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Role of excitatory amino acids in the ventral tegmental area for central actions of non-competitive NMDA-receptor antagonists and nicotine

T. H. Svensson, J. M. Mathé, G. G. Nomikos, and **B. Schilström**

Department of Physiology and Pharmacology,
Section of Neuropsychopharmacology, Karolinska Institutet,
Stockholm, Sweden

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Summary. The putative role of non-NMDA excitatory amino acid (EAA) receptors in the ventral tegmental area (VTA) for the increase in dopamine (DA) release in the nucleus acumbens (NAC) and the behavioural stimulation induced by systemically administered dizocilpine (MK-801) was investigated. Microdialysis was utilized in rats with probes in the VTA and NAC. The VTA was perfused with the AMPA and kainate receptor antagonist CNQX (0.3 or 1.0 mM) or vehicle and dialysates from the NAC were analyzed with high-performance liquid chromatography for DA. Forty min after onset of CNQX or vehicle perfusion of the VTA MK-801 (0.1 mg/kg) was injected subcutaneously (sc). Subsequently, typical MK-801 induced behaviours were assessed. The MK-801 induced hyperlocomotion was associated with a 50% increase of DA levels in NAC dialysates. Both the MK-801 evoked hyperlocomotion and DA release in the NAC were effectively antagonized by CNQX perfusion of the VTA. However, by itself the CNQX or vehicle perusion of the VTA did not affect DA levels in NAC or the rated behaviours. The results indicate that MK-801 induced hyperlocomotion and increased DA release in the NAC are largely elicited within the VTA via activation of non-NMDA EAA receptors, tentatively caused by locally increased EAA release. In contrast, the enhanced DA output in the NAC induced by systemic nicotine (0.5 mg/kg sc) was not antagonized by intra VTA infusion of CNQX (0.3 or 1.0 mM), but instead by infusion of the NMDA receptor antagonist AP-5 (0.3 or 1.0 mM) into the VTA, which by itself did not alter DA levels in the NAC. Thus, the probably indirect, EAA mediated activation of the mesolimbic DA neurons in the VTA by MK-801 and nicotine, respectively, seems to be mediated via different glutamate receptor subtypes.

Keywords: MK-801 – Locomotion – Dopamine – Ventral tegmental area – Glutamate receptors

Introduction

When administered systemically to rodents, non-competitive NMDA-receptor antagonists, such as dizocilpine (MK-801) evoke locomotor stimulation, but also head weaving, sniffing and, particularly in high doses, ataxia (Löscher et al., 1992). The hyperlocomotion by MK-801 appears to depend on endogenous dopamine (DA) since it can be antagonized by catecholamine depletion or by DA receptor antagonists (Clineschmidt et al., 1982; Criswell et al., 1993; Willins et al., 1993). The behavioural stimulation of the NMDA-receptor antagonists can also be antagonized by local, bilateral microinjections of the γ-amino-butyric-acid $(GABA)_B$ receptor agonist baclofen into the ventral tegmental area (VTA; Narayanan et al., 1996), which inhibits DA neuronal activity (Grace and Bunney, 1980). Thus, activation of midbrain DA neurons in the VTA appears to play a crucial rôle in the behavioural stimulation by systemically administered, non-competitive NMDA-receptor antagonists.

In accordance with the above findings and considerations, previous electrophysiological experiments have revealed that systemically, but not locally administered MK-801 as well as phencyclidine, another non-competitive NMDA-receptor antagonist, indeed activate VTA DA neuronal activity (French 1986; Pawlowski et al., 1990; Zhang et al., 1992; Murase et al., 1993). Specifically, the drugs induce a high frequency firing pattern in the DA cells, although burst firing was not increased (Mathé et al., 1996b; see Svensson et al., 1997), and recent microdialysis studies in freely moving animals have demonstrated that systemic MK-801 evokes a long-lasting increase in DA output within the terminal regions of the mesocorticolimbic and nigroneostriatal DA systems (Wolf et al., 1993; Wedzony et al., 1993; Mathé et al., 1996a; Miller and Abercrombie, 1996). These effects of NMDA-receptor antagonists have to be indirect, since unequivocal evidence demonstrates that various excitatory amino acid (EAA) agonists, when locally applied into the VTA augment central DA activity (Scarnati and Pacitti, 1982; Kalivas et al., 1989; Chergui et al., 1993; Schilström et al., 1997) and that microiontophoretic application of the EAA-antagonists does not affect the DA cellular activity. Thus, the stimulation of mesolimbic DA activity by MK-801 is probably mediated via afferents to DA neurons in the VTA.

Our electrophysiological recordings revealed an increased firing pattern in VTA DA cells induced by PCP or MK-801, which using the criteria of Grace and Bunney (1984) was interpreted as continuously bursting (Murase et al., 1993). However, this pattern was devoid of typical post-burst pauses as well as the characteristic, progressive decline in spike amplitude during each burst. Instead, our oscillographic recordings revealed a firing pattern almost identical to that seen after local application of quisqualate or kainate onto the DA neurons as we have previously observed (Chergui et al., 1993). Thus, the indirect stimulation of mesolimbic DA neurons by the systemically administered NMDA-receptor antagonists might reflect activation of AMPA and/or kainate receptors within the VTA, tentatively due to local release of EAAs, and this effect of the glutamate antagonists may secondarily cause both stimulation of DA release in the nucleus accumbens (NAC) as well as hyper-

locomotion. The present paper reviews our experiments to test this notion and includes a brief comparison with nicotine, which also stimulates mesolimbic DA activity and has therapeutic potential in movement related disorders, such as Parkinsons's disease and Tourette's disorder.

Experimental methods and design

Male rats, weighing 250–350 g were used both for biochemical and behavioural experiments. A dual-probe microdialysis technique was used in freely moving animals with local perfusion of the VTA with the AMPA and kainate receptor antagonist CNQX and simultaneous measurement of extracellular DA levels in the NAC during systemic administration of MK-801. Typical MK-801 induced behavioural effects, e.g. hyperlocomotion and head weaving were also assessed. The dual probe microdialysis experiments followed procedures described previously (Nisell et al., 1994). Briefly, following anesthesia with sodium pentobarbital, 60 mg/kg i.p., rats were mounted in a stereotaxic frame with their body temperature maintained at 37°C with a heating pad. In each rat one vertical probe of concentric type was implanted into the NAC and and a second vertical probe was implanted within the ipsilateral VTA. Dialysis occurred through a 2.25 mm semipermeable membrane for the NAC probe and through a 1.0 mm membrane for the VTA probe (copolymer of acrylonitrile and sodium methallyl sulphonate, i.d.= 0.24 mm, 40,000 Da molecular weight cutoff, AN69 Hospal). All experiments were conducted approximately 48 h after surgery. About 4 h after commencement of perfusion rats obtained either vehicle or CNQX through the VTA probe. Forty min later they were subcutaneously injected with MK-801 (0.1 mg/kg or saline, N = 5–7 rats per group). Microdialysis was performed using automated on-line sampling. Upon completion of experiments, rats were killed by an overdose of sodium pentobarbital, their brains were removed and stored in 5% formaldehyde and 25% sucrose. After sectioning on microtome and staining, each brain was examined for probe placement. DA as well as its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were determined by high-performance liquid chromatography (HPLC) with electrochemical detection (see Nomikos et al., 1994). Behavioural experiments started 10 min after systemic (MK-801) or local (CNQX) drug administration and involved continuous ratings of characteristic MK-801 induced behaviours by two independent observers. The time during which rats exhibited various behaviours was measured and included the following behaviours: Locomotor activity, ipsi- and contralateral turning, sniffing, head weaving and ataxia. Ataxia was defined as tottering of the hindquarters, abduction and dragging of hindlimbs, flat body posture, and loss of balance. Data were analyzed utilizing Turbochrom software (Perkin Elmer) and statistical evaluations were performed using the Statistica software suite (StatSoft Inc.). Temporal changes in DA output were assessed by one-or two-way ANOVA followed by the post-hoc Newman-Keuls test. (For methodological references, see Svensson et al., 1997).

Results and discussion

CNQX or vehicle perfusion of the VTA did not affect dialysate concentrations of DA, DOPAC or HVA. Systemic MK-801 administration (0.1 mg/kg) evoked a significant and progressive increase in DA levels in dialysates reaching a maximum of 154 ± 17% during the 80 min sampling period ($p < 0.001$, $n = 7$). Also dialysate levels of DOPAC and HVA increased significantly with a time course essentially parallel to that of DA. CNQX perfusion of the VTA at a concentration of 1 mM completely abolished MK-801 induced

increases in dialysate concentration of DA, whereas a CNQX concentration of 300 μM caused a partial antagonistic action. Also the MK-801 induced increases in DOPAC and HVA concentrations were antagonized. Locomotor activity or other behavioural signs were not evident before the injection of MK-801 (rats were resting or asleep), and neither vehicle or CNQX perfusion of the VTA or subcutaneous injections of 0.9% saline (1 ml/kg) caused any visible change in behaviour of the rats. In contrast, MK-801 administration during vehicle perfusion of the VTA caused long-lasting locomotor stimulation ($n = 10$), starting about 10 min after injection, with a duration of 84.0 $\pm$ 8.3 min. Also pronounced head weaving, sniffing and ipsi- and contralateral turning was observed, but only very limited ataxia (in 4 out of 10 animals). Perfusion of the VTA with CNQX caused significant reduction of the MK-801 induced locomotor stimulation at both concentrations. Specifically, the duration of the behavioural stimulation was significantly ($p < 0.001$) reduced from 84.0 $\pm$ 8.3 min to 20 $\pm$ 10 min (300 μM of CNQX) and to 13 $\pm$ 6 min (1 mM). Head weaving or ataxia were, in contrast, not affected.

Our results strongly support previous behavioural experiments in the rat indicating that the locomotor stimulation by low doses of MK-801 is dependent on DA in brain and that it is elicited within the VTA. Moreover, the augmented DA output in the NAC is in all probability related to, and dependent on, the previously demonstrated hyperactivity of the VTA DA neurons induced by MK-801. Since the effect was antagonized by local intra-VTA administration of CNQX, the evoked DA release seems to be critically dependent on activation of AMPA and/or kainate receptors in the VTA. These results suggest, in turn, that systemic MK-801 administration may increase EAA release in the VTA, which preliminary experiments actually indicate (Svensson et al., 1997). Our data thus clearly support the notion, that the locomotor stimulation by low doses of MK-801, i.e. 0.1 mg/kg in the rat, which is not associated with significant ataxia, is indeed causally related to an increased DA release in the NAC. The results are in consonance with data showing that systemic administration of the AMPA and kainate receptor antagonist GYKI 52466 also antagonizes MK-801 induced locomotor stimulation as well as the concomitantly increased DA turnover in the NAC (Bubser et al., 1995) and suggest, indirectly, that this antagonism may well take place within the VTA. Since other behavioural effects of MK-801, such as head weaving, were not antagonized by CNQX, these may largely be unrelated to the evoked DA release in the NAC. Recent results from our laboratory indicate that also the nicotine induced, increased DA output in the NAC may involve local glutamate release within the VTA (Schilström et al., 1997). This effect of systemic nicotine in contrast to MK-801, could be largely antagonized by infusion of the competitive NMDA-receptor antagonist, AP-5, into the VTA. In contrast, infusion of CNQX was ineffective. Consequently, the probably indirect, glutamate mediated VTA-DA neuronal activation by MK-801 and nicotine seems to be mediated via different glutamate receptor subtypes. Therefore, the two central stimulants induce clearly different firing patterns in the mesolimbic DA neurons: In contrast to the continuous, high frequency pattern caused by the NMDA receptor antagonist (cf. above),

nicotine essentially augments the physiological, variable burst firing pattern of the DA cells. In addition, systemic MK-801 has been reported to abolish burst firing in mesocortical DA neurons (Murase et al., 1993), in all probability reflecting their predominantly NMDA receptor mediated regulation (Kalivas et al., 1989). This effect may contribute to cognitive dysfunctions associated with the NMDA receptor antagonists that are not obtained with nicotine, which, if anything, may act as a cognitive enhancer.

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Authors' address: Prof. T. H. Svensson, Department of Physiology and Pharmacology, Section of Neuropsychopharmacology, Karolinska Institutet, S-17177 Stockholm, Sweden.

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