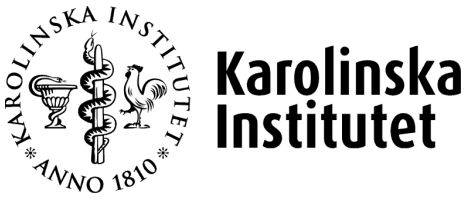
From the Department of Physiology and Pharmacology
Karolinska Institutet, Stockholm, Sweden

INFLUENCE OF KYNURENIC ACID ON DOPAMINE NEUROTRANSMISSION: IMPLICATIONS FOR SCHIZOPHRENIA

Alexandra Andersson



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INFLUENCE OF KYNURENIC ACID ON DOPAMINE NEUROTRANSMISSION: IMPLICATIONS FOR SCHIZOPHRENIA

THESIS FOR LICENTIATE DEGREE

By

Alexandra Andersson

Principal Supervisor:
Assoc. Professor Sophie Erhardt
Karolinska Institutet
Dept. of Physiology and Pharmacology

Co-supervisor:
Professor Göran Engberg
Karolinska Institutet
Dep. of Physiology and Pharmacology

Examination Board:
Professor Sven Ove Ögren
Karolinska Institutet
Dept. of Neuroscience

Assoc. Professor Karima Chergui
Karolinska Institutet
Dept. Of Physiology and Pharmacology

Professor Lars Oreland
Uppsala universitet
Dept. of Neuroscience

Till William

ABSTRACT

Kynurenic acid (KYNA) belongs to the kynurenines, a group of metabolically related compounds derived from the amino acid tryptophan. It is an antagonist of glutamate- and α7 nicotinic acetylcholine (α7nACh) receptors. Increased levels of KYNA have been observed in the cerebrospinal fluid and postmortem brains of patients with schizophrenia. An increased dopamine neurotransmission is suggested to underlie pivotal symptoms in schizophrenia and patients show an augmented dopamine response to amphetamine administration. Previous studies reveal that elevated levels of brain KYNA increase the firing activity of midbrain dopamine neurons. The aims of the present thesis were to investigate a) the receptors involved in the excitatory action of KYNA on midbrain dopamine neurons and b) the influence of elevated brain KYNA levels on the dopamine response to amphetamine.

Single unit recording techniques and microdialysis were used for measuring dopamine firing and terminal efflux, respectively. The excitatory effects on ventral tegmental area (VTA) dopamine neurons observed in rats with elevated levels of KYNA were mimicked by administration of 4-chlorokynurenine (4-CL-KYN). This compound is transformed *in situ* to 7-CL-KYNA, which specifically blocks the glycine-site of the NMDA-receptor without affecting α7nACh receptors. Further, administration of the selective NMDA receptor antagonist SDZ 220-581 also increased dopamine firing, while administration of the selective α7nACh receptor antagonist methyllycaconitin (MLA) was associated with a decreased VTA dopamine firing. These results argue that the excitatory effect on midbrain dopamine neurons by elevation of brain KYNA, is specifically related to a blockade of NMDA receptors.

Subchronic, but not acute, elevation of brain KYNA was associated with an enhancement of the amphetamine-induced dopamine response, with regard to both release and firing. This potentiated action of amphetamine on dopamine release appears related to an attenuated ability of the drug to inhibit firing of VTA dopamine neurons.

Altogether, the present results support the hypothesis that increased levels of brain KYNA, potentiate the amphetamine response in VTA dopamine neurons primarily through a blockade of glutamatergic receptors, rather than cholinergic receptors. A reduced responsiveness of VTA dopamine neurons toward the inhibitory action of amphetamine might partly explain the excessive dopamine efflux occurring in a situation of elevated brain KYNA. Given the similarities in amphetamine response between patients with schizophrenia and rats with subchronically elevated levels of KYNA, one may propose that the latter condition may serve as a valuable animal model of schizophrenia.

LIST OF PUBLICATIONS

I. Linderholm KR, ANDERSSON AS, Olsson S, Olsson E, Snoddgrass R, Engberg G, Erhardt S (2007) Activation of rat ventral tegmental area dopamine neurons by endogenous kynurenic acid: A pharmacological analysis. Neuropharmacology 53: 918-924.

II. Olsson SK*, ANDERSSON AS*, Linderholm KR, Holtze M, Nilsson-Todd LK, Schwieler L, Olsson E, Larsson K, Engberg G, Erhardt S (2009) Elevated levels of kynurenic acid change the dopaminergic response to amphetamine: Implications for schizophrenia. International Journal of Neuropsychopharmacology 12: 501-512.

* These authors contributed equally to this work.

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LIST OF ABBREVIATIONS

4-Cl-KYN	4-chloro-kynurenine
5-HT	serotonin
7-Cl-KYNA	7-chloro-kynurenic acid
α7nACh	α7 nicotinic acetylcholine
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	analysis of variance
AUC	area under the curve
COX	cyclooxygenase
CSF	cerebrospinal fluid
ECT	electroconvulsive therapy
EPS	extrapyramidal symptoms
GABA	γ-Aminobutyric acid
HPLC	high-performance liquid chromatography
IDO	indoleamine 2,3–dioxygenase
i.p	intraperitoneal
i.v	intravenous
ISI	interspike interval
IQ	intelligence quotient
KAT	kynurenine aminotransferase
KYNA	kynurenic acid
NaHCO3	sodium bicarbonate
NaCl	sodium chloride
NMDA	*N*-Methyl-D-aspartate
PCP	phencyclidine

PET	positron emission tomography
PFC	prefrontal cortex
PPI	prepulse inhibition
s.c	subcutaneous
SD	standard deviation
SEM	standard error of the mean
TDO	tryptophan 2,3–dioxygenase
TMS	transcranial magnetic stimulation
VTA	ventral tegmental area

1 INTRODUCTION

1.1 Schizophrenia

Schizophrenia was first identified as a discrete mental illness by Dr. Emil Kraepelin in 1887 and the illness itself is generally believed to having accompanied mankind through history. The disease is among the most disabling and economically devastating medical disorders, ranked by the World Health Organization as one of the top ten illnesses contributing to the global burden of disease. It is a chronic and severe brain disorder that emerges independently of sex and ethnical origin, with a prevalence of approximately 0.55-0.87% (Goldner et al., 2002; Perälä et al., 2007). Despite extensive research, its etiology and pathophysiology is largely unknown. Though, it is clear that both genetic and environmental factors are involved in the development of the disease. The onset of schizophrenia is a gradual deterioration that usually occurs in adolescence and early adulthood and is usually initiated with acute psychotic symptoms. Those close to the patient may though advert early warning signs long before the primary symptoms occur. Non-psychotic premorbid signs of neurological and behavior problems during early years may appear, such as delayed development of speech and motor function, as well as social withdrawal and decreased IQ scores (Jones et al., 1994; Woodberry et al., 2008). Average lifetime expectancy is 10-12 years less in patients with schizophrenia and is mainly related to cardiovascular diseases and a higher suicide rate. Death as a consequence of suicide is about ten times more common in patients compared to the general population (Brown, 1997). Moreover, an increased risk for psychiatric comorbidity, including major depression and substance abuse is also observed.

1.1.1 Symptoms

The clinical symptoms of schizophrenia are often manifested in a set of symptoms that may vary over time and likewise, vary between patients. Symptoms are commonly divided into three broad clusters, i.e. positive symptoms, negative symptoms and cognitive deficits (Andreasen, 1995). Positive symptoms, commonly referred to as psychotic symptoms, typically emerge as delusions and hallucinations and refer to features that appear. A *delusion* appears like an unshakable theory or belief in something false and impossible despite evidence of the contrary, often including

persecutory delusions involving bizarre ideas that someone is "out to get" the patient. A *hallucination* refers to a sensation or a sensory perception, which a person experiences in the absence of a relevant external stimulus. It can occur in any sensory modality (visual, auditory, olfactory, gustatory, tactile), although auditory hallucinations (hearing voices or some other sound) are the most common type of schizophrenia. Positive symptoms commonly also include some level of disorganized thinking and speech that often results in "word salad" or incoherent and incomprehensible speech.

Negative symptoms refer to a loss or absence of normal traits or abilities that healthy people normally own. Common negative symptoms include *affective flattening,* where the patient's range of emotional expression is clearly diminished, such as poor eye contact and reduced body language. Likewise, *anhedonia* (inability to experience pleasure), lack of motivation and poverty of speech, such as brief, empty replies frequently appear (Andreasen, 1995). Cognitive dysfunctions are related to impairments in thought processes, including a broad range of features where the patient displays difficulties in problem solving and abstract thinking. Correspondingly, deficit in memory and attention as well as poor judgment commonly emerge (Green et al., 2000). Positive symptoms are commonly episodic and vary over the course of the illness. The negative and cognitive symptoms appear to be more persistent over time, although a general decline of cognitive functions are associated with the time of onset of the illness (Mesholam-Gately et al., 2009; Sponheim et al., 2010). Predictors of poor treatment outcome are male gender, severity of negative symptoms and cognitive deficits, early age of onset as well as prolonged period of untreated illness (Perkins et al., 2005).

1.1.2 Treatment

Although an important element, antipsychotic drugs are far from the only treatment used for patients with schizophrenia. Commonly, supplemental therapies such as psychosocial or cognitive therapy are used in conjunction with drugs. In certain severe cases, some patients also respond to electroconvulsive therapy (ECT) or transcranial magnetic stimulation (TMS). These additional treatments can be essential for a full recovery, although antipsychotic drugs are the best tool right now for controlling symptoms, particularly the positive ones. The discovery of the antipsychotic properties of chlorpromazine (Delay et al., 1952) initiated new treatment options

and the development of antipsychotic medications constituted the primary pharmacological tool for the treatment of patients with schizophrenia.

1.1.3 Typical antipsychotics

The first generation of antipsychotic drugs (e.g. chlorpromazine and haloperidol) is often referred to as typical antipsychotic drugs. Generally these drugs reduce positive symptoms, whereas usually minor or no effects on negative and cognitive symptoms are generated (King, 1998). The clinical effects of the typical antipsychotics are generally believed to be due to their ability of blocking dopamine D2 receptors. Typical antipsychotic drugs are also associated with a relative high incidence of extrapyramidal side effects (EPS), such as *akathisia* (inner restlessness and a compelling need to be in constant motion), *parkinsonism* (tremor, rigidity, akinesia) and *tardive dyskinesia* (involuntary stereotypical movements of the mouth, face and tongue) (Marsden and Jenner, 1980).

1.1.4 Atypical antipsychotics

Atypical antipsychotics usually require lower occupancy of dopamine D2 receptors, with broader receptor profile and most of them own a high affinity for 5-HT2A receptors. Most atypical antipsychotics may not induce EPS at the same extent as typical antipsychotics but instead induce other severe adverse effects, such as heavy sedation and weight gain as well as insulin resistance, which may lead to diabetes and cardiovascular diseases. Clozapine is the most effective agent for the treatment of schizophrenia and is considered especially effective in patients with treatment-resistant schizophrenia and unlike other antipsychotics, owns the property of treating also negative and cognitive symptoms (Conley, 1998*)*. In addition, clozapine produces few or no EPS (Walker et al., 1997). It is also the only antipsychotic drug with a demonstrated significant reduction in suicidality. The side-effect agranulocytosis has however limited its use.

1.2 HYPOTHESES OF SCHIZOPHRENIA

1.2.1 The dopamine hypothesis of schizophrenia

When the dopamine hypothesis of schizophrenia was initially proposed 50 years ago, it postulated that schizophrenia is a manifestation of a hyperdopaminergic state in the brain (Carlsson and Lindqvist, 1963). The foundation of this hypothesis was mainly built on indirect pharmacological evidence and the discovery that antipsychotic drugs increase dopamine metabolism in animals and that the clinical effectiveness of these drugs, was directly correlated with their potency to block dopamine D2 receptors (Seeman and Lee, 1975). Conversely, following heavy doses or long-term administration, drugs that increase dopaminergic activity, such as amphetamine, can induce psychosis in healthy volunteers, (Snyder, 1973) and generally worsen symptoms in patients with schizophrenia (Snyder et al., 1974). Yet, for many patients the hypothesis fits poorly, particularly for those whose symptoms come on gradually and where the negative and cognitive symptoms are more prominent than the positive (Andreasen and Olsen, 1982). These patients also appear to be more resistant to treatment with antipsychotic drugs (Demjaha et al., 2012). Furthermore, the whole spectra of schizophrenia-like symptoms are rarely induced in healthy individuals when exposed to dopamine enhancing drugs (Snyder, 1973).

The inconsistency of the accumulated data commanded a modification of the dopamine hypothesis. Thus, a regional dopamine reactivity was suggested, where a specific prefrontal hypofunction due to deficits in D1 receptor-mediated signaling contributed to the negative symptoms and cognitive impairments, whereas an intermittent excess in subcortical dopamine hyperfunction involving D2 receptors was related to the emergence of psychotic states (Davis et al., 1991). This “dopamine imbalance theory” predominantly relied on animal studies and there was no direct evidence supporting either low dopamine levels in prefrontal cortex or enhanced subcortical levels of dopamine in patients with schizophrenia (Howes and Kapur, 2009). Subsequently it has become clear that these abnormalities are more complex and neuroimaging studies have revealed additional data, indicating an elevated presynaptic dopamine synthesis capacity in patients with schizophrenia (Hietala et al., 1995; Lindstrom et al., 1999).

Neurochemical models of schizophrenia based upon dopamine have had substantial empirical value in explaining key symptoms of schizophrenia, in particular the progress of positive

symptoms. Nevertheless, significant limitations with regard to the dopamine hypothesis remains and the underlying pathophysiology of schizophrenia may additionally involve other neurochemical systems. An alternative hypothesis reveals that symptoms in schizophrenia may reflect an underlying dysfunction or dysregulation in glutamatergic systems (Javitt, 2010).

1.2.2 The glutamate deficiency hypothesis of schizophrenia

The glutamate deficiency theory of schizophrenia proposes a hypofunction in glutamatergic transmission and is based on the findings that glutamate levels are decreased in the cerebrospinal fluid (CSF) of patients with schizophrenia (Kim et al., 1980). The "PCP model of schizophrenia" was formed upon the observation that drugs, blocking the N-methyl-D-aspartate (NMDA) receptor, such as phencyclidine (PCP), ketamine and other similar psychotomimetic compounds, possess the unique potential to reproduce the full symptomatic spectra of schizophrenia, in healthy individuals (Javitt and Zukin, 1991; Lahti et al., 2001). Compared to dopaminergic agents like amphetamine, which only induces psychoses (Snyder, 1973), PCP and ketamine provokes positive and negative symptoms, as well as the cognitive deficits (Javitt, 1987; Javitt and Zukin, 1991). Symptoms are also exaggerated or reawaken in patents with schizophrenia (Lahti et al., 2001; Malhotra et al., 1997). Further, brain imaging studies in humans reveal that ketamine administration to healthy individuals, increase striatal dopamine levels and potentiates the amphetamine-induced dopamine response, similar as in patients suffering from schizophrenia (Kegeles et al., 2000; Breier et al., 1997).

At the neurochemical level, PCP and ketamine interact with the PCP site of the NMDA receptor. Occupation of this specific site induces non-competitive inhibition of NMDA receptor-mediated transmission (Javitt and Zukin, 1991). A relationship regarding the dopamine hypothesis and the glutamate deficiency theory has been proposed. Thus, NMDA receptors regulate dopamine release. In particular, it is shown that longterm administration of NMDA receptor antagonists increase midbrain dopamine transmission (Jentsch et al., 1998) and decrease prefrontal cortex dopamine transmission concomitant with cognitive impairments in both rats (Jentsch et al., 1997b) and monkeys (Jentsch et al., 1997a). The regional dopaminergic imbalance seen in schizophrenia may thus be secondary to an underlying glutamatergic dysfunction (Javitt, 2007). In the midbrain, gamma-amino-butyric-acid (GABA) neurons exert an inhibitory influence on

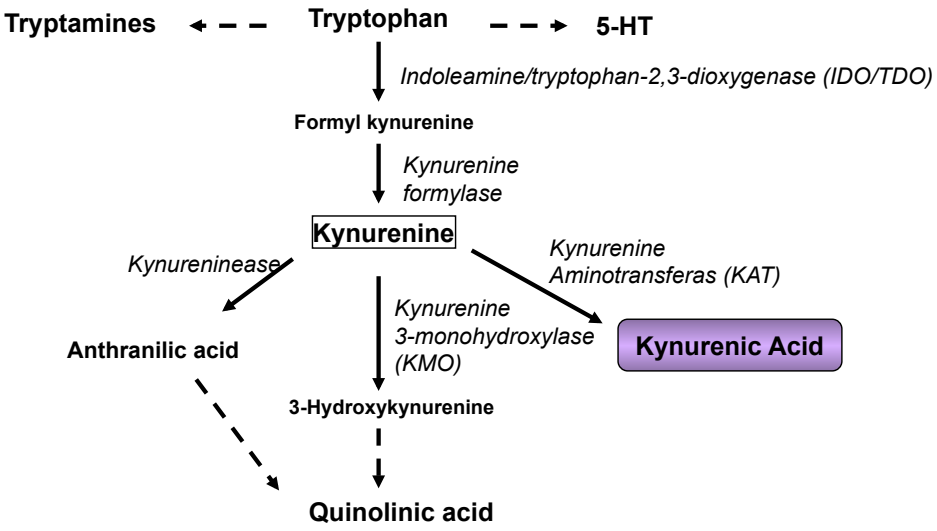
dopaminergic neurons (Erhardt et al., 2002). The enhancement of midbrain dopamine transmission following NMDA receptor antagonism, is hypothesized to be a consequence of decreased excitatory influence on GABA interneurons, relieving dopamine neurons in the ventral tegmental area (VTA) from this source of inhibitory tone (Zhang et al., 1993).

The glycine modulatory site of the NMDA receptor complex has been suggested as a promising target, although much research in this area is still needed. Thus, several clinical studies in patients with schizophrenia revealed beneficial effects on negative and cognitive symptoms when glycine was added to conventional antipsychotic treatment (Coyle and Tsai, 2004). Furthermore, complement of the potent full agonist D-serine, have also been shown to relieve positive symptoms (Tsai et al., 1998). These agents, including the anti-tuberculosis drug D-cycloserine, have also proven to be effective in several preclinical models, including reversal of PCP effects in both rodents (Javitt et al., 2004) and primates (Linn et al., 2007). Additionally, the antipsychotic drug clozapine, known for its superior efficacy in alleviating symptoms of schizophrenia, has been shown to retain partial agonistic properties at the glycine site of the NMDA receptor (Schwieler et al., 2008).

1.2.3 The kynurenic acid hypothesis of schizophrenia

Kynurenic acid (KYNA) was first discovered for more than 150 years ago (Liebig et al., 1853) and has been proven to retain important implications for the neurosciences. KYNA is synthesized by astrocytes and belongs to the kynurenines, a group of metabolically related compounds derived from the essential amino acid tryptophan (Leklem, 1971). The kynurenine pathway is the main route of tryptophan degradation, containing several enzymatic steps and responsible for approximately 95% of the metabolic breakdown of tryptophan (Schwarcz et al., 2012).

The kynurenine pathway is initiated through transformation of tryptophan into N-formylkynurenine by the enzyme indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO). N-formylkynurenine is further degraded by formamidase into kynurenine, the pivotal compound of the kynurenine pathway, which can be metabolized in three different routes (Schwarcz et al., 2012: see figure). To ultimately form KYNA, kynurenine undergoes irreversible transamination by four different kynurenine aminotransferases (KATs), where KAT II is suggested to be the main catabolic enzyme of KYNA in humans (Guidetti et al., 2007).



The kynurenine pathway

Neurochemically, KYNA is an antagonist of glutamate receptors, inhibiting all three ionotropic excitatory receptors, (e.g. NMDA-, kainate- and AMPA receptors) (Perkins and Stone, 1982). KYNA has a greater affinity for the obligatory glycine co-agonist site of the NMDA receptor, thus this glycine site constitutes a preferred molecular target (Parson et al., 1997). Owing to the competitive nature of the action of KYNA at this site, selective glycine receptor antagonists can substitute for KYNA (Maj et al., 1994), whereas agonists may counter the actions of KYNA (Birch et al., 1988; Russi et al., 1992). Furthermore, KYNA possesses antagonistic properties at the α7 nicotinic acetylcholine (α7nACh) receptor and the importance of an intact α7nACh receptor in cognitive processes, has been demonstrated in several studies during recent years (Albuquerque et al., 2009). Additionally, KYNA is known to exert neuroprotective properties against other kynurenine pathway metabolites, such as quinolinic acid and those of other excitotoxins (Zádori et al., 2012). The possibility of its therapeutic use therefore emerges regarding treatment of disorders in the central nervous system. Elevation of KYNA levels causes alterations in glutamatergic, cholinergic, and dopaminergic transmission, neurotransmitters frequently associated with symptoms of schizophrenia (Schwarcz et al., 2012). Recently, KYNA

also has been shown to posses regulating properties of GABA transmission (Beggiato et al., 2013; Beggiato et al., 2014). The "Kynurenic acid hypothesis of schizophrenia" derives from the findings that KYNA is elevated in the CSF (Erhardt et al., 2001) and postmortem brain (Schwarcz et al., 2001) of patients with schizophrenia. These observations were later supported by studies displaying elevated KYNA levels in the CSF in drug naïve and drug-treated patients (Nilsson et al., 2005; Linderholm et al., 2012) as well as in the post mortem brain (Sathyasaikumar et al., 2011) of other cohorts.

The relevance of elevated KYNA levels in the pathophysiology of schizophrenia has been maintained in several clinical and preclinical studies. Increased levels of midbrain dopamine concentrations are suggested to be involved in the creation of positive symptoms. In line with administration of exogenous NMDA receptor antagonists PCP and MK801 (French et al., 1993), KYNA increases the firing rate of midbrain dopamine neurons (Erhardt and Engberg, 2002; Nilsson et al., 2006). The opposite condition occurs when the levels of KYNA is reduced, leading to decreased firing rate of dopamine neurons (Schwieler et al., 2006) suggesting that KYNA owns the property to exert tonic regulation of midbrain dopamine neurons.

During the past decade the kynurenic acid hypothesis has been strengthened by a large number of experimental studies also, giving a robust support for the physiological significance of KYNA. Recent studies suggest that KYNA bi-directionally influences cortical and hippocampal glutamate release (Konradsson-Geuken et al., 2010) as well as modulates acetylcholine release (Zmarowski et al., 2009). Cognitive dysfunctions, such as impaired working memory and verbal learning are common in patients with schizophrenia. In experimental studies, pharmacologically elevated levels of KYNA reduce cortical and hippocampal levels of glutamate and cause impairment in visuospatial working memory and contextual learning memory in rodents (Chess and Bucci, 2006; Konradsson-Geuken et al., 2010). Prepulse inhibition (PPI), a behavior test of sensorymotor gating is further impaired in animals with elevated KYNA levels (Erhardt et al., 2004).

Endogenous KYNA emerges to control and regulate several transmitters, important for optimal brain functioning. Further research concerning the synthesis and influence of KYNA on various neurotransmitter systems, may provide new perspectives and bring light to new possibilities of developing better pharmacological tools in the treatment of schizophrenia.

2 SPECIFIC AIMS OF THE STUDY

1. To study the mechanism involved in the excitatory action of elevated levels of endogenous kynurenic acid on VTA dopamine firing.

2. To examine the influence of elevated brain kynurenic acid on amphetamine-induced dopamine transmission.

3 MATERIALS AND METHODS

3.1 ANIMALS

Male Sprague–Dawley rats (Scanbur BK, Sollentuna, Sweden) weighing minimally 180 g (at time of mini-pump implantation) and maximally 250 g (at the time of the experiment) were housed in groups of 3–4. Free access to food and water was provided. Environmental conditions were checked daily and maintained under constant temperature (25 °C) and humidity (40–60%) in a room with a regulated 12-h light/dark cycle (lights on 06:00 hours). Experiments were approved by and performed in accordance with the guidelines of the Ethical Committee of Northern Stockholm, Sweden. All efforts were made to minimize the number of animals used and their suffering.

3.2 SURGERY

To subchronically elevate endogenous KYNA concentration, rats were administered kynurenine for 6 d via two osmotic minipumps (volume 2 ml) with continuous flow of 10 µl/h. The osmotic pumps (2ML1 Alzet®, Cupertino, CA, USA) were filled under aseptic conditions with either vehicle (0.9% NaCl) or kynurenine (dissolved in deionized water; pH adjusted to 5.7 with NaHCO3) in a concentration equivalent to a dose of 90 mg/kg/d (at day of implantation). All solutions were passed through a sterile filter (Acrodisc Syringe Filter 13 mm with 0.2 µm Supor® membrane) before the filling of pumps. Rats were anaesthetized in a Plexiglas chamber filled continuously with 4.8% isoflurane in air using a vaporizer (Univentor 400 Anaesthesia Unit; Univentor Ltd, Zejtun, Malta) and positioned on a heating pad maintaining body temperature at 37 °C throughout the surgery (Temperature Control Unit, HB 101/2, AgnTho's AB, Lidingö, Sweden). Anesthesia was maintained using a nose cone delivering 2.4% isoflurane. Before surgery, 0.5 ml bupivacain (5 mg/ml) was administered subcutaneously (s.c.) to provide post-operative analgesia. The osmotic minipumps were inserted through an incision in the neck and placed s.c. on the back of the rats. To verify an effect of the kynurenine administration on KYNA concentration, blood was collected from the lateral tail vein at the end of surgery. These

samples were then compared with blood collected at day 6. After surgery rats were placed in single cages for 24 h before being reunited in groups of three. After 6 d, electrophysiological or microdialysis experiments were performed.

3.3 ELECTROPHYSIOLOGY

In electrophysiological experiments, rats were anaesthetized (chloral hydrate; 400 mg/kg i.p.) and mounted onto the ear bars of a conventional stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) so that the skull was set in a horizontal plane and the nose was secured using a clamp at the front of the frame. For i.v. administration, a cannula was inserted into a lateral tail vein and additional injections of chloral hydrate were given as needed to maintain a stable level of anesthesia. Drugs were also given through a lateral tail vein. Throughout the experiments, the body temperature of the animals was maintained at 37 ^{o}C by means of a thermostatic heating pad. The skull surface was exposed and a 3 mm burr hole was drilled with its center located approximately 3.0 mm anterior to the lambda and 0.7 mm lateral to the midline. Following careful removal of the dura, a glass microelectrode with a tip diameter of approximately 1-2 μm (filled with 0.5 M sodium acetate saturated with Pontamine Sky Blue) was lowered by means of a hydraulic micro drive (David Kopf Instruments, Tujunga, CA, USA) in to the region of VTA, according to the stereotaxic coordinates from the atlas of Paxinos and Watson (1998). The in vitro impedance of the electrode was generally 6-8 MΩ, measured at 135 Hz in 0.9% saline. Single unit potentials were passed through a high input-impedance amplifier and filters. The impulses were discriminated from background noise and fed into a computer, and simultaneously displayed on a digital storage oscilloscope, monitored on an audio monitor and on a strip chart recorder (Gould). All dopamine neurons were found 7.5-8.5 mm from the brain surface and fulfilled the neurophysiological characteristics (i.e. triphasic action potentials with a duration more than 2.0 ms, basal firing rates between 1 and 10 Hz and frequent occurrence of burst firing including progressively decreasing spike amplitude) previously described for dopamine neurons in the VTA (Grace and Bunney, 1984a,b). The inhibitory response to aversive stimuli (foot pinch) was also observed (Ungless et al., 2004). To further confirm that recordings had been made exclusively from dopamine neurons, the inhibitory action of a single dose of the dopamine agonist apomorphine (100 μg/kg, i.v.) was verified at the end of the experiments when

experiments allowed. Immediately after each electrophysiological experiment the rats were decapitated. Brains were rapidly removed and stored immediately at -70 ^{0}C for subsequent analysis of KYNA and 7-Cl-KYNA.

3.3.1 Experimental protocol for electrophysiological experiments in study I

In the first series of experiments, the firing activity of VTA dopamine neurons was analyzed 1-3.5 h after i.p. pretreatment with kynurenine or 4-Cl-KYN, by recording from a number of cells in each rat. The number of spontaneously firing VTA dopamine cells in the right hemisphere was calculated from the numbers of cells found per track (2-10 tracks per animal). The electrode was moved 0.1 mm between each track in a pattern to assure that cells were recorded from only once. No changes in firing pattern or in number of spontaneously active cells were observed with regard to the track number. In the second series of experiments the firing activity of VTA dopamine neurons was continuously monitored before, during and after i.v. injection of MLA or SDZ 220-581.

3.3.2 Data analysis of electrophysiological characteristics

The distribution of spikes was analyzed on line utilizing a Spike II software program. In order to avoid artifacts in the sampling procedure, the program was set to ignore time intervals below 20 ms. The onset of a burst was determined as an inter-spike interval shorter than 80 ms and the termination of a burst by the next interval longer than 160 ms (Grace and Bunney, 1984a,b). Cells were considered to be bursting if at least one interspike time interval of 100 recorded spikes was below 80 ms. The interspike time intervals were analyzed with regard to the percentage of spikes fired in bursts that occurred during a sampling of 100–500 spikes.

3.4 MICRODIALYSIS

Rats were anaesthetized as described above and mounted into a stereotaxic frame (Stoelting,Wood Dale, Il., USA). Before each guide cannula implantation the incisor bar was

adjusted so that the skull was set in a horizontal flat plane. Anesthesia was maintained using a nosecone, delivering 2.4% isoflurane throughout the surgery. The skull was exposed, and two shallow holes were drilled for insertion of anchor screws. A hole was drilled above the nucleus accumbens and following careful removal of the dura a guide cannula (MAB 9.IC, AgnTho's AB) was directed to the region of nucleus accumbens and fixed with stainless-steel screws and dental cement (Dentalon®, AgnTho's AB). Stereotaxic co-ordinates for the implantation of the guide cannula with reference to bregma and brain surface, respectively, were: AP +1.6, ML ±1.4, DV +6.2, meaning that the tip of the guide cannula was placed 2 mm above the vertical target for the final position of the microdialysis probe (Paxinos and Watson, 1998). Following surgery, 0.5 ml bupivacain (5 mg/ml) was administered s.c. to provide post-surgical analgesia. Rats were then allowed to recover, singly housed, for 24 h with food and water ad libitum.

On the day of experiment, microdialysis was performed in unanaesthetized freely moving rats. The guide was removed and a microdialysis probe (MAB 9.14.2, polyether sulphone, 2 mm dialysing length, 0.56 mm diameter and a 15 kDa cut-off membrane; AgnTho's AB) was inserted into the cannula. Rats were connected to microdialysis swivels. The probes were perfused with perfusion fluid CNS (CMA Microdialysis AB, Solna, Sweden) delivered via polyethylene tubing from an infusion pump (Univentor 864, Univentor Ltd) at a flow rate of 2 μl/min for dopamine analysis (in paper I Olsson), or 1 μl/min for KYNA/7-Cl-KYNA analysis (in paper II Linderholm). Thirty-minute fractions were collected using a microfraction collector (Univentor 820, Univentor Ltd) and manually injected (Rheodyne, Cotati, CA, USA) into a high-performance liquid chromatography (HPLC) system. To minimize the inter-individual variation due to differences in probe recovery, the dialysate concentrations were transformed to percent of baseline before statistical analysis. A stable baseline, consisting of three consecutive samples with a maximal variation of 10%, was usually obtained after 2–3 h and defined as 100%. Results for subsequent samples were calculated as percentages of this average basal release. In study II (Olsson), all rats were administered d-amphetamine intraperitoneally (i.p.) once a stable dopamine baseline was established and the dopamine concentration was measured for 180 min. One group of rats was pretreated with a single dose of kynurenine (5 mg/kg s.c.) 60 min prior to the microdialysis experiment and another group of rats were treated with kynurenine for 6 days (90 mg/kg/day) prior to the experiment.

In study I (Linderholm), rats were divided in two groups and administered with kynurenine (200 mg/kg, s.c., n = 10) or 4-Cl-KYN (25 mg/kg, s.c., n = 6) respectively, prior to the microdialysis experiment (as described above). Concentrations of in situ produced 7-Cl-KYNA and KYNA were measured in 30 or 40 min fractions, for 210 or 240 min respectively, to verify the effect of respectively precursor.

3.5 HISTOLOGY

At the end of the microdialysis experiment, rats were decapitated and brains rapidly removed and stored in 4% paraformaldehyde in phosphate buffer for at least 5 d. Serial coronal sections (50 μm) were made using a cryostat (Slee Medical GmbH, Mainz, Germany), and histological verification of probe placement was confirmed with reference to the stereotaxic atlas of Paxinos and Watson (1998). No distinction could be made between the core and the shell of the nucleus accumbens. Data are reported only from animals where probe membranes were correctly positioned in the nucleus accumbens.

3.6 ANALYSIS OF DOPAMINE

Separation of dopamine was achieved by reversed-phase liquid chromatography using a 55 mM sodium acetate buffer (pH 4.1, 10% methanol) with 0.8 mM octanesulfonic acid and 0.01 mM Na_2EDTA. The mobile phase was delivered by an HPLC pump (Bischoff Chromatography, Leonberg, Germany) through a ReproSil-Pur C18 column (4 × 150 mm, Dr Maisch GmbH, Ammerbuch, Germany) at a rate of 0.8 ml/min. Following separation, the analysate was first passed through a guard cell with an oxidizing potential of 50 mV. Samples were then quantified by sequential oxidation and reduction in a high-sensitivity analytical cell (ESA 5011; ESA Inc., Chelmsford, MA, USA) controlled by a potentiostat (Coulochem III; ESA Inc.) with an applied potential -200 mV for detection of dopamine. The signals from the detector were transferred to a computer for analysis (Datalys Azur, Grenoble, France). The retention time of dopamine was approximately 8 min.

3.7 PREPARATION OF BRAINS AND BLOOD FOR KYNA ANALYSIS

Brains were sonicated with homogenization medium (perchloric acid 0.4 M, $Na_2S_2O_5$, 0.1% and ethylene-diaminetetra-acetate (EDTA) 0.05%), which was added in the same amount as the weight of the brain before sonication. The samples were centrifuged at 20,000 g for 5 min, and 40 μl perchloric acid (70%) was added to the supernatant. Thereafter, the supernatant was centrifuged twice. Blood samples were centrifuged two times at 20 000 g for 5 min and 100 μl perchloric acid (70%) was added to the supernatant. Thereafter, the supernatant was centrifuged three times and stored at -18°C. Before analysis, samples were defrosted and one more time centrifuged.

3.8 ANALYSIS OF KYNURENIC ACID AND 7-CHLORO-KYNURENIC ACID

For analysis of KYNA and 7-Cl-KYNA, an isocratic reversed- phase high-performance liquid chromatography (HPLC) system was used, including a dual piston, high liquid delivery pump (Bischoff, Leonberg, Germany), a ReproSil-Pur C18 column (4 × 150 mm, Dr Maisch GmbH, Ammerbuch, Germany) and a fluorescence detector (Jasco Ltd, Hachioji City, Japan) with an excitation and emission wavelength of 344 nm and 398 nm, respectively (18 nm bandwidth). A mobile phase of sodium acetate (50 mM, pH 6.20, adjusted with acetic acid) and acetonitrile (7% or 10.0%, for KYNA or 7-Cl-KYNA, respectively) was pumped through the reversed-phase column at a flow rate of 0.5 ml/min. Samples of 30 ml were manually injected (Rheodyne, Cotati, CA, USA). Zinc acetate (0.5 M, not pH adjusted) was delivered post column by a peristaltic pump (P-500, Pharmacia, Uppsala, Sweden) at a flow rate of 0.10 ml/h. The signals from the fluorescence detector were transferred to a computer for analysis utilizing Datalys Azur (Grenoble, France). The retention time of KYNA or 7-Cl-KYNA was about 7 or 16 min, respectively.

3.9 STATISTICAL ANALYSIS

The statistical software package GraphPad Prism® 4.03 (GraphPad Software Inc., San Diego, CA, USA) was used. All data are expressed as mean ±S.E.M. In paper II, no differences between the naive control groups and the groups treated with saline for 6 d were observed in either of the tested parameters. For ease of presentation, all control data presented, were pooled into one control group. Dopamine release is presented as the percent of baseline and the effect of amphetamine administration on dopamine release is analyzed using one-way analysis of variance (ANOVA), compared to predrug value, or two-way ANOVA for repeated measures (time × treatment) followed by Bonferroni post-hoc test. Statistically significant differences in electrophysiological experiments and with regard to concentration of blood and whole brain KYNA, were established using Kruskal-Wallis analysis of variance followed by Mann-Whitney U-test. Differences in percent spikes fired in bursts, were established using Kruskal-Wallis analysis of variance followed by Mann-Whitney U-test or Wilcoxon signed rank test. Significance was assumed for all values where $P < 0.05$.

3.10 DRUGS AND CHEMICALS

The following drugs were used: chloral hydrate (Merck, Darmstadt, Germany), L-kynurenine sulfate salt (Sigma, St Louis, MO, USA), KYNA, d-amphetamine sulphate (Apoteksbolaget, Goteborg, Sweden); 4-Cl-KYN (kindly supplied by Vistagen Therapeutics, South San Francisco, CA, USA and dissolved in 7.5% (2-hydroxypropyl)-β-cyclodextrin); 7-Cl-KYNA and SDZ 220-581 (Tocris, Avonmouth, UK); isoflurane (Forene®, Abbott Scandinavia, Solna, Sweden); and MLA (Sigma, St.Louis,MO,USA). Chemicals used were as follows: zinc acetate, acetic acid (Sigma, St. Louis, MO, USA); pontamine sky blue (BDH Laboratory Supplies, UK), methanol, octanesulfonic acid, Na_2EDTA (Sigma), sodium acetate (Riedel-de Haen, Germany), perchloric acid (Kebo Laboratory, Stockholm, Sweden), and acetonitrile (Labasco, Partille, Sweden). Perfusion fluid composition (CMA Microdialysis AB, Solna, Sweden): NaCl, 147.0; KCl, 2.7; CaCl, 1.2; and $MgCl_2$, 0.85 (mM).

4 RESULTS AND DISCUSSION

4.1 PAPER I: ACTIVATION OF RAT VENTRAL TEGMENTAL AREA DOPAMINE NEURONS BY ENDOGENOUS KYNURENIC ACID: A PHARMACOLOGICAL ANALYSIS

Several studies reveal that elevated brain levels of endogenous KYNA increase firing of rat VTA dopamine neurons (Erhardt et al., 2001; Erhardt and Engberg, 2002; Nilsson et al., 2006; Schwieler et al., 2006).

Study I aimed to investigate the specific receptors involved in the excitation of VTA dopamine neurons, following pharmacologically elevated levels of endogenous KYNA. A series of electrophysiological experiments were used to examine the firing rate and burst firing activity of VTA dopamine neurons, following administration of selective antagonists at different KYNA inhibition sites. The synthetic compound 4-chlorokynurenine (4-Cl-KYN) is transformed in situ into 7-Cl-KYNA, a highly selective antagonist at the glycine-site of the NMDA receptor. It shares this property with KYNA (Hokari et al., 1996), but do not antagonize the α7nACh receptor. Further, methyllycaconitine (MLA), an antagonist of the α7nACh receptor, and the competitive NMDA receptor antagonist SDZ 220-581 were analyzed regarding the firing pattern of VTA dopamine neurons. Concentrations of in situ produced KYNA and 7-Cl-KYNA were measured utilizing microdialysis techniques in nucleus accumbens, following systemic administration of kynurenine or 4-Cl-KYN (Fig 1). Firing of VTA dopamine neurons was monitored 1 – 3.5 h after kynurenine (200 mg/kg, i.p.), 4-Cl-KYN (25 mg/kg, i.p.) or saline administration respectively. Pretreatment with kynurenine as well as with 4-Cl-KYN was associated with an increase in firing rate and burst firing activity (Table 1). Further, the number of dopamine cells found per track was almost doubled following administration of 4-Cl-KYN, indicating an activation of dopamine neurons that may be quiescent under normal conditions.

Table 1. Firing rate and spike distribution of dopamine neurons in the VTA

	Controls	**Kynurenine**	**4-chlorokynurenine**
		(5 mg/kg; s.c., 60 min)	(90 mg/kg/day; s.c., for six days)
	n = 15	n = 9	n = 13
Firing rate, Hz	4.0 ± 0.2	5.0 ± 0.2**	5.1 ± 0.2**
Mean % spikes in burst	26.7 ± 2.8	40.2 ± 4.0^{++}	43.1 ± 3.0^{+++}
Cells found per track	1.5 ± 0.2	2.1 ± 0.2	2.8 ± 0.4*

Intravenous pretreatment (1-3.5 h) with kynurenine (200 mg/kg, i.p.) or 4-Cl-Kyn (25 mg/kg, i.p.). Values represent means ±SEM from control rats (n=13) and rats treated with kynurenine (n=9) or 4-Cl-KYN (n=8). *P<0.05, **P<0.01 vs. corresponding control value (Mann-Whitney U-test). ++ P<0.01, +++ P<0.001 vs. corresponding control value (Wilcoxon signed rank test).

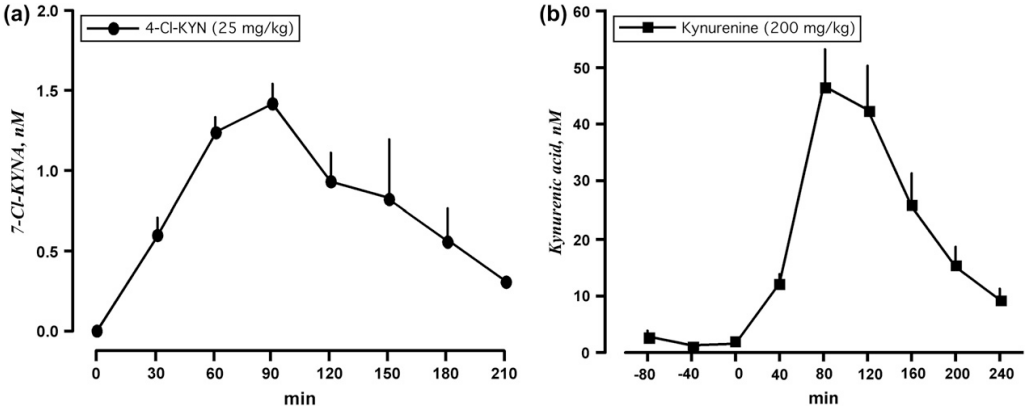


Figure 1. Concentrations of in situ produced 7-Cl-KYNA and KYNA were measured utilizing microdialysis techniques in nucleus accumbens, after systemic administration of 4-Cl-KYN (25 mg/kg, s.c., n=6; Fig. 1a) or kynurenine (200 mg/kg, s.c., n=10; Fig. 1b). Each value represents mean ± SEM.

Administration of the competitive NMDA receptor antagonist SDZ 220-581 (10 mg/kg, i.v.) was associated with an increase in firing rate of VTA dopamine neurons. The increase in frequency was typically not observed until 5 min after administration (Figures 2, 3a). Burst firing was increased 5 min after administration but not after 1, 10, 15 or 20 min (data not shown). A lower dose of SDZ 220-581 (5 mg/kg i.v.) failed to alter firing rate or burst firing activity of VTA dopamine neurons (data not shown).

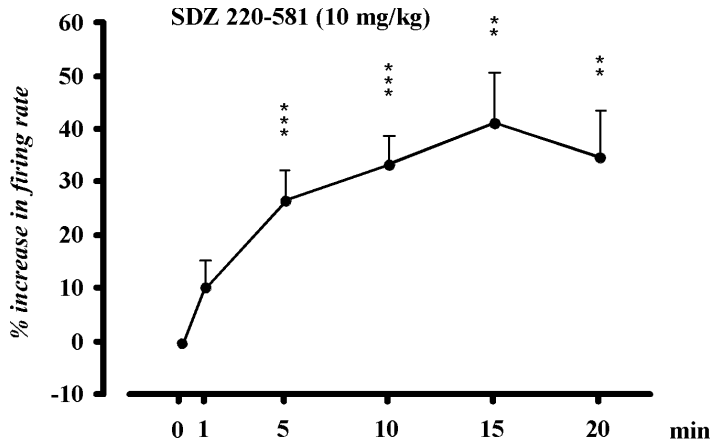


Figure 2. Time course depicting the increase in firing rate of VTA dopamine neurons after administration of SDZ 220-581(10 mg/kg, i.v., n = 4–7). **P < 0.01, ***P < 0.001 vs. corresponding control value (Mann-Whitney U-test)

Administration of the α7nACh receptor antagonist MLA (0.5–4 mg/kg, i.v) did not significantly change the firing rate or percent burst firing of VTA dopamine neurons (Figure 3b). However, a high dose of MLA (6 mg/kg) given i.p. significantly decreased firing rate and per cent burst firing of VTA dopamine neurons 10 min after administration (Figure 3c).

The results of the present study show that the excitatory effects on VTA dopamine neurons observed in rats with elevated levels of KYNA (by administration of kynurenine) were mimicked by administration of 4-CL-KYN and SDZ but not MLA. In consonance with a recent study (Wang et al., 2006) intravenous administration of MLA in various doses up to 4 mg/kg failed to alter firing rate or burst firing activity of VTA dopamine neurons. However, when the drug was injected i.p. in a high dose (6 mg/kg), a significant decrease in firing was observed. These data are in analogy with the excitatory action on VTA dopamine neurons observed by galantamine, an α7nACh receptor agonist (Schilstrom et al., 2007). Thus, the α7nACh receptor antagonistic action by KYNA appears highly unlikely to participate in the increase in firing rate and burst firing activity of VTA dopamine neurons.

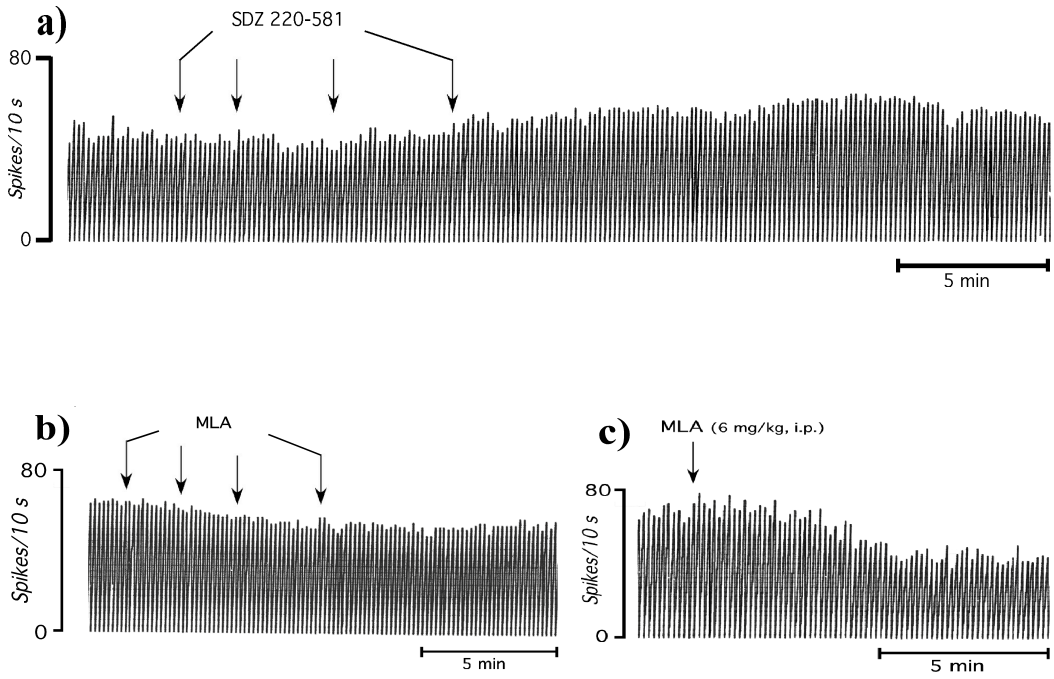


Figure 3. Extracellular recording from a dopamine neuron in the VTA showing the effect on firing rate by **(a)** SDZ 220-581 (1.25 + 1.25 + 2.5 + 5 mg/kg, injected i.v. at arrows), **(b)** MLA (0.5 + 0.5 + 1 + 2 mg/kg, injected i.v. at arrows) and **(c)** MLA (6 mg/kg, injected i.p. at arrow)

Altogether, the results of the present study clearly show that the excitatory effect on VTA dopamine neurons by elevated levels of KYNA is mediated via blockade of the NMDA receptor. In support of the hypothesis that KYNA functions through the glycine-site of the NMDA receptor to excite VTA dopamine neurons is the previously reported finding that the KYNA-induced increase in firing could be restored following administration of D-cycloserine, a partial agonist at the glycine-site of the NMDA receptor (Erhardt and Engberg, 2002).

Further, 7-Cl-KYNA a selective antagonist at the glycine-site of the NMDA without activity on the α7nACh receptor, produces the same magnitude of response of VTA dopamine neurons as

KYNA at equipotent concentrations. The excitatory action of VTA dopamine neurons by SDZ 220-581, a competitive antagonist at the glutamate recognition-site of the NMDA receptor, principally indicates that the well-known ability of KYNA to block also this site could participate in its excitatory effects on VTA dopamine neurons, although relatively high concentrations of endogenous brain KYNA would be required. The somewhat paradoxical increase in firing rate and burst firing activity of midbrain dopamine neurons following elevated KYNA levels, has been suggested to be related to inhibition of a tonic GABAergic input that normally dampens neuronal activity of these neurons (Erhardt and Engberg, 2002).

4.2 PAPER II: ELEVATED LEVELS OF KYNURENIC ACID CHANGE THE DOPAMINERGIC RESPONSE TO AMPHETAMINE: IMPLICATIONS FOR SCHIZOPHRENIA

The dopamine (dopamine) hypothesis of schizophrenia suggests that an enhanced dopaminergic transmission in brain limbic areas causes positive symptoms of the disorder, e.g. delusions and hallucinations. Although clinical support of the dopamine hypothesis has been sparse, positron emission tomography (PET) studies have revealed an abnormal, excessive dopamine release following administration of amphetamine in untreated patients with schizophrenia (Abi-Dargham et al., 1998; Laruelle and Abi-Dargham, 1999; Laruelle et al., 1996; Breier et al., 1997).

Amphetamine enters the brain where it increases extracellular dopamine. The large amount of dopamine efflux generates a negative feedback loop, leading to inhibition of cell firing in dopamine neurons (White, 1996). In study II, the aim was to investigate the amphetamine-induced effects on terminal dopamine release as well as on neuronal dopaminergic firing under conditions of acutely and subchronically elevated levels of brain KYNA. Hypothetically, if elevated levels of KYNA are one of the biological causes behind schizophrenia it might be a chronic condition. Kynurenine, the immediate precursor of KYNA, was administered to rats in a single-dose or subchronically via osmotic mini-pumps. The amphetamine-induced effects on dopamine transmission following elevated levels of brain KYNA were investigated, using in vivo extracellular single cell recordings of dopamine neurons in the VTA of anesthetized male Sprague Dawley rats. Also measurement of dopamine levels in the nucleus accumbens of awake, free moving rats were performed.

4.2.1 Effects on dopamine release following amphetamine administration

Rats were given a single dose (5 mg/kg, s.c) or subchronic administration (90 mg/kg/d for six days, s.c, minipumps) of L-kynurenine, in order to increase KYNA brain levels. A single dose of kynurenine enhanced brain KYNA levels 3-fold, whereas subchronic treatment with kynurenine was associated with a 2-fold increase in brain KYNA levels at day 6 compared to saline treated control rats (Table 2).

Table 2. Whole brain concentrations of kynurenic acid (nM)

	Controls	**Acute kynurenine**	**Subchronic kynurenine**
		(5 mg/kg; s.c., 60 min)	(90 mg/kg/day; s.c., for six days)
	n = 15	n = 9	n = 13
Kynurenic Acid (nM)	20.7 ± 3.5	66.1 ± 9.0***	40.6 ± 8.1*

Values represent mean±SEM. Statistics: *$p<0.05$, ***$p<0.001$ vs. controls (Kruskal-Wallis analysis of variance followed by Mann-Whitney U-test).

The extracellular dopamine levels in nucleus accumbens were measured by microdialysis and HPLC with electrochemical detektion. Data are presented as percent of baseline levels. The amphetamine-induced release of nucleus accumbens dopamine is summarized in figure 4. Systemic administration of amphetamine (2 mg/kg i.p.) was associated with a marked increase in dopamine release in the nucleus accumbens in control rats as well as in rats pretreated with a single dose of kynurenine (60 min prior to administration of amphetamine).

Maximal increase in dopamine release was obtained 60 min after the amphetamine injection (+374% in control rats and +461% in rats pretreated with a single dose of kynurenine) but showed no significant difference. Administration of amphetamine to rats subchronically treated with kynurenine was associated with a potentiated extracellular dopamine efflux. This effect reached its maximum 60 min after the amphetamine injection (+814%) and was significantly enhanced compared to control rats.

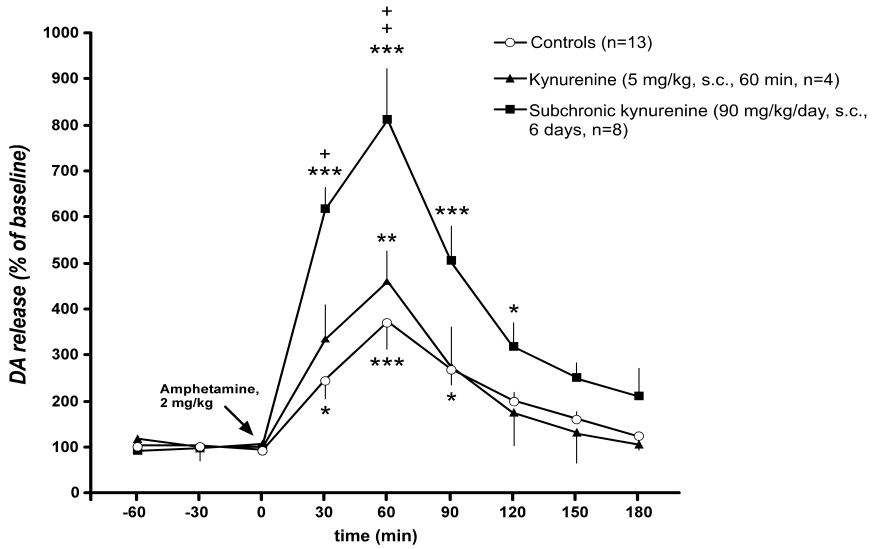


Figure 4. Effects of amphetamine (2 mg/kg i.p.) administration dopamine output in the nucleus accumbens in awake, freely moving rats. Each point represents the mean ±S.E.M percent of baseline (n=4-13). **p*<0.05 ***p*<0.01 ****p*<0.001 compared to pre-drug value (one way ANOVA followed Bonferroni multiple comparison test); + *p*<0.05 ++ *p*<0.001 between-group comparisons (two-way ANOVA for repeated measurements (time x treatment) followed by Bonferroni post-hoc tests).

These results show that subchronically, but not acutely, elevated levels of brain KYNA potentiate the amphetamine induced dopamine response in nucleus accumbens. The enhanced dopamine response following subchronic administration indicates that enhanced KYNA levels in the rat brain produces alterations in the dopamine system, leading to amphetamine-induced hyperresponsiveness, comparable to patients with schizophrenia (Abi-Dargham et al., 1998; Laruelle and Abi-Dargham, 1999; Laruelle et al., 1996; Breier et al., 1997). In line with previous studies (Rassoulpour et al., 2005), systemic administration of 5 mg/kg L-kynurenine s.c., was associated with a trend towards reduced (25%) striatal dopamine output.

4.2.2 Amphetamine effects on the firing activity of dopamine neurons

Firing activity and burst frequency of VTA dopamine neurons in rats pretreated with saline, a single dose of kynurenine (5 mg/kg s.c., 60 min) or with subchronic administration of kynurenine (90 mg/kg.d at day of surgery for 6 d) or saline was examined following increasing doses of amphetamine. No differences in response between the naive control groups and the groups treated with saline for 6 d were observed in either of the tested parameters. For ease of presentation, all control data presented, were thus pooled into one control group. A previous study demonstrates a relationship between basal firing of a single VTA dopamine neuron and the responsiveness of this neuron to amphetamine (White and Wang, 1984). Therefore, only recordings from dopamine neurons with similar frequency and burst firing activity within the groups were included. The effects of elevated brain KYNA levels on spontaneous firing rate and spike distribution are demonstrated in table 3.

Table 3. Effects of kynurenine on the firing rate and spike distribution of DA neurons in the VTA

	Controls	**Acute kynurenine** (5 mg/kg; s.c., 60 min)	**Subchronic kynurenine** (90 mg/kg/day; s.c., for six days)
	(33 neurons)	(32 neurons)	(19 neurons)
Firing rate (Hz)	4.3 ± 0.3	4.8 ± 0.4	6.1 ± 0.4**
Mean % burst firing	19.7 ± 39	37.2 ± 5.9*	37.9 ± 7.0*

Values represent mean±SEM from 24 control rats, 11 rats treated with acute kynurenine and 13 rats subchronically treated with kynurenine. Statistics: *p<0.05, **p<0.01 vs. corresponding control value (Kruskal-Wallis analysis of variance followed by Mann-Whitney U-test).

A single dose of 5 mg/kg L-kynurenine increased burst firing activity but did not change the firing rate of VTA dopamine neurons. Subchronic (90 mg/kg/day, s.c., for six days) pretreatment increased both firing rate and burst firing activity, in agreement with previous observations. Systemic administration of amphetamine was found to dose-dependently reduce firing rate and percent burst firing activity of VTA dopamine neurons. In control rats, the firing rate of nine VTA dopamine neurons out of 13 was totally suppressed following administration of 1.6 mg/kg

amphetamine (Figs 2, 3a). The dopamine neurons still firing following administration of 1.6 mg/kg amphetamine were quiescent after administration of 3.2 mg/kg (two neurons) or 6.4 mg/kg (two neurons). Rats pretreated with a single dose of kynurenine (5 mg/kg s.c., 60 min) did not change the inhibitory action of amphetamine (Fig 2). In rats with subchronically administered kynurenine (90 mg/kg.d at day of surgery for 6 d), larger doses of amphetamine were required to induce inhibition of firing rate and percent burst firing activity (Figure 5, 6b). Thus, 1,6 mg/kg amphetamine was not able to totally suppress firing in any dopamine neuron recorded from in these rats. Following 12.8 mg/kg amphetamine two neurons were still firing and bursting, one of them even at the dose 25.6 mg/kg and this neuron was not quiescent until apomorphine (100 mg/kg i.v.) was administered.

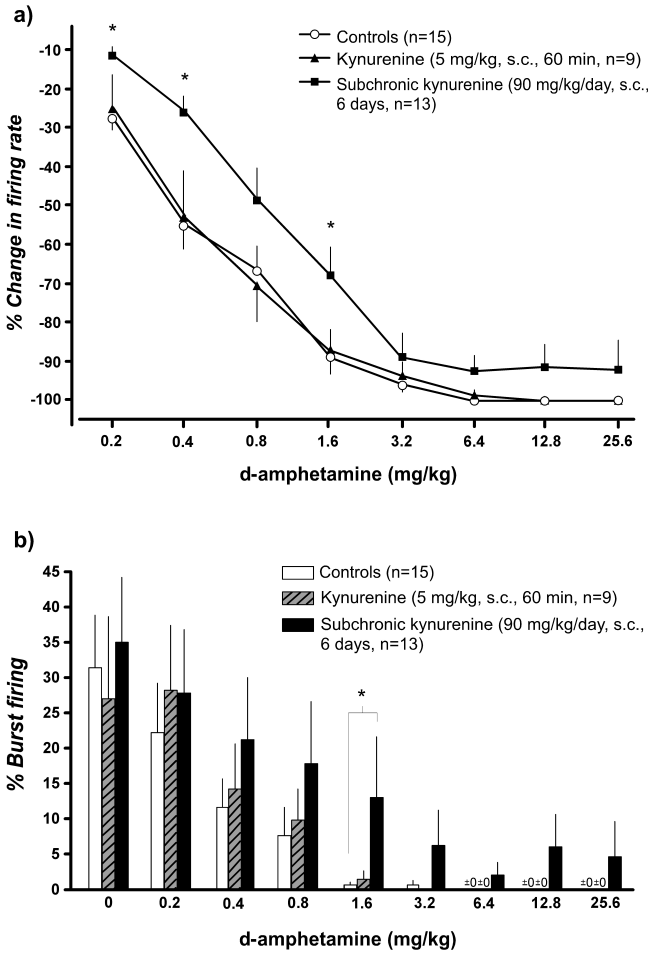


Figure 5. Cumulative dose-response curves illustrating the action of intravenously administered amphetamine on **(a)** the firing rate, and **(b)** the burst firing activity of VTA dopamine neurons in control rats and kynurenine treated rats, either a single dose or subchronically. Each point represents the mean ±SEM obtained from 8-15 neurons. *$p<0.05$, between-group comparisons (Kruskal-Wallis analysis of variance followed by Mann-Whitney *u* test).

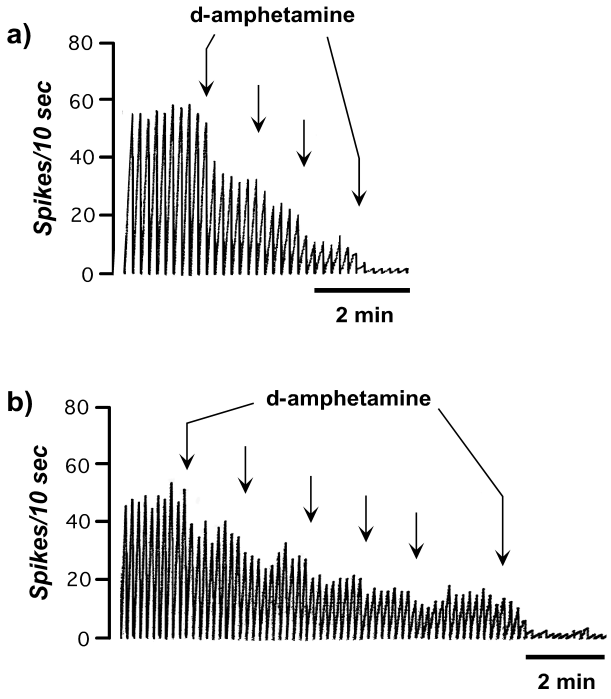


Figure 6. Extracellullar single recordings from dopamine neurons in the VTA following intravenous administration of D-amphetmine in cumulative doses (0.2+0.2+0.4+0.8+1.6+3.2 mg/kg at arrows) in **(a)** a control rat, and **(b)** a rat after subchronic treatment with kynurenine (90 mg/kg.d s.c., for 6 d).

5 GENERAL DISCUSSION

The major findings of the present thesis are that subchronic treatment with kynurenine, in contrast to a single dose of the compound, enhances the amphetamine-induced dopamine release in the rat nucleus accumbens. Given the augmented effect of amphetamine on dopamine neurotransmission seen in patients with schizophrenia (Abi-Dargham et al., 1998; Laruelle and Abi-Dargham, 1999; Laruelle et al., 1996; Breier et al., 1997), the results suggest that hypoglutamatergia, here induced by elevated levels of KYNA, may resemble a state similar to what is proposed in patients with schizophrenia. A rational explanation for the potentiated dopamine release may be an attenuated effect of amphetamine to inhibit firing of VTA dopamine neurons, in a situation of subchronically elevated brain KYNA.

A question of importance is whether the effects of KYNA are mediated via glutamatergic or cholinergic neurotransmission since KYNA is able to antagonize both the glycine site of the NMDA receptor and the α7nACh receptor at low concentrations. Here the excitatory effects on VTA dopamine neurons observed in rats with elevated levels of KYNA, were mimicked by pretreatment with the NMDA receptor antagonist SDZ, as well as with 4-Cl-KYN, which in situ is transformed to 7-Cl-KYNA, a specific antagonist at the glycine site of the NMDA receptor. The excitatory actions on VTA dopamine neurons by elevated KYNA levels are in accordance with the effects of other NMDA receptor antagonists. Thus, systemic administration of both noncompetitive NMDA receptor antagonists, such as PCP and MK 801 (French et al., 1991, 1993; Zhang et al., 1992) as well as antagonists at the D-serine/glycine-site of the NMDA receptor, e.g. L-701,324 (Schwieler et al., 2006), is associated with increased firing of midbrain dopamine neurons. In contrast, blockade of the α7nACh receptor was rather associated with decreased firing of VTA dopamine neurons. These results provide strong evidence that KYNA increases VTA dopamine firing, primarily through glutamatergic- rather than cholinergic mechanisms.

In conclusion**,** subchronic elevation of endogenous rat brain KYNA induces a markedly enhanced amphetamine-provoked dopamine release and hereby resembles the enhanced response to amphetamine seen in patients with schizophrenia (Abi-Dargham et al., 1998; Laruelle and Abi-Dargham, 1999; Laruelle et al., 1996; Breier et al., 1997). Although the underlying mechanisms need to be more thoroughly investigated, a reduced responsiveness of VTA dopamine neurons toward the inhibitory action of amphetamine might partly explain the excessive dopamine efflux. Given the similarities in amphetamine response between patients with schizophrenia and rats with subchronically elevated levels of KYNA, one may propose that the latter condition may serve as a possible animal model of schizophrenia.

Clearly, the kynurenine pathway has evolved to serve important regulatory functions in the mammalian brain and the current findings may serve to increase our understanding regarding the modulation of neuronal activity of midbrain dopamine neurons. The present results may also assist to open up novel perspectives and tools regarding the pharmacological treatment of schizophrenia and other dopamine-related diseases.

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