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Research report

Differential effects of single and repeated ketamine administration on dopamine, serotonin and GABA transmission in rat medial prefrontal cortex

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Abstract

Cognitive functions regulated by the prefrontal cortex are sensitive to changes in dopaminergic and serotoninergic transmission. The non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine influences dopaminergic transmission and induces psychotic symptoms in normal and schizophrenic individuals. This study examined the effect of single and repeated ketamine (25 mg/kg, i.p.) administration on extracellular levels of dopamine, GABA and the serotonin metabolite 5-hydroxyindoleacetic (5-HIAA) acid in the medial prefrontal cortex using in vivo microdialysis in conscious rat. In line with earlier studies, we observed a transient five-fold increase in dopamine release following single ketamine administration in drug naive animals. However, we also observed a two-fold increase in basal dopamine levels and an almost complete attenuation of the ketamine-induced increase in dopamine release in animals pre-treated with ketamine once daily for 7 days. Extracellular 5-HIAA levels were increased by ketamine in both drug naive and even more enhanced in ketamine-pre-treated animals but without a change in basal 5-HIAA levels. GABA levels were unaffected by either single or repeated ketamine administration. We demonstrate evidence for a differential effect of single and repeated ketamine administration on dopamine, serotonin and GABA transmission in the medial prefrontal cortex. We provide new evidence for a complex adaptation of neurotransmission following repeated NMDA receptor blockade whereby in the presence of increased basal dopamine levels the ketamine-induced increase in dopamine is attenuated and the increase in 5-HIAA is enhanced. It appears from our results that ketamine pre-treatment reduces the dynamics of dopaminergic transmission in the prefrontal cortex and may possibly alter the balance between dopamine and serotonin transmission. ©

Keywords: Dopamine; γ-Aminobutyric acid (GABA); 5-Hydroxyindoleacetic acid (5-HIAA); Ketamine; Microdialysis; Prefrontal cortex

1. Introduction

Ketamine is a dissociative anesthetic belonging to a group of non-competitive *N*-methyl-D-aspartate (NMDA) sub-type glutamate receptor antagonist [34]. Clinical use of ketamine and other related NMDA receptor antagonists such as phencyclidine (PCP) and dizocilpine (MK801) [34] induce a broad range of cognitive adverse effects including deficits in working memory, verbal fluency and vigilance tasks, and symptoms that resemble various aspects of psychoses such as schizophrenia [14]. Experimental administration of ketamine in low sub-anesthetic doses to schizophrenic patients precipitates a worsening in mental status with increased symptoms of psychosis reminiscent of the patients symptoms during their illness [15]. The capacity of the NMDA blocking drugs such as ketamine and PCP to induce psychosis makes these drugs valuable in evaluating experimental models of schizophrenia and glutamatergic dysfunction in this disease [8].

NMDA antagonists impair cognitive functions such as working memory in an alternation task [24]. In addition, NMDA receptor blockade by sub-anesthetic doses of ketamine interferes with sensory information processing in healthy humans volunteers [23]. Frontal cortex function as tested in rats by delayed alternation performance is highly sensitive to low doses of ketamine such that a single ketamine administration in a dose range of 10 to 30 mg/kg attenuates working memory in a dose-dependent fashion and this is blocked with antipsychotic drugs such

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as haloperidol or raclopride [39]. Dopamine transmission in the prefrontal cortex is of critical importance for working memory and an excess of dopaminergic transmission in this part of the brain interferes with such cognitive functions [42]. In line with this, in vivo microdialysis studies have shown that the acute ketamine-induced attenuation of working memory is associated with an increase in dopamine release in rat prefrontal cortex [39]. However, the adaptation of dopaminergic neurotransmission in the prefrontal cortex to repeated NMDA antagonism remains to be characterized.

The in vivo microdialysis technique [38] has been widely adopted to examine the effects of NMDA antagonism in the prefrontal cortex and other brain regions [9,22]. Low doses of both MK-801, PCP and ketamine increase extracellular dopamine levels in rat prefrontal cortex but not striatum and are associated with impaired cognitive functions such as delayed alternation performance [7,39,40]. In fact, there is strong evidence for a preferential cortical effect on dopaminergic transmission by sub-anesthetic doses of this group of non-competitive NMDA antagonists. Repeated ketamine pre-treatment has minor effects on spontaneous behavior in the rat and is associated with a behavioral supersensitivity to apomorphine-induced dopamine receptor stimulation [16]. We are not aware of studies of psychotropic effects of repeated ketamine administration in humans.

In addition to dopamine, serotonin and GABA have been suggested to be under the influence of NMDA receptor regulation. Thus, single NMDA antagonism by MK-801 increases extracellular 5-HIAA levels in the frontal cortex of drug naive animals [11,19]. Furthermore, NMDA receptor stimulation by NMDA itself increases GABA release from cultured cortex neurons [5], whereas NMDA blockade by a single MK-801 administration decreases those levels of mRNA encoding the GABA synthesizing enzyme glutamic acid decarboxylase$_{65}$ (GAD_{65}) [17]. However, apart from this the effect of NMDA blockade on the in vivo release of serotonin and GABA is largely unknown.

The ability of ketamine to induce schizophreniform psychosis implies that endogenous dysfunction or dysregulation of NMDA receptor-mediated transmission may occur in schizophrenia and contribute to the generation of symptoms, at least in addition to alteration of dopamine transmission. In this respect the effect of NMDA receptor antagonism on serotonergic and GABAergic transmission in the prefrontal cortex needs to be better explored. In addition, most studies have focused on the effects of single drug administration and knowledge of the effects of prolonged NMDA antagonism is unclear. This study was designed to investigate the effect of single and repeated ketamine administration on extracellular dopaminergic, serotonergic (as studied by monitoring 5-hydroxyindoleacetic acid (5-HIAA)) and GABAergic transmission in prefrontal cortex of the awake freely moving rats using in vivo microdialysis.

2. Materials and methods

2.1. Animals and in vivo microdialysis

Male Sprague-Dawley rats (mean b.wt 270–300 g; Alab AB, Sollentuna, Sweden) were maintained on a standard 12/12 light-dark cycle and allowed food and water ad libitum. The experimental protocols performed in the present study were approved by the Swedish Committee for Ethical Experiments on Laboratory Animals, license no. 199/92.

On the day of microdialysis probe implantation, rats were anesthetized (1.5% halothane/air mixture delivered at 1.4 l/min) and the dura was exposed through two burr holes (3 mm in diameter) in the skull and a microdialysis probe of concentric design (4 mm dialyzing membrane; CMA 12, CMA Mikrodialys AB, Stockholm) was stereotaxically implanted in the left prefrontal cortex (coordinates in mm from bregma: AP = 2.7, ML = 1.4, VD = 5.5) [26]. The dialysis probe was permanently fixed to the skull with stainless steel screws and methylacrylic cement. Local anesthetic was applied onto the surface adjacent to the cement to minimize postoperative pain. All animals were allowed to recover and 24 h following probe implantation the experiments were performed. A new microdialysis probe was used in each rat. The probes were perfused (2 μl/min) with Ringer solution (composition in mM: $CaCl_2$ 2.4, KCl 4, NaCl 148; pH 6).

On the day of the experiment, the rat was placed in a hemispheric bowl and the microdialysis probe was connected via a liquid swivel to a microinfusion pump (CMA 120 system 4, CMA Mikrodialys AB, Stockholm, Sweden) and was perfused (2 μl /min) with Ringer solution.

Dialysates were collected every 20 min starting 6 h after onset of probe perfusion. At the end of the microdialysis study, the animal was anesthetized with a high concentration of halothane and sacrificed. The brains were dissected out and frozen at −20°C and the placement of the probe was verified by cryostat sectioning followed by microscopic examination.

Each perfusate sample (40 μl) was divided into two parts and dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-HIAA (10 μl) and GABA (20 μl) were measured by means of HPLC (high pressure liquid chromatography) coupled to electrochemical detection [21].

Following the 6 h washout period the mean stable dopamine values from three consecutive samples were used to calculate the basal levels.

2.2. Biochemical analysis

Briefly, dopamine, DOPAC, HVA and 5-HIAA analysis was accomplished by injecting 4 μl sample onto a Sepstic microbore column (BAS, West Lafayette, IN, USA) perfused under isocratic conditions at the flow rate of 70

μl/min with a mobile phase containing 0.1 M sodium phosphate, 0.3 mM EGTA, 1.35 mM 1-octanesulfonic acid, 4% acetonitrile, 0.5% tetrahydrofurane, 0.1 M acetic acid at pH 4.0. The limit of sensitivity for dopamine was 2 fmol/sample. Serotonin (5-hydroxytryptamine) was not reliably measured with the available chromatography system.

The GABA assay employed in this study has been described previously in detail by Kehr and Ungerstedt [12]. Briefly, the assay was based on precolumn derivatization with *O*-phthaldialdehyde/*t*-butylthiol reagent and separation by reverse phase HPLC on a Nucleosil 3C18 column with electrochemical detection under isocratic conditions. The mobile phase was 0.15 M sodium acetate buffer, 1 mM EDTA, pH 5.4 and 50% acetonitril. The flow rate of the mobile phase was 0.8 ml/min. The limit of detection was 20 fmol/sample.

2.3. Protocol for drug treatments and materials

The animals were divided into three groups with six animals in each group. Groups 1 and 2 were pre-treated with saline intraperitoneally once daily for 7 days (day 1 to 7) while the third group received a daily intraperitoneal injection of ketamine (25 mg/kg) for 7 days (day 1 to 7). On the day of the microdialysis study (day 8) three successive stable basal samples were recorded and each animal received an intraperitoneal injection. Group 1 (saline-saline) received a saline injection following saline pre-treatment while groups 2 and 3 (saline-ketamine and ketamine-ketamine, respectively) received a ketamine injection following saline and ketamine pre-treatment, respectively. Ketamine hydrochloride (Ketalar®) was purchased from Parke-Davis Scandinavia AB.

2.4. Statistics

Data are reported as percentage of basal values (calculated as means of the three samples before injections). Two-factor ANOVA for repeated measurements and Student-Newman-Keuls post-hoc test was used to examine the possibility of significant differences ($P < 0.05$) between groups.

3. Results

3.1. Dopamine, DOPAC and HVA

Basal levels of dopamine were similar in both the saline-pre-treated groups while two-fold increase was observed in the ketamine-pre-treated group (Table 1). Ketamine administration in the saline-pre-treated group induced a rapid and prolonged increase in dopamine release which was maximal (five-fold) after 60 min and was still elevated (two-fold) for more than 2 h after the drug injection compared to saline-injected saline-pre-treated controls (Fig. 1). However, this increase was markedly attenuated in the ketamine-pre-treated group.

Table 1
Basal concentration (nM) of dopamine, DOPAC, HVA, 5-HIAA and GABA in rat left prefrontal cortex of control animals (drug naive) and following ketamine administration

Compound	Group 1 Saline-saline	Group 2 Saline-ketamine	Group 3 Ketamine-ketamine
Dopamine	0.55 ± 0.13	0.47 ± 0.07	1.31 ± 0.31 [a]
DOPAC	121 ± 29	89 ± 21	156 ± 24
HVA	311 ± 43	219 ± 23	337 ± 41
5-HIAA	732 ± 42	663 ± 40	726 ± 77
GABA	33.9 ± 2.7	35.3 ± 4.4	36.6 ± 2.3

Three groups ($n = 6$) are represented: (1) saline administration following saline pre-treatment, (2) ketamine administration following saline pre-treatment and (3) ketamine administration following ketamine pre-treatment. Values (means ± S.E.M.) are expressed as nanomolar and represent the mean of the three stable basal levels (60 min) prior to saline or ketamine administration (25 mg/kg; i.p.). Data presented for both groups 1 and 2 shows response following saline (7 days) and data presented for group 3 shows response following ketamine (7 days).
[a] Significant difference to saline-pre-treated animals ($P > 0.05$).

Basal extracellular DOPAC and HVA levels were similar in all three groups and remained stable over the time course of the experiment (Table 1, Figs. 2 and 3). DOPAC levels gradually increased (two-fold) following ketamine in the saline-pre-treated group (Fig. 2) and remained significantly elevated for 2 h. DOPAC levels only increased significantly 40 min following ketamine in the ketamine-pre-treated group and gradually returned to pre-treatment levels (Fig. 2). Ketamine increased HVA levels (up to

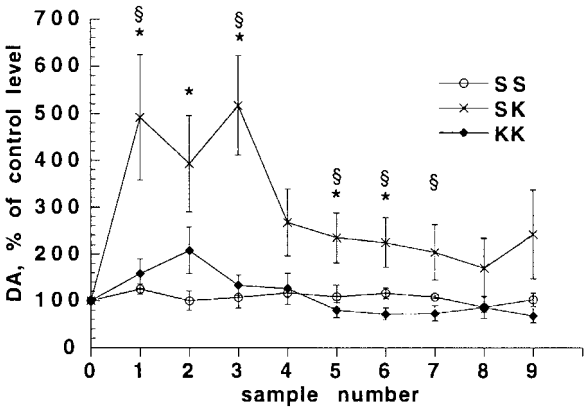

Fig. 1. Effect of ketamine administration (25 mg/kg i.p.) on extracellular dopamine in left prefronal cortex of drug naive or ketamine pre-treated (7 days, 25 mg/kg i.p. once daily). Samples were collected every 20 min following drug injections (numbers 1 to 9) and sample 0 represent basal levels prior to injections. Three groups ($n = 6$) are represented: (○) saline administration following saline pre-treatment (7 days), (×) ketamine administration following saline pre-treatment (7 days) and (♦) ketamine administration following ketamine pre-treatment (25 mg/kg i.p., daily for 7 days). * Significant ($P < 0.05$) difference between saline-ketamine (SK) and saline-saline (SS) groups. § Significant ($P < 0.05$) difference between saline-ketamine (SK) and ketamine-ketamine (KK) groups.

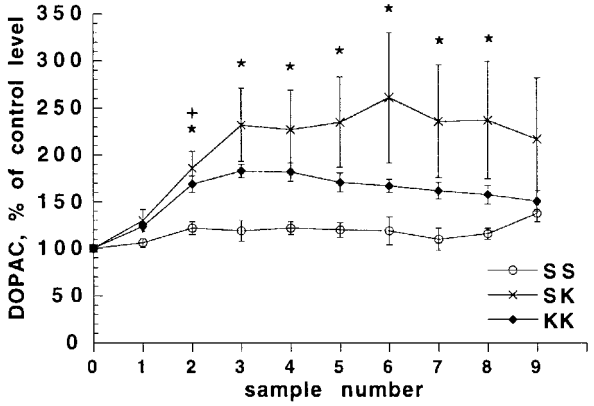

Fig. 2. Effect of ketamine administration (25 mg/kg i.p.) on extracellular DOPAC in left prefronal cortex of drug naive or ketamine pre-treated (7 days, 25 mg/kg i.p. once daily). Samples were collected every 20 min following drug injections (numbers 1 to 9) and sample 0 represent basal levels prior to injections. Three groups ($n = 6$) are represented: (○) saline administration following saline pre-treatment (7 days), (×) ketamine administration following saline pre-treatment (7 days) and (◆) ketamine administration following ketamine pre-treatment (25 mg/kg i.p., daily for 7 days). * Significant ($P < 0.05$) difference between saline-saline (SS) and saline-ketamine (SK) groups. + Significant ($P < 0.05$) difference between ketamine-ketamine (KK) and saline-saline (SS) groups.

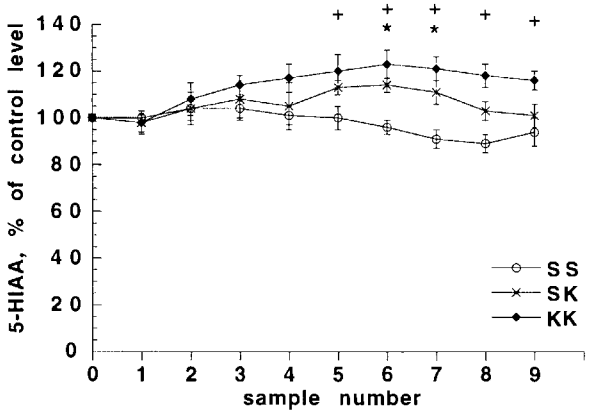

Fig. 4. Effect of ketamine administration (25 mg/kg i.p.) on extracellular 5-HIAA in left prefronal cortex of drug naive or ketamine pre-treated (7 days, 25 mg/kg i.p. once daily). Samples were collected every 20 min following drug injections (numbers 1 to 9) and sample 0 represent basal levels prior to injections. Three groups ($^{n} = 6$) are represented: (○) saline administration following saline pre-treatment (7 days), (×) ketamine administration following saline pre-treatment (7 days) and (◆) ketamine administration following ketamine pre-treatment (25 mg/kg i.p., daily for 7 days). * Significant ($P < 0.05$) difference between saline-saline (SS) and saline-ketamine (SK) groups. + Significant ($P < 0.05$) difference between saline-saline (SS) and ketamine-ketamine (KK) groups.

two-fold) following ketamine in both the saline-pre-treated and ketamine-pre-treated groups (Fig. 3).

3.2. 5-HIAA

Basal levels of 5-HIAA were similar in the three groups (Table 1). 5-HIAA gradually and transiently increased 120 and 140 min after ketamine in the saline-pre-treated group (Fig. 4) while a more prolonged increase in 5-HIAA levels was observed in the ketamine-pre-treated group.

3.3. GABA

Basal levels of GABA were similar in the three groups (Table 1) and there was no effect of ketamine on GABA levels in either the saline- or ketamine-pre-treated groups (Fig. 5).

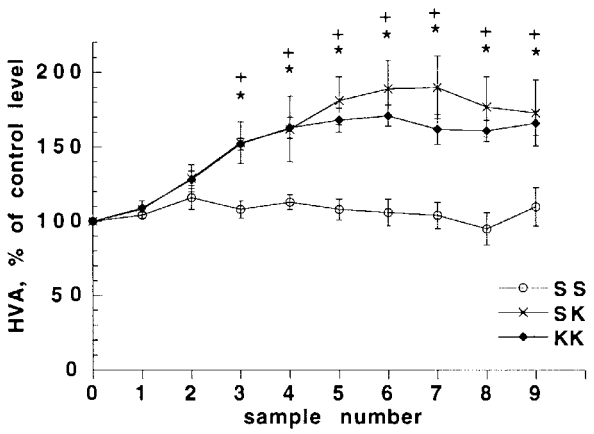

Fig. 3. Effect of ketamine administration (25 mg/kg i.p.) on extracellular HVA in left prefronal cortex of drug naive or ketamine pre-treated (7 days, 25 mg/kg i.p. once daily). Samples were collected every 20 min following drug injections (numbers 1 to 9) and sample 0 represent basal levels prior to injections. Three groups ($n = 6$) are represented: (○) saline administration following saline pre-treatment (7 days), (×) ketamine administration following saline pre-treatment (7 days) and (◆) ketamine administration following ketamine pre-treatment (25 mg/kg i.p., daily for 7 days). * Significant ($P < 0.05$) difference between saline-saline (SS) and saline-ketamine (SK) groups. + Significant ($P < 0.05$) difference between saline-saline (SS) and ketamine-ketamine (KK) groups.

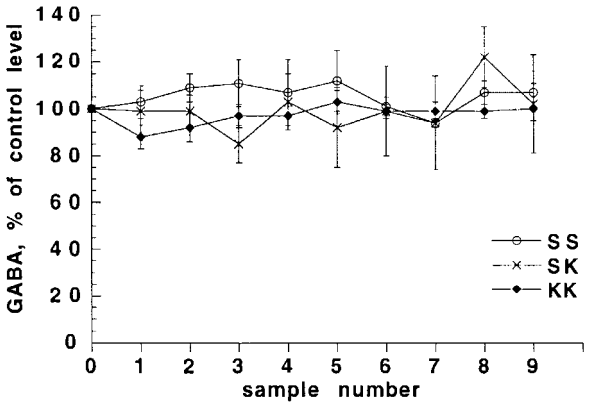

Fig. 5. Effect of ketamine administration (25 mg/kg i.p.) on extracellular GABA in left prefronal cortex of drug naive or ketamine pre-treated (7 days, 25 mg/kg i.p. once daily). Samples were collected every 20 min following drug injections (numbers 1 to 9) and sample 0 represent basal levels prior to injections. Three groups ($^{n} = 6$) are represented: (○) saline administration following saline pre-treatment (7 days), (×) ketamine administration following saline pre-treatment (7 days) and (◆) ketamine administration following ketamine pre-treatment (25 mg/kg i.p., daily for 7 days).

4. Discussion

In this study, we have shown that the non-competitive NMDA receptor antagonist ketamine modulates dopaminergic transmission in the prefrontal cortex of the awake rat. In addition, we provide indirect evidence for ketamine modulation of serotonin transmission as measured by the extracellular level of the serotonin metabolite 5-HIAA. We confirm that the extracellular in vivo level of dopamine is strongly increased 60 to 120 min following ketamine administration in drug naive rats. In addition, we provide new evidence for (a) a complete attenuation of this increase in the presence of increased basal dopamine levels and (b) an enhanced ketamine-induced increase in 5-HIAA, following ketamine pre-treatment (once daily for one week). Thus, we provide new evidence for a complex adaptation of dopamine and serotonin transmission in the prefrontal cortex following single and repeated NMDA blockade.

4.1. Basal extracellular neurotransmitter levels

Recent observations employing an identical microdialysis preparation to that used here show that extracellular dopamine levels in the prefrontal cortex are reduced by 48% and 50% by local perfusion with tetrodotoxin (TTX; 10 μM) and low Ca^{2+}, respectively, while a reduction of 33% and 20%, respectively, is observed in extracellular GABA levels (Franz, O'Connor and Ungerstedt, unpublished findings). Thus extracellular dopamine and GABA levels are both sodium and calcium sensitive and therefore to a large extent derived from impulse-dependent neuronal release.

4.2. The frontal cortex and psychosis

The frontal cerebral cortex displays physiological dysfunction in schizophrenia [3,41] and examinations of brain tissue from schizophrenic victims have provided evidence for neurobiological irregularities including abnormal excitatory neurotransmitter metabolism [36]. This is in line with the observation of lower than normal glutamate levels in the cerebrospinal fluid of schizophrenic patients [13] and decreased release of glutamate from synaptosomal preparations of brain tissue from schizophrenic victims [31]. Abnormal glutamate function in the frontal cortex is also implied in schizophrenia by changes in glutamate receptor levels [4].

4.3. Dopamine, NMDA receptors and schizophrenia

The function of the frontal cortex is important for the understanding of the mechanisms of action of NMDA receptor antagonists in inducing schizophreniform psychosis. The characterization of the psychotomimetic actions of NMDA antagonists was first facilitated by the increase in abuse of phencyclidine (PCP, angel dust) during late 1960s and 1970s [8]. PCP and later ketamine was observed to induce a psychotic state expressing both positive symptoms (hallucinations and delusions) and negative symptoms (emotional withdrawal, motor retardation) [8]. The psychotomimetic actions of PCP and ketamine-like drugs, which are potent NMDA antagonists, have become powerful tools in evaluating possible involvement of glutamatergic transmission in psychotic disorders such as schizophrenia [14,15] and psychosis like effects evoked in healthy volunteers [14]. Interestingly, in situ hybridization histochemistry in postmortal tissue sections of brains from schizophrenic victims show a region-specific abundance of an NMDA receptor sub-unit (NR2D) mRNA in the frontal cortex [1]. This may possibly be related to an abnormal NMDA receptor function in the frontal cortex in some schizophrenics. In addition, evidence have been provided for deficient growth of glutamatergic neurons in schizophrenic prefrontal cortex [30]. This is in agreement with deficient NMDA receptor regulation as reported by Akbarian and collaborators [1] and indicate deficient glutamate transmission in schizophrenic prefrontal cortex.

Dopaminergic transmission has for many years been in focus in theories of schizophrenia pathophysiology [6]. One argument for this is the psychotomimetic actions associated with prolonged administration of the dopamine releasing drug amphetamine. Accordingly, increased dopaminergic transmission is known to evoke psychotic reactions [18] and repeated doses of amphetamine also induce behavioral sensitization to dopamine receptor stimulation [28]. However, it has become increasingly evident that schizophrenic and other psychotic disorders are complex in nature and that a variety of neurotransmitters including glutamate are involved and interact with dopamine [10,35]. Thus, repeated NMDA receptor blockade is followed by behavioral tolerance in a continuous self-administration paradigm but most observations are from animal experiments [43]. Although NMDA antagonists are known to be abused by humans the cognitive effect of repeated doses remains obscure [43]. However, schizophrenics exposed to this class of drugs run a high risk of severe psychiatric morbidity [43].

In line with earlier findings [39], our results indicate that ketamine induces a transient increase in extracellular dopamine in the prefrontal cortex in drug naive animals over the first 1 to 2 h following drug injection. Similar effects are observed following administration of MK-801 [40] or PCP [7]. It is interesting to compare these findings with the transient psychotomimetic effects of this class of drugs in a clinical setting. Ketamine-induced psychosis-like cognitive deficits in healthy volunteers also last for 1 to 2 h [14]. However, occasionally a more complex temporal relationship is observed, particularly in relation to a worsening of psychosis in schizophrenics [15] and to recurrent psychotic episodes for several days after ketamine administration. In general, schizophrenics express a transient

dose-dependent worsening of psychosis within 1 h following ketamine administration and the symptoms are similar to those of active disease episodes [15]. Although the pharmacokinetics of ketamine may be different between rats and humans it is tempting to hypothesize that the rapid dose-dependent ketamine-induced psychotic reactions in schizophrenics are related to increased dopamine release in the frontal cortex. Thus, the increase in dopamine release following a single exposure to ketamine is in general agreement with the dopamine hypothesis of schizophrenia whereby the symptoms of schizophrenia are a manifestation of functional hyperactivity of mesocortical dopamine transmission.

4.4. NMDA receptor antagonism and dopamine release in the prefrontal cortex

Dopamine in the prefrontal cortex is derived from afferent mesocortical projections with cell bodies in the midbrain [2]. Prefrontal cortex dopamine release in vivo is stimulated by local non-NMDA receptor stimulation in the nerve terminal region, whereas NMDA receptor stimulation has a weak but unclear inhibitory effect [9]. The influence of cortical NMDA receptors on dopaminergic transmission in this brain region is of particular interest since dopamine receptors modulate working memory functions [42] which are affected in schizophrenia [25,33].

It this study we can confirm earlier findings [7,39,40] of a strong potentiation of dopamine release in prefrontal cortex by ketamine in drug naive animals. Unexpectedly, we also obtained new evidence for a complete attenuation of this increase following ketamine pre-treatment once daily for one week. However, ketamine pre-treatment increased base-line extracellular dopamine levels. Consequently, the absolute levels of dopamine measured the first few samples following ketamine injections was not significantly affected by the pre-treatment, but the drug-induced increase was due to the different base-line levels. It appears from our results that the dynamics of dopaminergic transmission in the prefrontal cortex may be reduced by the ketamine pre-treatment.

It is known that decreased dopamine receptor stimulation due to decreased endogenous levels of the ligand is followed by dopamine receptor sensitization [37]. It has also been shown that repeated administration of ketamine to rats produces a postsynaptic dopamine receptor sensitization as shown by apomorphine-induced behavioral activation [16], without any evidence for a change in dopamine turn-over in the striatum. The dopamine receptor sensitization reported in prefrontal cortex following repeated ketamine administration is interesting and remains to be explored in more detail. However, in addition to the attenuation of ketamine-induced dopamine release we also report an increase in basal dopamine levels in the ketamine-pre-treated animals. In light of this new finding it is unclear whether dopamine receptors in the prefrontal cortex are exposed to decreased or increased stimulation by endogenous dopamine. The attenuation of dopaminergic transmission in the prefrontal cortex following repeated exposure is accompanied by an attenuated ketamine-induced increase in DOPAC but not a concomitant change in basal pre-treatment DOPAC levels. HVA levels increase by ketamine following both pre-treatments. Consequently, measurements of dopamine metabolites do not provide additional support for changes in dopaminergic transmission by the ketamine pre-treatment.

Interestingly, dopamine transmission in the prefrontal cortex is of critical importance for working memory and an excess of dopaminergic transmission in this part of the brain interferes with such cognitive functions [42]. Thus, the increased basal levels of dopamine we have observed following repeated ketamine administration may possibly interfere with cognitive functions, something that needs to be further explored.

Additional aspects of monoaminergic transmission are also affected by ketamine. A concentration-dependent inhibition of high-affinity transport of both dopamine and serotonin into synaptosomes prepared from rat cerebral cortex has been reported [32], but this was only studied in tissue from reserpine-pre-treated animals without previous exposure to ketamine. The increased basal level of dopamine following ketamine pre-treatment may thus to some extent be due to remaining changes in cellular dopamine up-take mechanisms induced by the drug.

4.5. NMDA receptor antagonism and serotonin in the prefrontal cortex

Inhibition of neuronal serotonin uptake follows acute exposure of ketamine in synaptosomal preparation of rat cerebral cortex [32]. Acute MK-801 administration induces an increase in tissue levels of 5-HIAA in rat frontal cortex [19]. Behavioral effects indicate that 5-HT_{1A} receptors modulate the behavioral effect of NMDA antagonism by MK-801 [20]. More recently, evidence from in vivo microdialysis experiments suggest a specific NMDA receptor-mediated effect on serotonergic transmission in the prefrontal cortex. Thus, MK-801 elicits a TTX-sensitive increase in extracellular 5-HIAA in rat prefrontal cortex [11]. The present finding of a ketamine-induced increase in extracellular 5-HIAA in the drug naive group confirms this. In addition, we show that, in contrast to that observed for dopamine, DOPAC and 5-HIAA levels may be further increased by ketamine in the ketamine-pre-treated animals indicating a possible sensitization of serotonergic transmission to ketamine following repeated administration and suggests an altered serotonin to dopamine ratio following repeated ketamine in the prefrontal cortex in the chronically treated group. A disturbance in the serotonin to dopamine ratio may be relevant for psychotogenic properties of ketamine, since a decrease in dopamine and an increase in serotonin transmission in the frontal cortex are supposed to be important for cognitive defects observed in schizophrenia [29].

4.6. NMDA receptor antagonism and GABA in the prefrontal cortex

Cortical GABAergic transmission is also under local NMDA receptor control. PCP inhibits the NMDA-stimulated release of [^{3}H]GABA from cultured rat cortex neurons [5]. A single dose of MK-801 decreases the level of the mRNA GAD_{65} in rat frontal and cingulate cortex [17]. A decreased formation of another isoform of GAD namely GAD_{67} has also been observed within 2 days of continuous infusion of MK-801 [27]. Thus, these findings led us to expect a change in prefrontal cortical GABA release following single and repeated ketamine administration. Unexpectedly, the present investigation provided little evidence for a ketamine-induced change in extracellular GABA in the prefrontal cortex in the presence of robust changes in dopamine release. However, ketamine may influence cortical GABA release indirectly as regulated by local dopamine or serotonin receptors.

4.7. Conclusions

Using in vivo microdialysis in awake unrestrained animals we provide new evidence for a complex adaptation of dopaminergic and serotonergic transmission in rat prefrontal cortex following repeated ketamine-induced NMDA receptor blockade. Whereas ketamine-induced prefrontal cortical increase in dopamine release is completely attenuated by ketamine pre-treatment, serotonin activity, as reflected by 5-HIAA levels seems to be facilitated by prolonged NMDA receptor blockade. Our findings suggest that repeated ketamine administration may reduce the dynamics of cortical dopaminergic transmission. Thus, in contrast to dopamine levels during ketamine exposure, basal extracellular dopamine levels are increased by previous ketamine pre-treatment while basal 5-HIAA levels remains unchanged. Prefrontal cortical GABA release appears to be unaffected either by a single or repeated ketamine administration. It remains to be clarified to what extent the observed shift in ratio of serotonin to dopamine transmission in the prefrontal cortex is relevant for psychotropic effects following repeated ketamine administration to humans.

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