worthwhile. Since it is shown that NMDA receptor stimulation plays a critical role in learning processes and development (Collingridge & Singer, 1990; Danysz *et al.*, 1995) and TS is commonly associated with learning and attention disorders (Comings, 1995) it is possible that an impaired NMDA receptor activity is present in TS patients due to impaired production of its endogenous stimulants. It is also reported that the most commonly used neuroleptic in the treatment of TS, Haloperidol, also has a high affinity for NMDA receptors (Gallagher, 1998). QUINA levels in the plasma or CSF have not been measured in TS patients so far and this is an important area to invest time and funds in the light of these findings.

Our studies on ADHD confirmed that an impaired cortisol response is a feature of ADHD in its more established form seen in childhood. It also confirmed that ADHD and TS commonly associated with each other, showing a 56.25% incidence of TS in ADHD children (Clinical study 3) and a 77% incidence of ADHD among TS patients (Clinical study 1).

The data from Chapter 3 did not indicate any difference in the incidence of ASO positivity between TS patients and healthy controls. The hypothesis of GABHS playing a role in TS and associated neuropsychiatric disorders has been presented on observations made in younger subjects (children and adolescents) of recent onset disorders (refer to General Introduction). Our group of TS patients who were assessed for evidence of GABHS infection were not recent onset cases and they were of a wider age range mostly young adults. Other researchers have also recently reported similar negative results on examining the incidence of positive ASOT and other markers of GABHS infection in TS (Singer *et al.*, 1998; Murphy *et al.*, 1997), even in a study carried out on children with recent onset TS. These negative results confirm the suggestion by Swedo (1994) that this mechanism may influence TS only in genetically vulnerable individuals and that this is not a common pathophysiological mechanism associated with TS. Another recent studty

by Singer *et al.* (1997) suggest that the presence of antiphospholipid antibodies in TS represents an epiphenomenon rather than a pathophysiologic mechanism. A significant effect of GABHS immunity on DOI head shakes in mice was also not detected by the experiments presented in Chapter 3. Since the expression of neuropsychiatric features following exposure to GABHS have been suggested to be dependent on genetic vulnerability (Swedo, 1994) the negative results seen with these animal experiments may be due to the absence of genetic vulnerability in this particular group of mice used. It is worth further examining the hypothesis of GABHS in neuropsychiatric disorders, including TS using a suitable animal model and similar experiments in different strains of animals are suggested in order to develop a suitable animal model.

Suggestions for future studies

1. It was found that KYN does not directly bind or alter the binding of other ligands to the 5-HT2A receptors, but KYN may be acting via an unidentified receptor or one of the existing receptors in expressing its neuroactive properties. Therefore it is suggested that receptor profile studies should be done for KYN to identify a receptor via which it acts.

2. Further *in vitro* and *in vivo* studies to explore the inhibitory effect of QUINA on PI hydrolysis are suggested.

3. It is very important to develop a technique for the determination of plasma and CSF QUINA together with KYN in TS patients. A further study of the monthly plasma KYN levels in a group of TS patients and matched controls is also suggested, determining the plasma KYN levels monthly in all the patients and controls to examine the possible effect of monthly variation which may account for the results of different studies as discussed above. From my experience I feel that such studies would be very difficult due to the poor subject reliability in keeping their appointments and therefore such studies should recruit large numbers initially from multiple centres to give allowance for a high drop out rate.

4. The effects of almost all the drugs on the main drug metabolizing enzymes in the liver are well established. Similarly animal studies should be designed and carried out in future to determine the effects of the commonly used medications in TS for the treatment of tics or associated conditions (neuroleptics, SSRIs, TCAs and Clonidine), to examine the effect on TDO and IDO to detect how these medications affect the plasma and brain levels of different tryptophan metabolites, in the kynurenine and the serotonin pathways. Depending on the results of such experiments, the importance of studies on unmedicated patients should be decided.

5. Since urinary aMT6s excretion shows a wide inter-individual variation it is suggested that 24 hr urinary aMT6s levels should be determined in a larger group of TS patients and age and sex matched controls. These determinations should be done in the same subjects monthly over a period of one calendar year to determine whether there are monthly differences between patients and controls.

6. Further studies are suggested on larger groups of TS patients having reasonable subgroups within them with common associated conditions, especially OCD/B to determine the biochemical differences between these subgroups.

7. To further explore the lower plasma INF-γ levels seen in both TS patients and healthy individuals who are smokers, it is worth determining the degree of nicotine intake by means of plasma cotinine determination and then to examine the relationship with INF-γ. This will indicate the degree to which nicotine itself is responsible for the modulation of INF-γ production. It is also suggested that other interferons: INF-α and INF-β and TNFα which also have important effects on the kynurenine pathway by means of inducing IDO activity should be investigated in TS.

REFERENCES

Aardal-Eriksson E, Karlberg B E & Holm A C (1998) Salivary cortisol-an alternative to serum cortisol determinations in dynamic function tests. Clin. Chem. Lab. Med., *36*: 215-22

Abramson L Y & Seligman M E P (1977) Modelling psychopathology in the laboratory: History and rationale. *In* Psychopathology: Animal Model (Eds. J. D. Maser and M. E. P. Seligman), Plenum Press, New York, 2nd ed., *16* : 209-253,

Abwender D A, Como P G, Kurlan R, Parry K, Fett K A, Cui L, Plumb S & Deeley C (1996) School problems in Tourette's syndrome. Arch. Neurol., *53*: 509-511

Alberati-Giani, D, Ricciardi-Castagnoli, P, Kohler C & Cesura A M (1996) Regulation of the kynurenine metabolic pathway by interferon-gamma in murine cloned macrophages and microglial cells. J. Neurochem., *66*: 996-1004

Aldinio C, Mazzari S, Toffano G, Kohler C & Schwarcz R (1985) Effects of intracerebral injection of quinolinic acid and on serotonergic neurons in the rat brain. Brain Res., *341*: 57-65

Allen A J, Leonard H L & Swedo S E (1995) Case study: A new infection-triggered, autoimmune subtype of paediatric OCD and Tourette's syndrome. J. Am. Acad. Child Adolesc. Psychiatry *34*: 307-311

American Psychiatric Association (1995) Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV). Washington DC

Amin A H, Crawford T B B & Gaddum J H (1954) The distribution of substance P and 5-HT in the central nervous system of the dog. J. Physiol., *126*: 596-618

Andrade R, Malenka R C & Nicoll R A (1986) A G protein couples serotonin and GABA-B receptors to the same channels in hippocampus. Science, *234*: 1261-1265

Anyiwo C E, Obi C L & Nnaja N A (1989) Waning significance of anti-streptolysin O (ASO) titres in diagnosing streptococcal infections in Lagos, Nigeria. East Afr. Med. J., *66*: 636-40

Arendt J, Bojkowski C, Franey C, Wright J & Marks V (1985) Immunoassay of 6-hydroxymelatonin sulphate in human plasma and urine: abolition of the 24-hour rhythm with atenolol. J. Clinical Endocrinol. and Metab., *60*: 1166-1173

Arnt J, Hyttel J & Larsen J J (1984) The citalopram/5-HTP-induced head-shake syndrome is correlated to 5-HT2 receptor affinity and also influenced by other transmitters. Acta Pharmacol. Toxicol., *55*: 363-372

Badaway A A & Morgan C J (1982) Tryptophan and tryptophan pyrolase in haem regulation. Biochem. J., *206*: 451-460

Bandopadhya R & De Belleroche J (1991) Regulation of CCk release in cerebral cortex by N-methyl-D-aspartate receptors: sensitivity to APV, MK-801, kynurenate, magnesium and zinc ions. Neuropeptides, *18*: 159-163

Baran H, Cairns N, Lubec B & Lubec G (1996) Increased kynurenic acid levels and decreased brain kynurenine aminotransferase 1 in patients with Down syndrome. Life Sci., *58*: 1891-9

Barr Cathy L & Sandor Paul (1998) Current Status of Genetic Studies of Gilles de la Tourette Syndrome. Can. J. Psychiatry, *43*: 351-7

Beadle G W, Michell H K & Nyc J F (1947) Kynurenine as an intermediate in the formation of nicotinic acid from tryptophan. in *Neurospora*. Proc. Natl. Acad. Sci., *33*: 155-158

Beal M F, Matson W R, Swartz K J, Gamache P H & Bird E D (1990) Kynurenine pathway measurements in Huntington's disease striatum: evidence for reduced formation of kynurenic acid. J. Neurochem., *55*: 1327-1339

Becquet D, Faudon M & Hery F (1990) *In vivo* evidence for an inhibitory glutamatergic control of serotonin release in the cat caudate nucleus: involvement of GABA neurons. Brain Res., *519*: 82-8

Bennett J P & Snyder S H (1975) Stereospecific binding of d-lysergic acid diethylamide (LSD) to brain membranes: relationship to serotonin receptors. Brain Res., *94*: 523-544

Bennett J P & Snyder S H (1976) Serotonin and lysergic acid diethylamide binding in rat brain membrane. Relationship to postsynaptic serotonin receptors. Mol. Pharmacol., *12*: 373-389

Berg R (1985) A case of TS treated with nifedipine. Acta Psychiat. Scand., *72*: 400-401

Biederman J, Newcorn J & Sprich S (1991) Comorbidity of attention deficit hyperactivity disorder with conduct, depressive, anxiety, and other disorders. Am. J. Psychiatry, *148*: 564-577

Birch P J, Grossman C J & Hayes A G (1988) Kynurenic acid antagonises responses to NMDA via an action at the strychnine-insensitive glysine receptor. Eur. J. Pharmacol., *154*: 85-88

Blundell J E (1984) Serotonin and apetite. Neuropharmacology, *23*: 1537-1551

Boddeke H W G M, Fargin A, Raymond J R, Schoeffter P & Hoyer D (1992) Agonist/antagonist interactions with cloned human 5-HT1A receptors: variation in intrinsic activity studied in transfected HeLa cells. Naunyn Schmiedebergs Arch. Pharmacol., *37*: 257-263

Boehm U, Klamp T, Groot M & Howard J C (1997) Cellular responses to interferon-gamma. Ann. Rev. Immunol., *15* : 749-95

Bojkowski C J & Arendt J (1988) Annual changes in 6-sulphatoxymelatonin excretion in man. Acta Endorinologica, *117*: 470-476

Bojkowski C J, Arendt J, Shih M C & Markey S P (1987) Melatonin secretion in humans assessed by measuring its metabolite, 6-sulphatoxymelatonin. Clinical Chem., *33*: 1343-1348

Bornstein R A & Baker G B (1992) Urinary indoleamine in Tourette syndrome patients with obsessive-compulsive characteristics. Psychiatry Res., *41*: 267-74

Bosnan T (1978) Serotonin metabolism. *In* Serotonin in Health and Disease, Eds. Essman W B., SP Medical and Scientific Books, New York, *1*: 181-300.

Bouhelal R, Smounya L & Bockaert J (1988) 5-HT1B receptors negatively coupled with adenylate cyclase in rat substantia nigra. Eur. J. Pharmacol., *151*: 189-196

Boulton C S, Handley S L (1973) Factors modifying the head-twitch response to 5-hydroxytrptophan. Psychopharmacologia, *31*: 205-214

Bradbury A J, Kelly M E & Smith J A (1985) Melatonin action in the midbrain can regulate forebrain dopamine function both behaviourally and biochemically. *In* Advances in biosciences: The pineal gland: Endocrine Aspects, (Eds.,G. M. Brown & S. D. Wainwright), Pregamon Press, New York: pp 327-332

Brett P M, Curtis D, Robertson M M & Gurling H M (1995) The genetic susceptibility to Gilles de la Tourette syndrome in a large multiple affected British kindred: linkage analysis excludes a role for the genes coding for dopamine D1, D2, D3, D4, D5 receptors, dopamine beta hydroxylase, tyrosine hydroxylase. Biol. Psychiatry, *37*: 533-40

Brett P, Robertson M, Gurling H & Curtis D (1993) Failure to find linkage and increased homozygosity for the dopamine D3 receptor gene in Tourette's Syndrome. Lancet, *341*: 1225

Brittain R T & Handley S L (1967) Temperature changes produced by the injection of catecholamines and 5-hydroxytrptamine into the cerebral ventricles of the conscious mouse. J. Physiol., *192*: 805-813

Brodie T G & Shore P A (1957) A concept for a role of serotonin and norepinephrine as chemical mediators in the brain. Ann N Y Acad Sci.****

Brown R R, Borden E C, Sondel P M & Lee C M (1987) Effects of interferons and interleukin-2 on tryptophan metabolism in humans, *In* Progress in tryptophan and Serotonin Research 1986, (Eds., Bender D A, Joseph M H, Kochen W & Steinhart H) de Gruyter, Berlin, pp 19-26

Bruun R D & Budman C L (1996) Risperidone as a treatment for Tourette's syndrome. J. Clin. Psychiatry, *57*: 29-31

Bruun R D (1984) Gilles de la Tourette's syndrome: an overview of clinical experience. J. Am. Acad. Child Psychiatry, *23*:126-133

Butler I J, Koslow S H, Siefert W E, Caprioli R M & Singer H S (1979) Biogenic amine metabolism in Tourette Syndrome. Ann. Neurol., *6*: 34-36

Byrne G I, Lehman L K, Kirschbaum J G, Borden E C, Lee C M & Brown R R (1986) Introduction of tryptophan degradation in vitro and in vivo: a gamma interferon-stimulated activity. J. Interferon Res., *6*: 389-396

Carlin J M, Borden E C & Byrne G I (1989) Enhancement of indoleamine 2,3-dioxygenase activity in cancer patients receiving interferon-beta Ser. J. Interferon Res., *9*: 167-73

Carlin J M, Borden E C, Sodel P M & Byrne G I (1987) Biologic response modifier-induced indoleamine 2,3-dioxygenase activity in human peripheral blood mononuclear cell cultures. J. Immunol., *139*: 2414-2418

Carr J E.& Bailey J S (1996) A brief behavior therapy protocol for Tourette Syndrome. J. Behav. Ther. Exp. Psychiatry, *27*: 33-40

Cavallini M C, Macciardi F, Pasquale L, Bellodi L & Smeraldi E (1995) Complex segregation analysis of obsessive compulsive and spectrum related disorders. Psychiatr. Genet. *5*: 31

Cheng Y C & Prusoff W H (1973) Relationship between inhibition constant(Ki) and the concentration of the inhibitor which cause 50 percent inhibition(I50) of an enzymatic reaction. Biochem. Pharmacol., *22*: 3099-3108

Coccaro E F & Astill J L (1990) Central serotonergic function in parasuicide. Progr. Neuropsychopharmacol. Biol. Psychiatry., *14*: 663-674

Coccaro E F (1989) Central serotonin and impulsive aggression. Br. J. Psychiatry, *155 (suppl. 8)*: 52-62

Cohen H N, Hay I D, Annesley T M, Beastall G H, Wallace A M, Spooner R, Thomson J A, Eastwood P & Klee G G (1982) Serum immunoreactive melatonin in boys with delayed puberty. Clinical Endocinol., *17*: 517-521

Collier H O J, Hammond M D, Schneider C (1974) Biogenic amines and head twitches in mice during ethanol withdrawal. Br. J. Pharmacol., *51*: 130-311

Collingridge G L & Singer W (1990) Excitatory amino acid receptors and synaptic plasticity. Trends Pharmacol. Sci., *11*: 290-296

Comings D E, Wu H, Chiu C, Ring R H, Dietz G & Muhleman D (1996) Polygenic inheritance of Tourette syndrome, stuttering, ADHD, conduct and oppositional defiant disorder. The additive and subtractive effects of the three dopaminergic genes-DRD2, DbH and DAT1. Am. J. Med. Gen. (Neuropsych. Genet.) *67*: 264-288

Comings D E, Muhleman D, Dietz G, Dino M, LeGro R & Gade R (1993) Association between Tourette's syndrome and homozygosity at the dopamine D3 receptor gene. (Letter). Lancet, *341*: 906

Comings D E, Comings B G, Muhleman D, Dietz G, Shahbahrami B, Tast D and *others (1991) The dopamine D2 receptor locus as a modifying gene in neuropsychiatric disorders. JAMA 266: 1793-800

Comings D E (1990a) Blood serotonin and tryptophan in Tourette syndrome. Am. J. Med. Gen., *36*: 418-430

Comings D E (1990b) Tourette syndrome and human behaviour. Hope Press, Duarte, California

Comings D E & Comings B G (1985) Tourette syndrome: clinical and psychological aspects of 250 cases. Am. J. Hum. Gen., *37*: 435-450

Comings D E, Comings B G, Devor E J & Cloninger C R (1984) Detection of major gene for Gilles de la Tourette syndrome. Am. J. Hum. Genet., *36*: 586-600

Conn P J & Sander-Bush E (1986) Regulation of serotonin-stimulated phosphoinositide hydrolysis: relation to the serotonin 5-HT2 binding site. J. Neurosci., *6*: 3669-3675

Conn P J & Sander-Bush E (1985) Serotonin-stimulated phosphoinositide turnover: mediation by the S2 binding site in rat cerebral cortex but not in subcortical regions. J. Pharmacol. Exp. Ther., *234*: 195-203

Conn P J & Sander-Bush E (1984) Selective 5-HT2 antagonists inhibit serotonin stimulated phosphatidyl inositol metabolism in cerebral cortex. Neuropharmacology, 23: 993-996

Cook E H (1990) Autism: review of neurochemical investigation. Synapse, *6*: 292-308

Cooper J R, Bloom E F & Roth R H (1996) Serotonin (5-Hydroxytryptamine and Histamine) and Histamine. *In* The Biochemical Basis of Neuropharmacology, (Eds. Jack R. Cooper, Floyd E. Bloom & Robert H. Roth), Oxford University Press, New York. 7th Edition

Cooper J (1970) The Leyton Obsessional Inventory. Psychol. Med., *1*: 48-64

Corbett J A & Turpin G (1990) Tics and Tourette's syndrome *In* Child and Adolescent Psychiatry, Modern Approaches, (Eds. Rutter M & Hersov L), 2nd Edition, Blackwell Scientific Publications, Oxford, pp. 516-525

Corbett J A, Mathews A M, Connell P H & Shapiro D A (1969) Tics and Gilles de la Tourette's syndrome: a follow-up study and critical review. Br. J. Psychiatry, *115*: 1229-1241

Corne S J, Pickering R W & Warner B T (1963) A method for assessing the effects of drugs on central actions of 5-hydroxytryptamine. Br. J. Pharmacol., *20*: 106-120

Csernansky J G, Poscher M & Faull K F (1992) Serotonin (5-HT) in schizophrenia. *In* Serotonin in Major Psychiatric Disorders, (Eds. E F Coccaro and D L Murphy), American Psychiatric Press, Washington, D.C.

Curry K, Magnuson D S, McLennan H & Peet M J (1986) Acridinic acid transmitters acting at a new antagonist of amino acid induced excitations of central neurones. Neurosci. Lett., *66*: 101-105

Curtis D, Robertson M M & Gurling H M (1992) Autosomal dominant gene transmission in a large kindred with Gilles de la Tourette syndrome. Br. J. Psychiatry, *160*: 845-9

Curzon G (1990) Serotonin and apetite. Ann. N. Y. Acad. Sci., *600*: 521-531

Dahlstrom A & Fuxe K (1965) Evidence for the existence of monoamine containing neurone in the central nervous system. Demonstration of monoamine in the cell bodies of brain stem neurons. Acta Physiol. Scand., *232*: 1-55

Dandrea T, Tu B, Blomberg A, Sandstrom T, Skold M, Eklund A & Cotgreave I (1997) Differential inhibition of inflammatory cytokine release from cultured alveolar macrophages from smokers and non-smokers. Hum. Exp. Toxicol., *16* : 577-88

Danysz W, Zajaczkowski W & Parsons C G (1995) Modulation of learning processes by ionotropic glutamate receptor ligands. Behav. Pharmacol., *6*: 455-474

Danysz W, Fadda E, Wroblewski J T & Costa E (1989) Kynurenate and 2APV interact with multiple binding sites of the NMDA sensitive glutamate receptor domain. Neurosci. Lett., *96*: 340-344

Datta S P, Brown R R, Borden E C, Sondel P M & Trump D L (1987a) Interferon and interleukin 2 induced changes of tryptophan and neopterin metabolism: possible markers for biologically effective doses. Proc. Am. Assoc. Cancer Res., *28*: 338

Datta S P, Brown R R, Schiller J H, Storer B E & Borden E C (1987b) Neopterin (NP) levels in cancer patients treated with human recombinant interferon (IFN). Abstract, Annual Meeting of the International Soc. for Interferon Research, Washinton D. C., November 2-6

Davies ***(1986) A new potent and selective NMDA antagonist. Depression of central neuron responses, affinity for [3H]-D-AP5 binding sites on brain membranes and anti-convulsant activity. Brain Res., *382*: 169

De Vivo M & Maayani S (1986) Characterzation of the 5-h-HT1A receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in guinea pig and rat hippocampal membranes. J. Pharmacol. Exp. Ther., *238*: 248-253

Dell A, Anton S M, Gauntt C J, Crossley C A, Clark W A & Cunningham M W (1991) Autoimmune determinants of rheumatic carditis: localization of epitopes in human cardiac myosin. Eur. Heart J., *12* (*suppl. D*): 158-62

Devor E J (1984) Complex segregation analysis of Gilles de la Tourette syndrome: further evidence for a major locus mode of transmission. Am. J. Hum. Genet., *36*: 704-9

Di Chiara G, Camba G & Spano P F (1971) Evidence for inhibition by brain serotonin of mouse killing behaviour in rats. Nature, *233*: 272-273

Dure L S & Tucker D M (1997) Tourette syndrome. Curr. Opin. Neurol., *10*: 153-159

Dursun S M, Farrar G, Handley S L, Rickards H, Betts T, Corbett J A (1994) Elevated plasma kynurenine in Tourette Syndrome. Mol. Chem. Neuropathol. *21*: 55-59

Dursun, S.M. & Handley, S.L. (1993) The effects of a_2-antagonists on the inhibition of 1-(2,5-dimethoxy 4-iodophenyl)-2-amino propane (DOI) induced head-shakes by $5HT_{1A}$ agonists in the mouse. Br. J. Pharmacol., *109*: 1046-1052.

Dursun, S.M. (1992) An investigation into the pharmacology of tics and tic-like movements. Ph.D. thesis, University of Aston, Birmingham.

Eastman C L, Guilarte T R & Lever J R (1992) Uptake of 3-hydroxykynurenine measured in rat brain slices and in a neuronal cell line. Brain Res., *584*: 110-116

Eastman C L & Guilarte T R (1990) The role of hydrogen peroxide in the in vitro cytotoxicity of 3-hydroxykynurenine. Neurochem., *15*: 1101-1107

Eastman C L & Guilarte T R (1989) Cytotoxicity of 3-hydroxykynurenine in a neuronal hybrid cell line. Brain Res., *495*: 225-231

Eison, A. S., Freeman, R. P., Guss, V. B., Mullins, U. L. & Wright, R. N. (1995). Melatonin agonists modulate 5-HT2A receptor mediated neurotransmission: behavioural and biochemical studies in the rats. J. Pharmacol. Exp. Ther., *273*: 304-308

Eisenberg L, Ascher E & Kanner L (1959) A clinical study of Gilles de la Tourette's disease (maladie des tics) in children. Am. J. Psychiatry, *115*: 715-723

Eldridge R, Sweet R, Lake R, Ziegler M & Shapiro A K (1977) Gilles de la Tourette's syndrome: clinical, genetic, psychologic and biochemical aspects in 21 selected families. Neurology, *35*: 115-127

El-Sewedy S M (1989) Pharmacology of the kynurenine pathway. *In* Quinolinic acid and kynurenines, (Ed. T. W. Stone), CRC Press, Boca Raton, FL, pp.101-112

Evans****(1982) The effect of series of ω-phosphonic-α-carboxylic amino acids on electrically evoked and amino acid induced responses in isolated spinal cord preparations. Br. J. Pharmacol., *75*: 65

Faux E J (1966) Gilles de la Tourette syndrome, social psychiatric management. Arch. Gen. Psychiatry, *14*: 139-142

Fellenberg A J, Phillipou G, Seamark R F (1981) Urinary 6-sulphatoxymelatonin excretion and melatonin production rate: studies in sheep and man. *In* Pineal function, (Eds. C. D. Mathews and R. F. Seamark), Elsevier Press, Oxford, pp. 143-149,

Fernstrom J D & Wurtman R J (1972) Brain serotonin content: physiological regulation by plasma neutral amino acids. Science, *178*: 414-416

Fernandez-Guasti A, Ahlenius S, Hjorth S & Larsson K (1987) Separation of dopaminergic and sertonergic inhibitory mechanisms in the mediation of estrogen-induced lordosis behaviour in rat. Pharmacol. Biochem. Behav., *27*: 93-98

Fillion G, Fillion M P, Spirakis C, Bahers J M & Jacob J (1976) 5-HT binding to synaptic membranes from rat brain. Life Sci., *18*: 65-74

Fink K, Bonisch H & Gothert M (1990) Presynaptic NMDA receptors stimulate noradrenaline release in the cerebral cortex. Eur. J. Pharmacol., *185*: 115-117

Foster A C, Vezzani A, French E D & Schwarcz R (1984) Kynurenic acid blocks neurotoxicity and seizures induced in rats by the related brain metabolite quinolinic acid. Neurosci. Lett., *48*: 273-278

Foster A C, Collins J F, Schwarcz R (1983) On the excitotoxic properties of quinolinic acid, 2,3-piperidine dicarboxylic acids and structurally related compounds. Neuropharmacol. *22* : 1331-1342

Fudge M W, Murphy T K & Goodman W K(1996) Frequency of D8/17 and antistreptococcal antibodies in patients with childhood onset obsessive compulsive disorder and /or Tourette's syndrome. J. Invest. Med., *44* : 39A

Fukui Shinsuke, Schwarcz Robert, Rapoport Stanley I (1991) Blood-Brain Barrier Transport of Kynurenines: Implications for Brain Synthesis and Metabolism. J. Neurochem., *56*: 2007-2017

Fuller R W (1990) Serotonin receptors and neuroendocrine responses. Neuropsychopharmacology, *3*: 495-502

Gaddum J H & Picarelli Z P (1957) Two kinds of tryptamine receptor. Br. J. Pharmacol., *12*: 323-328

Gal E M & Sherman A D (1980) L-Kynurenine: its synthesis and possible regulatory function in brain. Neurochem. Res., *5*: 223-239

Gal E M, Sherman A D (1977) Synthesis and metabolism of L-kynurenine in rat brain. J. Neurochem., *30*: 607-613

Gallagher M J, Huang H & Lynch D R (1998) Modulation of the N-methyl-D-aspartate receptor by haloperidol: NR2B-specific interactions. J. Neurochem., *70*: 2120-8

Gardner C R (1985) Pharmacological studies of the role of serotonin in animal models of anxiety. *In* Neuropharmacology of Serotonin, (Ed., A R Green), Oxford University Press, United Kingdom, *19*: 345-356

Gaynor C M, Rickards E H G, Kariyawasam S H, Sidey F M, Corbett J A, Robertson M M & Handley S L (1997) Increased plasma kynurenine in Tourette syndrome may be due to induction of IDO. J. Psychopharmacol. *11 (suppl.)*: A23

Geletner J, Pauls D L, Leckman J, Kidd K K & Kurlan R (1994) D2 dopamine receptor alleles do not influence severity of Tourette's syndrome. Results from four large kindreds. Arch. Neurol., *51*: 397-400

Geletner J (1995) The dopamine transporter gene (SLC6A3): primary linkage mapping and linkage studies in Tourette syndrome. Genomics, *30*: 459-463

Gershon S C & Baldessarini R J (1980) Motor effects of serotonin in the central nervous system. Life Sci., *27*: 1435-1451

Giedd J N, Rapoport J L, Leonard H L, Richter D & Swedo S E (1996) Case study-acute basal ganglia enlargement and obsessive-compulsive symptoms in an adolescent boy. J. Am. Acad. Child Adolesc. Psychiatry, *35*: 913-915

Glaze D G, Jankovic J & Frost J D (1982) Sleep in Gilles de la Tourette syndrome: disorder of arousal. Neurology, *32*: 586-592

Glennon R A, McKenney J D, Lyon R A & Titeler M (1986) 5-HT1 and 5-HT2 binding characteristics of 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane analogues. J. Med. Chem., *29*: 194-199

Godfrey P P, Wilkins C J, Tyler W & Watson S P (1998) Stimulatory and inhibitory actions of excitatory amino acids on inositol phosphate metabolism in rat cerebral cortex. Br. J. Pharmacol., *95*: 131-138

Goetz, C. G. (1986). Tics: Gilles de la Tourette's syndrome. *In* Handbook of clinical neurology: extrapyramidal disorders, (Eds., P. J. Vinken, G. W. Bruyn & H. L. Klawans), Elsevier Publishers, Amsterdam, *5*: 627-639

Golombek D A, Pevet P & Cardinali D P (1996). Melatonin effects on behaviour: possible mediation by the central GABAergic system. Neurosci. Biobehav. Rev., *20*: 403-412

Goldstein J A (1984) Nifedipine treatment of Tourette's Syndrome. J. Clin. Psychiatry, *45*: 360

Golstein D, Sielaff K M, Storer B E, Brown R R, Datta S P, Witt P L, Teitelbaum A P, Smalley R V & Borden E C (1989) Human biological response modification by interferon in the absence of measurable serum concentrations: a comparative trial of subcutaneous and intravenous interferon-βser. J. Nat. Cancer Inst., *81*: 1061-1068

Gonzales R A & Moerschbaecher J M (1989) A phencyclidine recognition site is associated with N-methyl-D-aspartate inhibition of carbachol-stimulated phosphoinositide hydrolysis in rat cortical slices. Molec. Pharmacol., *35*: 787-794

Gordon B R (1995) Future immunotherapy: what lies ahead? Otolaryngol. Head Neck Surg., *113*: 603-5

Gorkin V Z (1996) Monoamine oxidase. Pharmacol. Rev., *18*: 115-120

Gorzalka B B, Mendelson S D & Watson N V (1990) Serotonin receptor subtypes and sexual behaviour. Ann. N. Y. Acad. Sci., *600*: 435-445

Goust J M & Bierer B E (1998) Cell-mediated immunity. *In* Introduction To Medical Immunology, 4th edition, (Ed. Gabriel Virella), published by Marcel Dekker INC, New York, pp 187-216

Gray Barry M (1991) Streptococcal Infections. *In* Bacterial Infections of Humans: Epidemiology and Control, Second Edition, (Eds. Alfred S, Evans & Philip S Brachman), Plenum Publishing Corporation, New York, United States of America, pp 639-673

Green A R, O'Shaughnessy K, Hammond M, Schachter M & Grahame-Smith D G (1983) Inhibition of 5-HT-mediated behaviour by the putative 5-HT2 agonist piperone. Neuropharmacology, *22*: 573-578

Greenwood B M (1987) The host's response to infection. *In* Oxford Textbook of Medicine, 2nd edition, (Eds. Weatherall D J, Lendingham J G G & Warrell D A), Oxford University Press (Oxford medical publications) Oxford, UK, pp 5.174-5.177

Grice D E, Leckman J F, Pauls D L, Kurlan R, Kidd K K, Pakstis A J Chang F M, Buxbaum J D, Cohen D J & Geletner J (1996) Linkage disequillibrium between an allele at the dopamine D4 receptor locus and Tourette syndrome by the transmission-disequilibrium test. Am. J. Hum. Genet., *59*: 644-52

Haber S N, Kowall N W, Vonsattel J P, Bird E D & Richards E P (1986) Gilles de la Tourette's Syndrome: a postmortem neuropathological and immunohistochemical study. J. Neurol. Sci., *75*: 225-241

Handley S L & Dursun S M (1992). Serotonin and Tourette's syndrome: movements such as headshakes and wet-dog-shakes may model human tics. Adv. Biosci., *85*: 235-253

Handley S L, Dunn R L, Waldron G & Baker J M (1980) Tryptophan, cortisol and puerperal mood. Br. J. Psychiatry, *136*: 498-508

Handley S L & Miskin R C (1977) The interaction of some kynurenine pathway metabolites with 5-hydroxytryptophan and 5-hydroxytryptamine. Psychopharmacology, *51*: 305-309

Handley, S. L. & Miskin, R. C. (1977) The interaction of some kynurenine pathway metabolites with 5-Hydroxytryptophan and 5-Hydroxytryptamine. Psychopharmacology, *51*, 305-309

Handley S L (1970) Study of the pharmacological actions of noradrenaline and related compounds injected into the brains of mice. Ph.D. Thesis, University of Aston, Birmingham.

Harting P, Kao H T, Macchi M, Adham N, Zgombick J, Weinshank R & Branchek T (1990) The molecular biology of serotonin receptors. An overview. Neuropsychopharmacology, *3*: 335-347

Heaton J C P, Handley S L (1989) Comparison of DOI-induced scratching and head-twitch behaviours: differential action of 8-OH DPAT. Br. J. Pharmacol. *98*: 687

Herrling P L (1985) Pharmacology of the cortico-caudate EPSP in the cat: evidence for its mediation by quisqualate-or kainate-receptor. Neuroscience, *14*: 417-426

Hesslinger C, Kremmer E, Hultner L, Ueffing M & Ziegler I (1998) Phosphorylation of GTP cyclohydrolase 1 and modulation of its activity in rodent mast cells. GTP cyclohydrolase 1 hyperphosphorylation is coupled to high affinity IgE receptor signalling and involve protein kinase C. J. Biol. Chem., *273*: 21616-22

Heyes M P, Morrison P F (1997) J Neurochem 68: 280-288*

Heyes M P (1993) Metabolism and neuropathological significance of quinolinic acid and kynurenic acid. Biochem. Soc. Trans., *21*: 83-89

Heyes M P, Saito K, Major E O, Milstein S, Markey S P, Vickers J H (1993) A mechanism of quinolinic acid formation by brain in inflammatory neurologic disease: Attenuation of synthesis from L-tryptophan by 6-chloro-tryptophan and 4- chloro-3-hydroxyanthranilate. Brain, *116*: 1425

Heyes M P, Saito K, Jacobowitz D, Takikawa O, Markey S P & Vickers J H (1992a) Poliovirus induces indoleamine-2-3-dioxygenase and quinolinic acid synthesis in macaque brain. FASEB J., *6*: 2977-2989

Heyes M P, Saito K & Markey S P (1992b) Human macrophages convert L-tryptophan into the neurotoxic quinolinic acid. Biochem. J., *283*: 633-635

Heyes M P, Brew B, Martin A, Markey S P, Price R W, Bhalla R B & Salazar A (1991a) Cerebropinal fluid quinolinic acid concentrations are increased in accquired immune deficiency syndrome. Adv. Exp. Med. Biol., *294*: 687-690

Heyes M P, Brew B, Martin A, Price R W, Salazar A M, Sidtis J J, Yergey J A, Mourdian M M, Sadler A E, Keilp J, Rubinow D & Markey S P (1991b) Quinolinic acid in cerebropinal fluid and serum in HIV-1 infection: relationship to clinical and neurological status. Ann. Neurol., *29*: 202-209

Heyes M P, Swartz K J, Markey S P & Beal M F (1991c) Regional brain and cerebropinal fluid quinolinic acid concentrations in Huntington's disease. Neurosci. Lett., *122*: 265-269

Heyes M P & Lackner A (1990) Increased cerebropinal fluid quinolinic acid, kynurenic acid and L-kynurenine in acute septicemia. J. Neurochem., *55*: 338-341

Heyes M P, Wyler A R, Devinsky O, Yergey J A, Markey S P & Nadi N S (1990b) Quinolinic acid concentrations in brain and cerebropinal fluid of patients with intractable complex partial seizures. Epilepsia, *31*: 172-177

Heyes M P, Rubinow D, Lane C & Markey S P (1989) Cerebrospinal fiuid quinolinic acid concentrations are increased in acquired immune deficiency syndrome. Ann. Neurol., *26*: 275-277

Heyes M P (1987) Hypothesis: a role for quinolinic acid in the neuropathology of glutaric aciduria type 1. Can. J. Neurol. Sci., *14*: 441-443

Heyliger S O, Goodman C B, Ngong J M, Soliman K F (1998) The analgesic effects of tryptophan and its metabolites in the rat. Pharmacol. Res., *38*: 243-50

Hirata F & Hayaishi O (1971) Possible participation of superoxide anion in the intestinal tryptophan 2,3-dioxygenase reaction. J. Biol. Chem., *246*: 7825-7826

Hollmann M & Heinemann S (1994) Cloned glutamate receptors. Annu. Rev. Neurosci., *17*: 31-108

Hoyer D, Clarke D E, Fozard J R, Harting P R, Martin G R, Mylecharane E J, Saxena P R & Humphrey P P A (1994) International union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacol. Rev., *46*: 157-203

Hoyer D & Schoeffter P (1988) 5-HT1D receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in calf substantia nigra. Eur. J. Pharmacol., *147*: 145-147

Hoyland V J, Shilliton E E & Vogt M (1970) The effect of parachlorophenylalanine on the behaviour of cats. Br. J. Pharmacol., *40*: 659-667

Huber C, Troppmair J, Fuchs D, Hausen A, Lang A, Niederwierser D, Reibnegger G, Swetly P, Wachter H & Margreiter R (1985) Immune response-associated production of neopterin- release from macrophages under control of lymphokines. Transplant Proc., *17*: 582-585

Huber C, Batchelor J R, Fuchs D, Hausen A, Lang A, Niederwierser D, Reibnegger G, Swetly P, Troppmair Jr. & Wachter H (1984) Immune response associated production of

neopterin: release from macrophages primarily under control of interferon-gamma. J. Exp. Med., *160*: 310-316

Huber C, Fuchs D, Hausen A, Margreiter R, Reibnegger G, Spielberger M & Wachter H (1983) Pteridines as a new marker to detect human T-cells activated by allogenic or modified self major histocompatibility complex (MHC) determinants. J. Immunol., *130*: 1047-1050

Humphrey P P A, Harting P & Hoyer D (1993) A proposed new nomenclature for 5-HT receptors. Trends in Pharmacol. Sci., *14*: 223-236

Husby G., van de Rijn I., Zabriskie J. B., Abdin Z H & Williams R C (1976) Antibodies reacting with cytoplasm of subthalamic and caudate nuclei neurons in chorea and acute rheumatic fever. J. Exp. Med., *144*: 1094-1110

Ignatowicz E, Vezzani A M, Rizzi M & D'Incalci M (1991) Nerve cell death induced in vivo by kainic acid and quinolinic acid does not involve apoptosis. Neuroreport., *2*: 651-654

Incagnoli T & Kane R (1983) Developmental perspectives of the Gilles de la Tourette's syndrome. Perceptual and motor skills *57*: 1271-1281

Insel T R, Zohar J, Benkelfat C & Murphy D L (1990) Serotonin in obsessions, compulsions, and the control of aggressive impulses. Ann. N. Y. Acad. Sci., *600*: 574-586

Iversen S D (1984) 5-HT and anxiety. Neuropharmacology, *23*: 1553-1560

Jacob J & Girault J (1979) 5-Hydroxytryptamine. *In* Body temperature regulation, drug effects and therapeutic implications, (Eds. P Lomax & E Schonbaum), Marcel Dekker, New York and Basel, pp. 183-230

Jellinger K & Riederer P (1981) Brain neurotransmitter amines in cerebral ischemia and stroke. *In* Transmitter Biochemistry of Human Tissue, (Eds. P. Riederer & E. Usdin), Macmillan, London, U. K, pp. 25-42

Johnson M D, Whetsell W O Jr & Crowley W R (1985a) Quinolinic acid stimulates luteinising hormone secretion in female rats: evidence for involvement of NMDA-preferring receptors. Exp. Brain Res., *59*: 57-61

Johnson M D, Whetsell W O Jr & Crowley W R (1985b) Quinolinic acid stimulates luteinising hormone secretion through a serotonin-dependent mechanism. Exp. Brain. Res., *59*: 62-67

Jones R L, McGreer P L & Greiner A C (1969) Metabolism of exogenous melatonin in schizophrenic and non- schizophrenic volunteers. Clinica. Chimica. Acta, *26*: 281-285

Jope R S & Li X (1989) Inhibition of inositol phospholipid synthesis and norepinephrine-stimulated hydrolysis in rat brain slices by excitatory amino acids. Biochem. Pharmacol., *38* : 589-596

Joseph M H, Baker H F & Lawson A M (1978) Positive identification of kynurenine in rat and human brain. Biochem. Soc. Trans., *6*: 123-126

Kalisch B E (1994) Picolinic acid protects against quinolinic acid-induced depletion of NADPH diaphorase containing neurons in the rat striatum. Brain. Res., *668*: 1-8

Keita H, Lepouse C, Henzel D, Desmonts J M & Mantz J (1997) Riluzole blocks dopamine release evoked by N-methyl-D-aspartate, kainate and veratridine in the rat striatum. Anesthesiology, *87*: 1164-71

Kennett G A & Curzon G (1991) Potencies of antagonists indicate that 5HT1C receptors mediate the 1-3 (chlorophenyl piperazine-induced hypophagia. Br. J. Pharmacol., *103*: 2016-2020

Kennett G A & Curzon G (1991) Potencies of antagonists indicate that 5-HT$_{1C}$ receptors mediate the 1,3 (chlorophenyl) piperazine-induced hypophagia. Br. J. Pharmacol., *103*: 2016-2020

Kennett G A, Wood M D, Grewal G S, Forbes I, Gadre A, Blackburn T P (1994) In vivo properties of SB200646A, a 5-HT2C/2B receptor antagonist. Br. J. Pharmacol., *111*: 798-802

Kidd K K & Pauls D L (1982) Genetic hypothesis for Gilles de la Tourette Syndrome. Raven Press, New York., pp. 243-9

Kidd K K, Prusoff B A & Cohen D J (1980) Familial pattern of Gilles de la Tourette Syndrome. Arch. Gen. Psychiatry *37*: 1336-9

Kiessling L S, Marcotte A C & Culpepper L (1993) Antineuronal antibodies in movement disorders. Paediatrics *92*: 39-43

Kiessling L S, Marcotte A C & Culpepper L (1994) Antineuronal antibodies: Tics and Obsessive-Compulsive Symptoms. Dev. Behav. Paed., *15*: 421-5

Kim H S, Park I S & Park W K (1998) NMDA Receptor antagonists enhance 5-HT2 receptor-mediated behaviour, head-twitch response, in mice. Life. Sci., *63*: 2305-2311

Kim J P & Choi D W (1987) Quinolinate neurotoxicity in cortical cell culture. Neurosci., *23* : 423-432

King J A, Barkley R A & Barrett S (1998) Attention-deficit hyperactivity disorder and the stress response. Biol. Psychiatry, *44*: 72-4

Kitay (1954) Pineal lesions and precocious puberty: a review. J. Clinical Endocrinol. Metab., *14*: 622-625

Klein D C (1978) The pineal gland : a model of neuroendocrine regulation, *In* 'The Hypothalamus', Eds. Reichlin S, Baldessarini R J & Martin J D, Raven Press, New York, pp 303-327

*Knox et al (1970

Knowles R G, Pogson C I & Salter M (1989) Application of control analysis to the study of amino acid metabolism. *In* Control of metabolic prscesses, 9Eds. Cornish-Bowden A, Richard J, Westerhoff H V & Goldbeter A), Plenum Press, New York, pp. 377-384

Koella W P (1988) Serotonin and sleep. *In* Neuronal Serotonin, (Eds. N N Osborne & M Hamon), John Wiley & Sons Ltd., New York, pp. 153-170

Krebs M O, Desce J M, Kemel M L, Gauchy C, Geneheu G, Cheramy A & Glowinski J (1991) Glutamatergic control of dopamine release in the rat striatum: evidence for presynaptic NMDA receptors on dopaminergic nerve terminals. J. Neurochem., *56*: 81-85

Kurlan, R. (1997) Future direction of research in Tourette syndrome. Neurol. clini. of North Amer., *15*: 451-455

Lakke, J. P. W. F. & Wilmink, J. T. (1985). A case of Gilles de la Tourette's syndrome with midbrain involvement. J. Neurol. Neurosurg. Psych., *48*: 1293-1296

Lapin I P (1978) Stimulant and convulsive effects of kynurenines injected into brain ventricles in mice. J. Neural. Trans., *42*: 37-43

Lapin I P (1983) Antagonism of kynurenine-induced seizures by picolinic, kynurenic and xanthurenic acids. J. Neural. Transm., *56*: 177-85

Lapin I P (1981) Kynurenines and seizures. Epilepsia, *22*: 257-265

Lapin I P (1989a) Behavioural and convulsant effects of kynurenines. *In* Quinolinic acid and kynurenines, (Ed. Stone T. W), CRC Press, Boca Raton, pp. 193-211

Lapin I P (1989b) hypothermic effect*

Lapin I P, Mutovkina L G, Ryzov I V & Mirzaev S (1996) Anxiogenic activity of quinolinic acid and kynurenine in the social interaction test in mice. J. Psychopharm., *10*: 246-249

LeBars D (1988) Serotonin and pain. *In* Neuronal Serotonin, (Eds. N N Osborne & M Hamon), John Wiley & Sons Ltd., New York, pp. 171-229

Leckman J F, Riddle M A, Hardin M T, Ort S I, Swartz K L, Stevenson J & Cohen D J (1989) The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. J. Am. Acad. Child Adolesc. Psychiatry, *28*: 566-573

Leckman J F, Riddle M A, Berrettini W H, Anderson G M, Hardin M T, Chappel P, Bissette G, Nemeroff C B, Goodman W K & Cohen D J (1988) Elevated CSF Dynorphin A [1-8] in Tourette's syndrome. Life Sci., *43*: 2015-2023

Leckman J F, Detlor J, Harcherik D F, Ort S I, Shaywitz B A & Cohen D J (1985) Short and long-term treatment of Tourette's disorder with clonidine: A clinical perspective. Neurology, *35*: 343-351

Lees A. J., Robertson M., Timble M R & Murray N. M. F. (1984) A clinical study of Gilles de la Tourette syndrome in the United Kingdom. J. Neurol. Neurosurg. Psychiatry *47*: 1-8

Lehmann J, Schaefer P, Ferkany J W & Coyle J T (1983) Quinolinic acid evokes [^{3}H] acetylcholine release in striatal slices: medation by NMDA-type excitatory amino acid receptors. Eur. J. Pharmacol., *96*: 111-115

Lendvai, B., Shershen, H., Lajtha, A., Santha, E., Baranyi, M., & Vizi, E. S. (1996). Differential mechanisms involved in the effect of nicotinic agonists DMPP and lobeline to release [3H]5-HT from rat hippocampal slices. Neuropharmacology, *35*:1769-1777

Leonard Brian E (1995) Stress and the Immune System: Immunological aspects of depressive illness. *In* Stress, the Immune System and Psychiatry. (Eds. B. Leonard & K. Miller), John Wiley & Sons Ltd., New York.

Leysen J. E., Niemegeers C. J. E., Van Nueten J. M. & Laduran P. M. (1982) [^{3}H] Ketanserin (R41468) a selective 3H ligand for serotonin2 receptor binding sites. Binding properties, brain distribution, and functional role, Mol. Pharmacol., *21*: 301-314.

Lilic D, Cant A J, Abinun M, Calvert J E & Spickett G P (1997) Cytokine production differs in children and adults. Paediatr. Res., *42*: 237-40

Lombroso P J, Scahill L, King R A, Lynch K A, Chappell P B, Peters B S, McDougle C J & Leckman J F (1995) Risperidone treatment of children and adolescents with chronic tic disorders: a preliminary report. J. Am. Acad. Child Adolesc. Psychiatry, *34*: 1147-1152

Lucki I, Nobler M S & Frazer A (1984) Differential actions on two behavioral models of serotonin receptor activation in the rat. J. Pharmacol. Exp. Ther., *228*: 133-9

Luthman J, Vanerman E, Fredriksson G & Fornstedt-Wallin B (1996) Regulation of quinolinic acid in the normal rat brain by kynurenine pathway precursors. *In* Recent Advances in Tryptophan Research, (Eds. Graziella Allergri Fillipini G A *et al.*), Plenum Press, New York. pp. 229-239

Mangoni A (1974) The 'Kynurenine Shunt' and Depression. Advances in Biochem. Psychopharmacol., *11*: 293-298

Mann J J, Arango V & Underwood M D (1990) Serotonin and suicidal behavior. Ann. N. Y. Acad. Sci., *600*: 476-485

Marazziti D, Macchi E, Rotondo A, Placidi G F & Cassano G B (1988) Involvement of serotonin systems in bulimia. Life Sci., *43*: 2123-2126

Mason G A, Bissette G & Neneroff C B (1983) Effects of excitotoxic amino acids on pituitary hormone secretion in the rat. Brain Res., *289*: 366-369

Mataro M, Garcia-Sanchez C, Junque C, Estevez-Gonzalez·A & Pujol J (1997) Magnetic resonance imaging measurement of the caudate nucleus in adolescents with Attention-Deficit Hyperactivity Disorder and its relationship with neuropsychological and behavioral measures. Arch. Neurol., *54*: 963-968

McCrea K A, Ensor J E, Nall K, Bleecker E R & Hasday J D (1994) Altered cytokine regulation in the lungs of cigarette smokers. Am. J. Respir. Crit. Care Med., *150*: 696-703

McCreary A C & Handley S L (1995) Kynurenine potentiates the DOI head shake in mice. J. Psychopharmacol., *9*: 69-70

McEntee W J & Crook T H (1991) Serotonin, memory and the aging brain. Psychopharmacology, *103*: 143-149

McKinney W T & Bunney W E (1969) Animal models of depression: Review of evidence and implications for research. Arch. Gen. Psychiatry, *21*: 240-248

McPerson G. A. (1983) A practical computer-based approach to the analysis of radioligand binding experiments. Computer Programs Biomed. *17*: 107-113

Meltzer H Y & Lowy M T (1987) The serotonin hypothesis of depression. *In* Psychopharmacology: The Third Generation of Progress, Eds. H. Y. Meltzer, Raven Press, New York, pp. 513-526

Mendelson S D, Lee N & Gorzalka B B (1987) Intraventricular administration of L-kynurenine and kynuramine facilitates lordosis in the female rat. Eur. J. Pharmacol., *142*: 447-452

Meyerson B J & Malmnas C O (1978) Brain monoamines and sexual behavior. *In* Biological Determinants of Sexual Behavior, ed., J S Hutchinson, Wiley & Sons Ltd., New York, pp. 521-554

Miguez, J. M., Martin, F. J. & Aldegunde, M. (1997). Melatonin effects on serotonin synthesis and metabolism in the striatum, nucleus accumbens, and dorsal and median raphe nuclei of rats. Neurochem. Res., *22*: 87-92

Miguez, J. M., Martin, F. J., Lema, M. & Aldegunde, M. (1996). Changes in serotonin level and turnover in discrete hypothalamic nuclei after pinealectomy and melatonin administration to rats. Neurochem. Int., *29*: 651-658

Miossec P (1997) Cytokine induced autoimmune disorders. Drug Saf., *17*: 93-104

Miwa S, Watanabe Y & Hayaishi O (1985) 6R-L-erythro-5,6,7,8-tetrahydrobiopterine as a regulator of dopamine and serotonin biosynthesis in the rat brain. Arch. Biophy., *239*: 234-41

Moir A T B & Eccleston D (1968) The effect of precursor loading in the cerebral metabolism of 5-hydroxyindoles. J. Neurochem., *15*: 1093-1108

Monteleone P, Catapano F, Fabrazzo M, Tortorella A & Maj M (1998) Decreased blood levels of tumour necrosis factor-alpha in patients with obsessive-compulsive disorder. Neuropsychobiology, *37*: 182-5

Montgomery M A, Clayton P J & Friedhoff A J (1982) Psychiatric illness in Tourette syndrome patients and first degree relatives. *In* Advances in Neurology, (Eds. A. J. Friedhoff & T. N. Chase), Raven Press, New York, *35*: 335-339

Moroni F, Lombardi G, Carla V, Pellegrini D, Carassale G L & Cortesini C (1986) Content of quinolinic acid and other tryptophan metabolites increase in brain regions of rats used as experimental models of hepatic encephalopathy. J. Neurochem., *46*: 869-874

Moroni F, Lombardi G, Carla V & Moneti G (1984a) The excitotoxin quinolinic acid is present and unevenly distributed in the rat brain. Brain Res., *285*: 352-355

Moroni F, Lombardi G, Moneti G & Aldinio C (1984b) The excitotoxin quinolinic acid is present in the brain of several animal species and its cortical content increases during the ageing process. Neurosci. Lett., *47*: 51-55

Moss H B, Vanyukov M M & Martin C S (1995) Salivary cortisol responses and the risk for substance abuse in prepubertal boys. Biol. Psychiatry, *38*: 547-55

Mount H, Quirion R, Chaudieu I & Boksa P (1991) Inhibitory and potantiating influences of glycine on N-methyl-D-aspartate-evoked dopamine release from cultured rat mesencephalic cells. Mol. Pharmacol., *39*: 205-210

Murphy T K, Goodman W K, Fudge M W, Williams R C Jr, Ayoub E M, Dalal M, Lewis M H, Zabriskie J B (1997) B lymphocyte antigen D8/17: a peripheral marker for childhood-onset obsessive-compulsive disorder and Tourette's syndrome. Am. J. Psychiatry, *154*: 402-7.

Nakai M, Klarica M, Fage D, Carter C (1998) The pharmacology of native N-methyl-D-aspartate receptor subtypes: different receptors control the release of different striatal and spinal transmitters. Prog. Neuropsychopharmacol. Biol. Psychiatry, *22*: 35-64

Naoi M, Ishiki R, Nomura Y, Hasegawa S & Nagatsu T (1987) Quinolinic acid: an endogenous inhibitor specific for type B MAO in human brain synaptosomes. Neurosci. Lett., *74*: 232-236

Nisell, M., Marcus, M., Nomikos, G. G. & Svensson,T. H. (1997). Differential effects of acute and chronic nicotine on dopamine output in the core and shell of the rat nucleus accumbens. J. Neural Transm., *104*:1-10

Noble E P, Sincini E, Bergman D, Bruggencate G T (1989) Excitatory amino acids inhibit stimulated phosphoinositide hydrolysis in the rat prefrontal cortex. Life Sci., *44* : 19-26

Nothen M M, Cichon S, Hemmer S, Hebebrand J, Remschmidt H, Lehmkuhl G and others (1994) Human dopamine D4 receptor gene: frequent occurrence of a null allele and observation of homozygosity. Hum. Mol. Genet. *3*: 2207-12

Nutt D J & George D T (1990) Serotonin and anxiety. Neurobiol. Anxiety, *3*: 189-221

Ogawa T, Matson W R, Beal M F, Myers R H, Bird E D, Milbury P, Saso S (1992) Kynurenine pathway abnormalities in Parkinson's disease. Neurology, *42*: 1702-6

Olivier B, Moss J, Tulp M, Schipper J & Bevan P (1989) Modulatory action of serotonin in aggressive behaviour. *In* Behavioural Pharmacology of 5-HT, (Eds. P. Bevan, A. R. Cools & T. Archer), Lawrence Erlbaum Associates, Hillsdale, N.J, pp. 89-115

Orlikow A B, Prakhye I B & Ryzov I V (1991) Kynurenine in blood plasma and DST in patients with endogenous anxiety and endogenous depression. Biol. Psychiatry, *36*: 97-102

Orlikow A & Ryzov I (1991) Caffeine-induced anxiety and increase of kynurenine concentrations in plasma of healthy subjects: a pilot study. Biol. Psychiatry, *29*: 391-396

Osborne T B, Mendel L B (1914) Amino acids in nutrition and growth. J. Biol. Chem., *17*: 325-349

Pakstis A J, Heutink P, Pauls D L, Kurlan R, van de Wetering B J, Leckman J F, Sandkuyl L A, Kidd J R, Breedveld G J, Castiglione C M, Weber J, Sparkes R S, Cohen D J, Kidd K K, Oostra B A (1991) Progress in the search for genetic linkage with Tourette syndrome: an exclution map covering more than 50% of the autosomal genome. Am. J. Hum. Genet., *48*: 281-94

Pauls D L, Pakstis A J, Kurlan R, Kidd K K, Leckman J F, Cohen D J, Kidd J R, Como P, Spakes R (1990) Segregation and linkage analyses of Tourette's syndrome and related disorders. J. Am. Acad. Child Adolesc. Psychiatry, *29*: 195-203

Pearson S J & Reynolds G P (1991) Determination of 3-hydroxykynurenine in human brain and plasma by high-performance liquid chromatography with electrochemical detection. Increased concentrations in hepatic encephalopathy. J. Chromatogr., *565*: 436-440

Peet M J, Curry K, Magnuson D S & McLennan H (1986) Ca^{2+}-dependent depolarisation and burst firing of rat CA1 pyramidal neurones induced by N-methyl-D-aspartatic acid and quinolinic acid: antagonism by 2-amino-5-phosphonovaleric and kynurenic acids. Can J Physiol. Pharmacol., *64*: 163-168

Perkins M. N. & Stone T. W. (1982) An iontophoretic investigation of the ations of convulsant kynurenines and their interactions with the endogenous excitant quinolinic acid. Brain Res., *247*: 184-187

Peroutka S J & Snyder S H (1979) Multiple serotonin receptors: differential binding of [^{3}H]LSD and [^{3}H]spiroperidol. Mol. Pharmacol., *16*: 687-695

Pitman R K, Green R C, Jenike M A & Mesulam M M (1987) Clinical comparison of Tourette's syndrome and obsessive-compulsive disorder. Am. J. Psychiatry, *144*: 1166-1171

Pittaluga A & Raiteri M (1992) NMDA and non-NMDA receptors regulating hippocampal norepinephrine release. I. Location on axon terminals and pharmacological characterization. J. Pharmacol. Exp. Ther., *260*: 232

Pittaluga A, Vaccari D, Raiteri M (1997) The 'kynurenate test', a biochemical assay for putative cognition enhancers. J. Pharmacol. Exp. Ther., *283*: 82-90

Plaznik A, Kostowsky W & Archer T (1989) Serotonin and depression: old problems and new data. Prog. Neuropsychopharmacol. Biol. Psychiatry, *13*: 623-633

Price R A, Kidd K K, Cohen D J, Pauls D L, Leckman J F (1985) A twin study of Tourette Syndrome. Arch. Gen. Psychiatry, *42*: 815-20

Pucilowski O & Kostowsky W (1983) Aggressive behavior and the central serotonergic systems. Behav. Brain Res., *9*: 33-48

Rang H P, Dale M M & Ritter J M (1995) The endocrine system. *In* Pharmacology, 3rd Edition, pp. 417-453

Rapport M M & Green A A (1947) Purification of the substance which is responsible for vasoconstricter activity of serum. Fed. Proc., *6*: 184

Rauch S L, Baer L, Cosgrove G R & Jenike M A (1995) Neurosurgical treatment of Tourette's syndrome: a critical review. Comp. Psychiatry, *36*: 141-156

R'ecasens M, Mayat E, Guiramand J (1991) Excitatory amino acid receptors and phosphoinositide breakdown: facts and perspectives. Current Aspects of the Neurosciences, (Ed. N. N. Osborne), The Macmillan Press Ltd, Vol. *3*

Regeur L, Pakkenberg B, Fog R, Pakkenberg H (1986) Clinical features and long-term treatment with pimozide in 65 patients with Gilles de la Tourette syndrome. J Neurol Neurosurg Psychiatry 49: 791-795

Reinhard Jr. J F, Erickson J B (1994) Quinolinic acid in neurological disease: opportunities for novel drug discovery. *In* Advances in Pharmacology, Academic Press, *30:* 85-126

Reynolds G P, Pearson S J, Halket J & Sandler M (1988) Brain quinolinic acid in Huntington's disease. J. Neurochem., *50*: 1959-1960

Reynolds I J, Harris K M & Miller R J (1989) NMDA receptor antagonists that bind to the strychnine-insensitive glysine site and inhibit NMDA-induced Ca^{++} fluxes and [^{3}H]GABA release.Eur. J. Pharmacol., *172*: 9-17

Richardson B P(1990) Serotonin and nociception. Ann. N Y Acad. Sci., *600*: 511-520

Rifkind A B, Gillette P N, Song C S (1970) Induction of hepatic δ-amino laevulinate synthetase by oral contraceptive steroids. J. Clinical Endocrinolo., *30* : 330-335

Robertson M M, Schnieden V & Lees A J (1990) Management of Gilles de la Tourette syndrome using sulpiride. Clin. Neuropharmacol., *13*: 229-235

Robertson, M. M. (1989) The Gilles de la Tourette syndrome: the current status. Br. J. Psych., *154*: 147-149

Robertson, M. M., Trimble M R, Lees A J (1988) The psychopathology of the Gilles de la Tourette Syndrome: a phenomenological analysis. Br. J. Psychiatry, *152*: 383-390

Rogawski M A (1993) Therapeutic potential of excitatory amino acid antagonists: channel blockers and 2,3-benzodiazepines. Trends Pharmacol. Sci., *14*: 325-331

Rose W C (1957) The amino acid requirements of adult man. Nutr. Abstr. Rev., *27*: 631-647

Sainio E L (1997) The role of adrenal hormones in the activation of tryptophan 2,3-dioxygenase by nicotinic acid in rat liver. Methods Find Exp. Clin. Pharmacol., *19*: 465-70

Rothe F, Wolf G, Fischer S, Hass P, Keilhoff G & Abicht K (1993) Quinolinate and kainate facilitate magnesium penetration into brain tissue. Neuroreport, *4*: 205-207

Roy A & Linnoila M (1988) Suicidal behavior, impulsiveness and serotonin. Acta Psychiatr. Scand., *78*: 529-535

Saito K, Nowak T S Jr, Markey S P, Heyes M P (1992) Mechanism of delayed increases in kynurenine pathway metabolism in damaged brain regions following transient cerebral ischemia. J. Neurochem., *60*: 180-192

Sandler M, Ruthven C R (1969) The biosynthesis and metabolism of catecholamines. Progr. Med. Chem., *6*: 200-265

Sandor P (1995) Clinical management of Tourette's syndrome and associated disorders. Can. J. Psychiatry, *40*: 577-583

Sandyk, R. & Kay, S. R. (1991) The relationship of pineal calcification and melatonin secretion to the pathophysiology of tardive dyskinesia and Tourette's syndrome. Intern. J. Neurosc., *58:* 215-247

Sawada M, Sugimoto T, Matsuura S, Nagatsu T (1986) 6R-tetrahydrobiopterine increases the activity of tryptophan hydroxylase in rat raphe slices. J. Neurochem., *47*: 1544-7

Schildkraut J J (1965) The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am. J. Psychiatry, *122*: 509-522

Schimidt N A (1998) Salivary cortisol testing in children. Issues Compr. Pediatr. Nurs., *20*: 183-90

Schimke R T, Sweeney E W & Berlin C M (1965) The roles of synthesis and degradation in the control of rat liver tryptophan pyrrolase. J. Biol. Chem., *240*: 322-331

Schneider H P & McCann S M (1970) Mono-and indolamines and control of LH secretion. Endocrinology, *86*: 1127-1133

Schoeffter P, Waeber C, Palacios J M & Hoyer D (1988) The 5-hydroxytryptamine 5-HT1D receptor subtype is negatively coupled to adenylate cyclase in calf substantia nigra. Naunyn Schmiedebergs Arch. Pharmacol., *337*: 602-608

Scheard M H (1988) The effect of pCPA on behavior in rats: relation to brain serotonin and 5-HIAA. Brain Res., *15*: 524-528

Schutz G, Beato M & Feigelson P (1973) Messenger RNA for hepatic tryptophan oxygenase, its partial purification, its translation in a heterologous cell-free system and its control by glucocorticoid hormones. Proc. Natl. Acad. Sci., *70*: 1218-1221

Schwarcz, R., Ceresoli G, Guidetti P (1996) Kynurenine metabolism in the rat brain in vivo. in Recent Advances in Tryptophan research, (Eds. Graziella Allegri Fillippini et al.,), Plenum Press, New York.

Schwarcz R (1993) Metabolism and function of brain kynurenines. Biochem. Soc. Trans., *21*: 77-82

Schwarcz, R., Okuno E & White R J (1988) Basal ganglia lesions in the rat: effects on quinolinic acid metabolism. Brain Res., *490*: 103-109

Schwarcz, R., Bush, G. S., Foster, A. C. & French, E. D. (1984) Seizure activity and lesions after intrahippocampal quinolinic acid injection. Exp. Nerol., *84*:1-17

Schwarcz R & Kohler C (1983) Differential vulnerability of central neurones of the rat to quinolinic acid. Neurosci. Lett., *38*: 85-90

Schwarcz R Whetsell W O Jr & Mangano R M (1983) Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain. Science, *219*: 316-318

Schweitzer J W & Freidhoff A J (1988) Gilles de la Tourette syndrome. *In* Receptors and Ligands in Neurological Disorders, (Eds. Sen A K & Lee T), Cambridge University Press, Cambridge, pp. 64-79

Seeburg (1993) The molecular biology of mammalian glutamate receptor channels. Trends Neurosci., *16*: 325-343

Shapiro A K & Shapiro E S (1981) Tourette syndrome history and present status. *In* Adv. Neurology: Gilles de la Tourette syndrome, (Eds. Freidhoff A J & Chase T N), Raven Press, New York, *35*: 17-23

Shapiro A K, Shapiro E S, Bruun R D & Sweet R D (1978) Gilles de la Tourette syndrome. Raven Press, New York.

Shytle, R. D., Borlongan, C. V. & Sanberg, P. R. (1995). Nicotine blocks kainic acid-induced wet dog shakes in rats. Neuropsychopharmacology, *13*: 261-264

Silman R E, Leone R M, Hooper R J L & Preece M A (1979) Melatonin, the pineal gland and human puberty. Nature, *282*: 301-303

Singer H S, Giuliano J D, Hansen B H, Hallett J J, Laurino J P, Benson M, Kiessling L S (1998) Antibodies against human putamen in children with Tourette syndrome. Neurology, *50*: 1618-24

Singer H S & Walkup J T (1991) Tourette syndrome and other tic disorders: Diagnosis, pathophysiology and treatment. Medicine, *70*: 15-32

Speciale C, Ungerstedt U, Schwarcz R (1988) Effect of kynurenine loading on quinolinic acid production in the rat: studies in vitro and in vivo. Life Sc., *43* : 777-786

Stahl S M (1997) Psychopharmacology of antidepressants. Martin Dunitz Ltd, London.

Staines Norman A, Brostoff Jonathan, James Keith (1993) Introducing Immunology, second edition, Mosby-Yearbook Europe Limited, England

Sternbach H (1991) The serotonin syndrome. Am. J. Psychiatry, *148*: 705-713

Stevens H (1964) The syndrome of Gilles de la Tourette and its treatment: report of a case. Med. Ann. of the District of Columbia, *33*:277-279

Stone T W (1993) Neuropharmacology of quinolinic and kynurenic acids. Pharmacol. Rev., *45*: 310-379

Stone T W (1991) The endogenous antagonist kynurenic acid: Effects at kainate receptors. *In* Excitatory amino acids (Eds. Meldrum B S, Moroni F, Simon R P & Woods J H) Raven Press, New York, pp. 361-365

Stone T W, Connick J H (1985) Quinolinic acid and other kynurenines in the central nervous system. Neurosciences, *15*: 597-617

Stone T W, Perkins M N (1981) Quinolinic acid: A potent endogenous excitant at amino acid receptors in CNS. Eur. J. Pharmacol., *72*: 411

Sugano N, Shimada K, Ito K & Murai S (1998) Nicotine inhibits the production of inflammatory mediators in U937 cells through modulation of nuclear factor-kappaB activation. Biochem. Biophys. Res. Commun., *252*: 25-8

Summers, K. L., Lippiello, P. & Giacobini, E. (1996). A microdialysis study of the effects of the nicotinic agonist RJR-2403 on cortical release of acetylcholine and biogenic amines. Neurochem. Res., *21*: 1181-1186

Swartz K J, Matson W R, Macgarvey U, Ryan E A & Beal M F (1990) Measurement of kynurenic acid in mammalian brain extracts and cerebrospinal fluid by high-performance liquid chromatography with fluorometric and coulometric electrode array detection. Anal. Biochem., *185*: 363-376

Swedo S E, Leonard H L & Kiessling L S (1994) Speculations on antineuronal antibody mediated neuropsychiatric disorders of childhood. Paediatrics, *93*: 323-326

Swedo S E, Leonard H L, Mittleman B B, Allen A J, Rapoport J L, Dow S P, Kanter M E, Chapman F & Zabriskie J (1997) Identification of children with paediatric autoimmune neuropsychiatric disorders associated with streptococcal infections by a marker associated with rheumatic fever. Am. J. Psychiatry, *154*: 110-2

Swedo S. E.(1994) Grand Rounds at the Clinical Center of the National Institute of Health. Sydenham's Chorea: A Model for Childhood Autoimmune Neuropsychiatric Disorders. JAMA, *272*: 1789-1791

Takikawa, O., Yoshida, R., Kido, R. & Hayaishi, O. (1986) Tryptophan degradation in mice initiated by indoleamine –2,3-dioxygenase. J. Biol. Chem., *261*: 3648-3653

Tappia P S, Troughton K L, Langley-Evans S C & Grimble R F (1995) Cigarette smoking influences cytokine production and antioxidant defences. Clinic. Sci., *88*: 485-489

Tenen S S (1967) The effect of p-chlorophenylalanine, a serotonin depletor, on avoidance acquisition, pain sensitivity and related behaviour in the rat. Psychoharmacology, *10*: 204-219

Tonohiro T, Kaneko T, Tanabe M & Iwata N. (1997) Picolinic acid and indole-2-carboxylic acid: two types of glycinergic compounds modulate motor function differentially. Gen Pharmacol (England) *28*: 555-60

Tsuzuki K, Iino M & Ozawa S (1989) Changes in calcium permeability caused by quinolinic acid in cultured rat hippocampal neurones. Neurosci. Lett., *105*: 269-274

Tucker D M, Leckman J F & Scahill L (1996) A putative poststreptococcal case of OCD with chronic tic disorder, not otherwise specified. J. Am. Acad. Child Adolesc. Psychiatry, *35*: 1684-1691

Tumour necrosis factor-alpha and lipopolysaccharide enhance interferon-induced tryptophan degradation and pteridine synthesis in human cells. Biol. Chem. hoppe. Seyler., *370*: 1063-9

Tuomisto J & Mannisto P (1985) Neurotransmitter regulation of anterior pituitary hormones. Pharmacol. Rev., *37*: 249-332

Undenfriend S, Titus E & Weissbach H (1955) The identification of 5-hydroxy-3-indoleacetic acid in normal urine and a method for its assay. J. Biol. Chem., *216*: 499-505

Van Praag H M, Asnis G M, Kahn R S, Brown S L, Korn M, Friedman J M & Wetzler S (1990) Monoamines and abnormal behavior: a multi-aminergic perspective. Br. J. Psychiatry, *157*, 723-734

Van Woert M J, Rosenbaum D & Enna S J (1982) Overview of pharmacological approaches to therapy for Tourette syndrome. *In* Gilles de la Tourette syndrome, (Eds. Freidhoff A J & Chase T N), Raven Press, New York, pp. 369-376

Vecsei L & Beal M F (1991) Comparative behavioral and pharmacological studies with centrally administered kynurenine and kynurenic acid in rats. Eur. J. Pharmacol., *196*: 239-46

Vecsei L & Beal M F (1990) Intracerebroventricular injection of kynurenic acid, but not kynurenine, induces ataxia and stereotyped behaviour in rats. Brain Res. Bull., *25*: 623-627

Vezzani A, Stasi M A, Wu H Q, Castiglioni M, Weckermann B & Samanin R (1989) Studies on the potential neurotoxic and convulsant effects of increased blood levels of quinolinic acid in rats with altered blood brain barrier permeability. Exp. Neurol., *106*: 90-8

Vialli M & Erpsamer V (1933) Cellule enterocromaffin e cellule basigranulose acidofile nei vertebrati.2.Zellforsch. Mikrosk. Anat., *19*: 743

Vivian V M, Brown R R, Price J M & Reynolds M S (1966) Some aspects of tryptophan and niacin metabolism in young women consuming a low tryptophan diet supplementation with niacin. J. Nutr., *88*: 93-99

Walkup J T, LaBuda M C, Singer H S, Brown J & Riddle M A, Hurko O (1996) Family study and segregation analysis of Tourette syndrome: evidence for a mixed model of inheritance. Am. J. Hum. Genet., *59*: 684-93

Walkup J T, Leckman J F, Price R A, Hardin M, Ort S I & Cohen D J (1988) The relationship between obsessive-compulsive disorder and Tourette's Syndrome: a twin study. Psychopharmacol. Bull., *24*: 375-9

Walsh H A, Daya S & Whiteley C G (1994) Mode of inhibition of rat liver tryptophan 2,3-dioxygenase by melatonin. J. Pineal. Res., *16*: 188-192

Wang Y & White T D (1998) Effect of protein kinase C activation on N-methyl-D-aspartate-evoked release of adenosine and [^{3}H]Norepinephrine from rat cortical slices. J. Pharmacol. Exp. Ther., *285*: 105-109

Wei E.T. (1981) Pharmacological aspects of shaking behaviour produced by TRH, AG-3-5 and morphine withdrawal. Fedn. Proc. *40*: 1491-1496

Weiss S, Sebben M, Kemp D E & Bockaert J (1986) Serotonin 5-HT1 receptors mediate inhibition of cyclic AMP production in neurons. Eur. J. Pharmacol., *120*: 227-230

Werner E R, Werner- Felmayer , Fuchs D, Hausen A, Reibnegger G, Wachter H (1989) Parallel induction of tetrahydrobiopterin biosynthesis and indoleamine 2,3-dioxygenase activity in human cells and cell lines by interferon-gamma. Biochem. J., *262*: 861-6

Werner E R, Kauffmann M H, Fuchs D, Hausen A, Reibnegger G, Schweiger M & Wachter H (1987) Interferon-gamma induced degredation of tryptophan by human cells in vitro. Biol. Chem. Hoppe-Seyler, *368*: 1407-1412

Werner-Felmayer G, Werner E R, Fuchs D, Hausen A, Reibnegger G, Wachter H (1990) Neopterin formation and tryptophan degradation by a human myelomonocyte cell line (THP-1) upon cytokine treatment. Cancer Res., *50*: 2863-7

Werner-Felmayer G, Werner E R, Fuchs D, Hausen A, Reibnegger G, Wachter H (1989) : a potent endogenous excitant at amino acid receptors in CNS. Eur. J. Pharmocol., *72*: 411

Werner-Felmayer G, Werner E R, Fuchs D, Hausen A, Reibnegger G, Wachter H (1989) Tumour necrosis factor-alpha and lipopolysaccharide enhance interferon-induced tryptophan degradation and pteridine synthesis in human cells. Biol. Chem. hoppe. Seyler., *370*: 1063-9

Werner E R, Werner- Felmayer , Fuchs D, Hausen A, Reibnegger G, Wachter H (1989) Parallel induction of tetrahydrobiopterin biosynthesis and indoleamine 2,3-dioxygenase activity in human cells and cell lines by interferon-gamma. Biochem. J., *262*: 861-6

Wetzler S, Kahn R S, Asnis G M, Korn M & Van Praag H M (1991) Serotonin receptor sensitivity and aggression. Psychiatr. Res., *37*: 271-279

Whitton PS; Richards DA; Biggs CS; Fowler L J (1994) N-methyl-D-aspartate receptors modulate extracellular 5-hydroxytryptamine concentration in rat hippocampus and striatum in vivo. Neurosci. Lett., *169*: 215-8

Whitton PS; Biggs CS; Pearce BR; Fowler LJ (1992) MK-801 increases extracellular 5-hydroxytryptamine in rat hippocampus and striatum in vivo. J. Neurochem., *58*: 1573-5

Wolf H (1974) Studies on tryptophan metabolism in man. Scand. J. Clin. Lab. Invest., *136* (*Suppl.*): 33

Wolf S S, Jones D W, Michael B K, Gorey J G, Lee K S, Hyde T M, Coppola R & Weinberger R (1996) Tourette Syndrome: Prediction of phenotypic variation in monozygotic twins by caudate nucleus D2 receptor binding. Science, *273*: 1225-1227

Wolfensberger M, Amsler U, Cuenod M, Foster A C, Whestsell W O Jr & Schwarcz R (1984) Identification of quinolinic acid in rat and human brain tissue. Neurosci. Lett., *41*: 247-252

Yamamoto S & Hayaishi O (1967) Tryptophan pyrrolase of rabbit intestine. J. Biol. Chem., *242*: 5260-5266

Yoshida, R., Oku, T., Imanishi, J., Kishida T. & Hayaishi, O. (1986) Interferon: a mediator of indoleamine 2,3-dioxygenase induction by lipopolysaccharide, poly(I), poly(C), and pokeweed mitogen in mouse lung. Arch. Biophys. *249*: 596-604

Zisapel N & Laudon M (1982) Dopamine release induced by electrical field stimulation of rat hypothalamus in vitro inhibition by melatonin. Biochemistry Biophysics Res. Communic., *104*: 1610-1616

Zohar J & Insel T R (1987) Obsessive-compulsive disorders: psychobiological approaches to diagnosis, treatment and pathophysiology. Biol. Psychiatry, *22*: 667-687