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ACTIVATION OF DOPAMINERGIC NEUROTRANSMISSION IN THE MEDIAL PREFRONTAL CORTEX BY *N*-METHYL-D-ASPARTATE STIMULATION OF THE VENTRAL HIPPOCAMPUS IN RATS

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Abstract—Many behavioral functions—including sensorimotor, attentional, memory, and emotional processes—have been associated with hippocampal processes and with dopamine transmission in the medial prefrontal cortex (mPFC). This suggests a functional interaction between hippocampus and prefrontal dopamine. The anatomical substrate for such an interaction is the intimate interconnection between the ventral hippocampus and the dopamine innervation of the mPFC.

The present study yielded direct neurochemical evidence for an interaction between ventral hippocampus and prefrontal dopamine transmission in rats by demonstrating that subconvulsive stimulation of the ventral hippocampus with *N*-methyl-D-aspartate (NMDA; 0.5 μg/side) activates dopamine transmission in the mPFC. Postmortem measurements revealed that bilateral NMDA stimulation of the ventral hippocampus, resulting in locomotor hyperactivity, increased the homovanillic acid/dopamine ratio, an index of dopamine transmission, in the mPFC; indices of dopamine transmission in any of five additionally examined forebrain regions (amygdala, nucleus accumbens shell/core, lateral prefrontal cortex, caudate putamen) were unaltered. *In vivo* microdialysis measurements in freely moving rats corroborated the suggested activation of prefrontal dopamine transmission by demonstrating that unilateral NMDA stimulation of the ventral hippocampus increased extracellular dopamine in the ipsilateral mPFC.

The suggested influence of the ventral hippocampus on prefrontal dopamine may be an important mechanism for hippocampo–prefrontal interactions in normal behavioral processes. Moreover, it indicates that aberrant hippocampal activity, as found in neuropsychiatric diseases, such as schizophrenia and mood disorders, may contribute to disruption of certain cognitive and emotional functions which are extremely sensitive to imbalanced prefrontal dopamine transmission. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: dopamine, *in vivo* microdialysis, metabolites, postmortem neurochemistry, *N*-methyl-D-aspartate, functional interaction.

Interactions between hippocampus and prefrontal cortex (PFC) have recently received growing interest, both for their role in normal memory functions and for their possible relevance concerning the pathophysiology of neuropsychiatric diseases, such as schizophrenia and depression (Bertolino et al., 1998; Csernansky et al., 1998; Fletcher, 1998; Grace, 2000; Laroche et al., 2000; Mayberg et al., 2000; Simons and Spiers, 2003; Rocher et al., 2004; Jodo et al., 2004). Moreover, if considered together, behavioral studies, investigating the effects of manipulations of the hippocampus and of prefrontal dopamine (DA) transmission in rats, suggest that hippocampal activity and prefrontal DA may interact in several behavioral processes. For example, the DA-dependent drive of locomotion by activity of the ventral hippocampus (VH; Yang and Mogenson, 1987; Wu and Brudzynski, 1995; Bardgett and Henry, 1999; Bast et al., 2001b–d; Bast and Feldon, 2003), which has mainly been associated with a stimulatory influence of ventral hippocampal activity on the meso-accumbens DA system (Blaha et al., 1997; Brudzynski and Gibson, 1997; Brenner and Bardgett, 1998; Legault and Wise, 1999; Legault et al., 2000; Taepavarapruk et al., 2000; Mitchell et al., 2000; Moss et al., 2003), may also involve an enhancement of DA transmission in the medial PFC (mPFC), given that DA in the mPFC facilitates locomotor activity (Beninger et al., 1990; Bast et al., 2002b; Pezze et al., 2003). Additionally, both the hippocampus and prefrontal DA transmission have been implicated in attentional (Robbins, 2000, 2002; Le Pen et al., 2003) and in anxiety- and fear-related processes (Bast et al., 2001a,c, 2003; Zhang et al., 2001; Pezze et al., 2003; Wiltgen and Fanselow, 2003; Bannerman et al., 2004; Maren and Holt, 2004; Pezze and Feldon, 2004; Shah et al., 2004; Wall et al., 2004). Finally, disconnection of the VH from prefrontal DA transmission impaired the use of one-trial place memory (Seamans et al., 1998), an aspect of episodic-like memory depending on hippocampal synaptic plasticity (Steele and

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Abbreviations: AMYG, amygdala; ANOVA, analysis of variance; CP, caudate putamen; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HIAA, 5-hydroxyindolacetic acid; HPLC, high-performance-liquid-chromatography; HVA, homovanillic acid; lPFC, lateral prefrontal cortex; mPFC, medial prefrontal cortex; NAC, nucleus accumbens; NMDA, *N*-methyl-D-aspartate; PFC, prefrontal cortex; SQRT, square-root; VEH, vehicle; VH, ventral hippocampus; VTA, ventral tegmental area; 5-HT, serotonin.

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Morris, 1999; Morris et al., 2003; Bast et al., 2004). In addition to the behavioral evidence, the close anatomical interconnection between hippocampus and prefrontal DA innervation suggests that both may functionally interact. This interconnection has been thoroughly characterized in rats and involves direct ventral hippocampal projections to the mPFC, terminating in close apposition to dopaminergic terminals, and indirect projections of the VH to the origin of the prefrontal DA innervation in the ventral tegmental area (VTA; Thierry et al., 2000).

Despite the above reviewed behavioral and anatomical evidence, neurochemical studies investigating hippocampal interactions with DA systems have mainly focused on the effect of ventral hippocampal stimulation on DA transmission in the NAC (Blaha et al., 1997; Brudzynski and Gibson, 1997; Legault and Wise, 1999; Legault et al., 2000; Taepavarapruk et al., 2000; Mitchell et al., 2000; Moss et al., 2003). Here, we report direct neurochemical evidence that subconvulsive stimulation of the VH by *N*-methyl-D-aspartate (NMDA, 0.5 μg/side) activates DA transmission in the mPFC of Wistar rats. Experiment 1 examined the effects of bilateral NMDA stimulation of the VH on postmortem neurochemical measures of DA transmission in six forebrain areas and found evidence for a selective increase of DA utilization in the mPFC. The suggested activation of prefrontal DA transmission by ventral hippocampal stimulation was then corroborated in experiment 2 which demonstrated that unilateral NMDA stimulation of the VH increases extracellular DA concentrations in the ipsilateral mPFC using *in vivo* microdialysis in freely moving rats.

EXPERIMENTAL PROCEDURES

Subjects

Male Wistar rats (Zur:WIST[HanIbm]; Research Unit Schwerzenbach, Schwerzenbach, Switzerland), weighing about 250 g and about 10 weeks old at the time of surgery, were used for the experiments (experiment 1, 18 rats; experiment 2, 19 rats). Rats were housed under a reversed light/dark cycle (lights on: 19:00–07:00 h) in a temperature- (21±1 °C) and humidity- (55±5%) controlled room and allowed free access to food and water. Before surgery, rats were housed in groups of four per cage; after surgery, they were individually caged. Beginning 3 days before surgery and thereafter until the beginning of the experiments, all rats were handled daily. All experimental procedures were carried out in the dark phase of the cycle. The Principles of Laboratory Animal Care (NIH publication no. 86–23, revised 1996) and Swiss regulations for animal experimentation were followed. All efforts were made to minimize the number of animals used and their suffering.

Implantation of infusion and microdialysis guide cannulae

Rats were anesthetized with Nembutal (sodium pentobarbital, 50 mg/kg; Abbott Laboratories, North Chicago, IL, USA), and their head was placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). After application of a local anesthetic (lidocaine spray), the scalp was incised to expose the skull, and bregma and lambda were aligned in the same horizontal plane. The rats were implanted with infusion guide cannulae (26 gauge) aiming at the following coordinates (in mm) above the VH: −5.2 posterior and ±5.0 lateral to bregma, and 5.0 ventral from dura. Infusion cannulae were implanted bilaterally for experiment 1 and unilaterally into the left hemisphere for experiment 2. Rats in experiment 2 were additionally implanted with a microdialysis guide cannula (MAB 4.9.IC; Microbiotech, Stockholm, Sweden) aiming at the following coordinates above the left mPFC (in mm): 3.0 anterior and 0.6 lateral to bregma, and 2.0 ventral to skull surface. The guide cannulae, three to four small anchor screws, and, in experiment 2, a screw to attach the swivel for the microdialysis measurements were fixed by dental cement. The infusion guide cannulae were closed with stylets (34 gauge) projecting 0.5 mm into the brain, and the microdialysis guides were closed with flush dummy probes (MAB 4.9.IC; Microbiotech, Stockholm, Sweden) to prevent occlusion. At least 6 days were allowed after surgery before start of the experiments.

Intracerebral microinfusion

Rats were manually restrained and the stylets removed from the guide cannulae. Infusion cannulae (34 gauge, stainless steel), connected via flexible polyetheretherketone (PEEK) tubing to 10-μl Hamilton microsyringes mounted on a microinfusion pump (KD Scientific or WPI sp200i, Holliston, MA, USA), were then inserted into the guide cannulae. The tips of the infusion cannulae protruded 1.5 mm from the guide cannulae into the VH, thus aiming at a final dorsoventral coordinate of 6.5 mm below dura. NMDA (0.5 μg; $C_5H_9NO_4$; Sigma, Switzerland) in 0.5 μl vehicle (0.9% saline) or 0.5 μl vehicle (VEH) only per side were infused into the VH at a rate of 0.5 μl/min. To allow for absorption of the infusion bolus by the brain tissue, infusion cannulae were left in the brain for 60 s after infusion before being replaced by the stylets. NMDA solutions (1 mg/ml) for infusions were freshly prepared on the day of infusion. The dose of NMDA (0.5 μg/ 0.5 μl) was based on previous studies, in which it yielded marked behavioral effects without inducing convulsions when bilaterally infused into the VH (Bast et al., 2001d; Zhang et al., 2001, 2002).

High-performance-liquid-chromatography (HPLC) analysis: general procedures

A HPLC system coupled with an amperometric electrochemical detector (Decade; Antec, Leyden, The Netherlands) was used to determine concentrations of monoamines and metabolites in tissue and microdialysis samples. Samples were manually injected via a seven-port injection valve (Model 7725i; Rheodyne, Berkeley, CA, USA) and a 100-μl (experiment 1) or 50-μl (experiment 2) injection loop, and separated on a reversed-phase column (experiment 1: 100×3 mm glass column, Chromsphere 5B; Varian, Palo Alto, CA, USA; experiment 2: 125×3 mm glass column, Nucleosil 120–3 C18; Knauer, Berlin, Germany). Column and detector cell were maintained at 30 °C by a column oven as part of the electrochemical detector. An HPLC pump (Waters 515; Waters, Milford, MA, USA) connected to a pulse dampener and a degasser was used to pump the mobile phase (see below) through the system. The working potential of the electrochemical glassy carbon flow cell (VT-03; Antec) was +0.75 V vs an Ag/AgCl reference electrode. Chromatograms were recorded and analyzed with a PC equipped with dedicated software (experiment 1: Millenium; Millipore Corporation, Bedford, MA, USA; experiment 2: Chromeleon; Dionex, Olten, Switzerland). Substance amounts which yielded a detector signal corresponding to three times noise level were considered at detection limit.

Experiment 1: postmortem study of alterations in forebrain DA transmission after NMDA stimulation of the VH

Postmortem neurochemistry was used to simultaneously assess changes in DA transmission in several forebrain sites following bilateral NMDA stimulation of the VH (0.5 μg/side) in Wistar rats. This hippocampal manipulation induces marked behavioral effects