Dopamine Depresses Excitatory Synaptic Transmission Onto Rat Subicular Neurons Via Presynaptic D1-Like Dopamine Receptors

JOACHIM BEHR,1 TENGIS GLOVELI,1 DIETMAR SCHMITZ,2 AND UWE HEINEMANN1

1Department of Neurophysiology, Institute of Physiology, University Hospital Charité, Humboldt University Berlin, 10117 Berlin, Germany; and 2Department of Cellular and Molecular Pharmacology, University of California, San Francisco, California 94141-0450

Received 10 November 1999; accepted in final form 22 March 2000

Behr, Joachim, Tengis Gloveli, Dietmar Schmitz, and Uwe Heinemann. Dopamine depresses excitatory synaptic transmission onto rat subicular neurons via presynaptic D1-like dopamine receptors. J Neurophysiol 84: 112–119, 2000. Schizophrenia is considered to be associated with an abnormal functioning of the hippocampal output. The high clinical potency of antipsychotics that act as antagonists at dopamine (DA) receptors indicate a hyperfunction of the dopaminergic system. The subiculum obtains information from area CA1 and the entorhinal cortex and represents the major output region of the hippocampal complex. To clarify whether an enhanced dopaminergic activity alters the hippocampal output, the effect of DA on alveus- and perforant path–evoked excitatory postsynaptic currents (EPSCs) in subicular neurons was examined using conventional intracellular and whole cell voltage-clamp recordings. Dopamine (100 μM) depressed alveus-elicited (S)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor–mediated EPSCs to 56 ± 8% of control while perforant path–evoked EPSCs were attenuated to only 76 ± 7% of control. Dopamine had no effect on the EPSC kinetics. Dopamine reduced the frequency of spontaneous miniature EPSCs without affecting their amplitudes. The sensitivity of subicular neurons to the glutamate receptor agonist (S)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid was unchanged by DA pretreatment, excluding a postsynaptic mechanism for the observed reduction of excitatory synaptic transmission. The effect of DA on evoked EPSCs was mimicked by the D1 receptor agonist SKF 38393 and partially antagonized by the D1 receptor antagonist SCH 23390. While the D2 receptor agonist quinolone failed to reduce the EPSCs, the D2 receptor antagonist sulpiride did not block the action of DA. The results indicate that DA strongly depresses the hippocampal and the entorhinal excitatory input onto subicular neurons by decreasing the glutamate release following activation of presynaptic D1-like DA receptors.

INTRODUCTION

Although the hippocampal complex is not classified as a part of the dopaminergic neuronal system, previous studies have shown that dopamine (DA) acts as a neurotransmitter in the hippocampus (Hsu 1996; Otmakhova and Lisman 1999). This idea was given support by anatomical and biochemical studies suggesting that the hippocampal area CA1 and the subiculum receive a dense mesencephalic dopaminergic projection from the ventral tegmental area (VTA) (Descarries et al. 1987; Gasbarri et al. 1994; Verney et al. 1985) and express high levels of D1- and D2-like DA receptors (Bruinink and Bischoff 1993; Fremeau et al. 1991; Martres et al. 1985; Meador-Woodruff et al. 1994).

The subiculum controls most of the entorhinal-hippocampal output. It receives strong input from area CA1 (Amaral et al. 1991; Finch and Babb 1981; Tamamaki and Nojyo 1990) and layer III of the entorhinal cortex (Steward and Scoville 1976; Witter 1993) and relays information to other regions of the subicular complex, to the deep and superficial layers of the entorhinal cortex, as well as to a variety of distant cortical and subcortical structures (Witter 1993; Witter and Groenewegen 1990; Witter et al. 1990). The subicular projection to the nucleus accumbens has received increasing attention as alterations of its activity seem to be involved in schizophrenia (Gray 1998; Gray et al. 1995a; Greene 1999; Joyce 1993; Mogenson et al. 1993). The subicular output onto the nucleus accumbens appears to functionally interact with a substantial dopaminergic projection from the VTA, thereby balancing the activity of the nucleus accumbens (Blaha et al. 1997; Brudzynski and Gibson 1997; Harvey and Lacey 1996; Nicola et al. 1996; Totterdell and Smith 1989; Wu and Brudzynski 1995). As DA hyperfunction has been implicated in schizophrenia (Gray et al. 1995b; Joyce 1993; Joyce and Meador-Woodruff 1997) the present study examines how DA modulates the synaptic excitability of subicular neurons. Using intracellular- and whole cell voltage-clamp recordings, the results obtained indicate that DA strongly suppresses glutamatergic hippocampal and entorhinal neurotransmission onto subicular neurons by activation of presynaptic D1-like DA receptors.

METHODS

Slice preparation

The experiments were performed on horizontal slices containing the entorhinal cortex, the subiculum, and the hippocampal formation obtained from adult 180- to 230-g female Wistar rats. The rats were decapitated under deep ether anesthesia, the brains were quickly removed, and 400-μm-thick slices were prepared with a Campden vibroslicer (Loughborough, UK). The slices were transferred to an interface recording chamber continuously perfused with an aerated (95% O₂, 5% CO₂), prewarmed (34°C) artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 1.25...
Na<sub>2</sub>PO<sub>4</sub>, 26 NaHCO<sub>3</sub>, 3 KCl, 1.6 CaCl<sub>2</sub>, 1.8 MgSO<sub>4</sub>, and 10 glucose at pH 7.4.

**Electrophysiological recordings**

Recordings from subicular neurons were made exclusively in the cell band in extension of the pyramidal cell layer in area CA1 within close proximity to the perforating fibers of the perforant path. Discontinuous single-electrode voltage-clamp recordings were performed with conventional sharp microelectrodes by using a SEVC amplifier (SEC10L, NPI Electronic, Tamm, Germany). Only cells with resting potentials more negative than -60 mV and with overshooting action potentials (prior to QX-314 diffusion) were accepted. Electrodes were pulled from borosilicate glass (1.2 mm OD, resistance 50–80 MΩ) and filled with 2.5 M K-acetate. In all experiments we additionally included 50 mM QX-314 in the recording electrode to minimize effects on intrinsic K<sup>+</sup> currents following application of DA. After clamping the cell close to the resting membrane potential, we optimized the gain, capacitance compensation, and switching frequency (25–35 kHz). Stimulation of afferent fibers in the alveus or the perforant path was performed with glass-insulated bipolar platinum wire electrodes (tip diameter 50 μm, tip separation 100–200 μm). Synaptic potentials were evoked following electrical stimulation every 10 s (intensity: 1–5 V; duration: 0.05 ms). Local application of glutamate agonist onto subicular dendrites was conducted with electrodes pulled from borosilicate glass (2.0 mm OD, 1.0 mm ID, 3 μm tip diam) using high pressure (1.5 bar) application of 20 ms duration.

Miniature excitatory postsynaptic currents (mEPSCs) were recorded in whole cell voltage-clamp mode using a continuous feedback patch-clamp amplifier (EPC-7, HEKA, Lambrecht, Germany). Electrodes were pulled from borosilicate glass (2.0 mm OD, 1.0 mm ID, 3–8 MΩ resistance). Intraperitoneal solutions contained (in mM) 135 Cs-glucanote, 6 CsCl, 2 MgCl<sub>2</sub>, 10 HEPES, and 1 QX-314; CsOH was used to adjust pH to 7.2. Whole cell recordings were obtained by lowering patch electrodes into the subcellular cell layer while monitoring current responses to 10-mV voltage pulses and applying suction to form >Ω seals. Access resistance was monitored throughout each experiment, and only recordings with access resistance of <15 MΩ were considered acceptable for analysis. Access resistance (not compensated) was repeatedly checked during the experiments, and recordings showing an increase of >20% were rejected. Neurons were voltage-clamped at ~70 mV for recordings of mEPSCs.

Signals were filtered at 3 kHz, digitized at 9–12 kHz by a TIDA interface card (Battele, Frankfurt, Germany) or an ITC-16 interface (Instrutech, Great Neck, NY), and subsequently stored on an IBM-compatible computer. Peak amplitudes of evoked EPSCS were measured from an average of 8–10 sweeps. All data were analyzed off-line using TIDA software (HEKA, Lambrecht/Pfalz, Germany). Detection of individual mEPSCs was done off-line using ISO2/AN3A3 (MFK, Niedernhausen, Germany). Statistical evaluation was performed on the means ± SE by applying a Student’s t-test (Origin 4.1, Microcal). Significance level was set to P < 0.05.

**Compounds**

The following drugs were bath-applied: bicuculline methiodide (BCM) (SIGMA, Deisenhofen, Germany), 5 μM; 2-amino-5-phosphonoovalic acid (APV) (Research Biochemicals, Natick, MA), 60 μM; 6-nitro-7-sulamoylbenzof[b]quinoline-2,3-dione (NBQX; a gift from NOVO Nordisk), 10 μM; 3-N-[1-(s)-,3-(4-dichlorophenyl)ethyl]amino-2-(s)-hydroxypropyl-P-benzyl-phosphonic acid (CGP55842A; a gift from CIBA-GEIGY, Basel), 2 μM; dopamine-HCl, 100 μM; (±)-sulpiride, 20 μM; (R)-+7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1 h-3-benzazepine hydrochloride [(+)-(S)-SC-23390], 20 μM (all from Research Biochemicals, Natick, MA); (±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol (SFK 38393 hydrobromide), 20 μM; (4aR-trans)-

**RESULTS**

Voltage-clamp recordings were used to examine the effect of DA (100 μM) on stimulus-evoked glutamate receptor-mediated EPSCs in subicular neurons. GABA<sub>A</sub> receptor–mediated inhibitory postsynaptic currents (IPSCs) were blocked by bicuculline (5 μM), and GABA<sub>B</sub> receptor–mediated IPSCs were eliminated by CGP 55845A (2 μM) and by QX-314—containing intracellular solutions. AMPA/kainate receptor (in the following indicated as AMPAR)–mediated responses were isolated by application of APV (60 μM) while N-methyl-D-aspartate (NMDA) receptor (NMDAR)–mediated EPSCs were recorded in the presence of NBQX (10 μM).

Dopamine reduces alveus and perforant path–evoked EPSCs

Stimulation of alvear fibers evokes a glutamatergic postsynaptic response in subicular neurons consisting of a NBQX-sensitive AMPAR-mediated response and a strong NMDAR-mediated component that is predominantly APV sensitive (Behr et al. 1998). In addition, subicular cells express a small APV-insensitive NMDAR-mediated component (Hetka et al. 1998) that shows an attenuated Mg<sup>2+</sup> blockade reminiscent to recently described neocortical NMDAR-mediated EPSCs (Fleidervish et al. 1998). Bath application of DA for 10 min reversibly depressed AMPAR- and NMDAR-mediated EPSCs in subicular neurons evoked by stimulation of alvear fibers, which represent the dominant hippocampal input from area CA1. Both glutamatergic components were decreased by the same extent: While the AMPAR-mediated EPSCs declined to 56 ± 8% (mean ± SE, n = 9) of control, the NMDAR-mediated response attenuated to 54 ± 11% (n = 4) of control (Fig. 1, A and B). The effect of DA could not be prevented by intracellular perfusion of the cell in patch-clamp recording technique (n = 4).

In addition to the alvear input from area CA1, the subiculum receives an excitatory monosynaptic input from the entorhinal cortex via the perforant path projection (Behr et al. 1998; Steward and Scoville 1976; Witter 1993) (Fig. 2A). As both pathways excite subicular neurons and determine the activity of the hippocampal output, we extended our investigation examining the effect of DA on the excitatory entorhinal input. As previously described, stimulation of perforant path fibers originating in layer III of the entorhinal cortex evokes a glutamatergic postsynaptic response in subicular neurons consisting of an AMPAR- and NMDAR-mediated component (Behr et al. 1998). Using drop application of glutamate onto layer III of the entorhinal cortex to elicit orthodromic activation of subicular cells, we had ascertained that perforant path–evoked responses in subicular cells are not due to the antidromic activation of subiculo-entorhinal axons. Comparing the kinetics of perforant path–evoked AMPAR- and NMDAR-medi-
Fig. 1. Effect of dopamine (DA) on alveus-evoked (S)-alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptor-mediated excitatory postsynaptic currents (EPSCs) and on AMPA-evoked currents. A: isolated AMPAR-mediated EPSCs elicited in the presence of 2-amino-5-phosphonovaleric acid (APV; 60 μM), bicuculline (5 μM), and CGP55845A (2 μM) were reduced by DA (100 μM). B: isolated NMDAR-mediated EPSCs evoked in the presence of 6-nitro-7-sulfamoylbenzof[1,2,3]oxazine-2,3-dione (NBQX; 10 μM), bicuculline (5 μM), and CGP55845A (2 μM) were similarly reduced by DA. There was no DA-induced difference in the kinetics of the isolated AMPAR-mediated EPSCs (C) and NMDAR-mediated EPSCs (D) before and after application of DA. E: DA (100 μM) had no effect on the membrane inward current induced by pressure application of the glutamate receptor agonist AMPA (1 mM), indicating that the depression of EPSCs by DA is not mediated by a postsynaptic action on glutamate receptors.

Depression of EPSCs is mediated by a D1-like DA receptor

Dopamine receptors can be subdivided into two pharmacologically and biochemically distinct classes (Civelli et al. 1993; Sibley 1995), the D1- and the D2-like DA receptors. To elucidate the DA receptor subtype responsible for the depression of excitation, first we examined the effects of D1 and D2 receptor agonists and antagonists on alveus-evoked AMPAR-mediated EPSCs (Fig. 3). Like DA, SFK 38393 (20 μM), an agonist of D1-like DA receptors, decreased the EPSC peak amplitude to 73 ± 8% of control (n = 6). Although DA attenuated EPSCs to 56 ± 8% of control (n = 9), the effects were not significantly different (P = 0.16). In contrast, the D2 receptor agonist quinolinol failed to suppress EPSCs even when applied at higher concentrations (up to 400 μM; n = 6). Consistent with this result, the D2 receptor antagonist sulpiride (20 μM) did not prevent the DA-induced depression to 57 ± 8% of control (n = 9), which is not different from the observed decline in the absence of any antagonist (56 ± 8%, n = 9). However, the D1 receptor antagonist SCH 23390 (20 μM) did not completely block the depressant action of DA (82 ± 7% of control, n = 8), despite the fact that this antagonist was used at concentrations exceeding the ones applied by other groups (Harvey and Lacey 1996; Nicola et al. 1996). Finally, we examined the DA receptor involved in the attenuation of perforant path-evoked EPSCs. As shown for alveus-evoked EPSCs, application of the D1 agonist SFK 38393 (20 μM) mimicked the effect of DA and attenuated AMPAR-mediated EPSCs to 80 ± 8% of control (n = 5, data not shown).

The mechanism by which DA exerts its effect on excitatory synaptic transmission may involve a decrease in presynaptic glutamate release and/or a decreased postsynaptic sensitivity to glutamate. To clarify the site of action, we first examined the kinetics of the isolated alveus-evoked AMPAR- and NMDAR-mediated EPSCs before and after application of DA. There was no DA-induced difference in the decay time, making a modulation of glutamate receptors by competitive interaction with binding sites or a shunting of the EPSCs in the dendrites unlikely. The decay time constants of AMPAR- and NMDAR-mediated EPSCs before and after application of DA were 7.8 ± 0.63 ms versus 8.2 ± 0.65 ms (n = 5) and 43.5 ± 10.7 ms versus 39.8 ± 7.0 ms (n = 4), respectively, when monoeexponentially fitted (Fig. 1, C and D). In addition, the sensitivity of subicular neurons to pressure application of AMPA (100–1,000 μM) was unchanged by DA (100 μM) pretreatment (151 ± 24 pA vs. 137 ± 9 pA, n = 7, paired t-test analysis, P = 0.58), suggesting a presynaptic mechanism for the observed effect (Fig. 1E).

To further determine whether a pre- or postsynaptic mechanism is involved, we recorded miniature EPSCs (mEPSCs) in the presence of the Na+ channel blocker tetrodotoxin (TTX; 1 μM) in whole cell voltage-clamp recordings to study the effect of DA receptor activation on spontaneous glutamate release (Fig. 4). Miniature EPSCs are believed to represent the release of single neurotransmitter packets. Analysis of the mEPSC frequency before and after application of DA provides information about possible changes in the presynaptic release process, while changes in the amplitudes of the miniature currents reflect postsynaptic alterations in receptor properties including their number at the synapse. DA (100 μM) prolonged the mean inter-event interval from 198 ± 57 ms to 1.149 ± 0.249 s (n = 4, paired t-test analysis, P < 0.05). The
amplitude distribution of mEPSCs remained unaltered by DA (9.61 ± 0.60 pA vs. 9.12 ± 0.86 pA), suggesting that DA reduces glutamate release onto subicular neurons. A purely postsynaptic origin for the more than 80% decrease of mEPSC frequency in the presence of DA can be excluded because, as calculated for the cell shown in Fig. 2, a 56% decrease of mEPSC amplitude (percentage reduction of evoked AMPAR-mediated EPSCs) would increase the mean inter-event interval by only 27%, from 409 ± 18 ms to 562 ± 36 ms.

Although the reduction in mEPSC frequency strongly suggests a presynaptic action of DA, the excitatory synapses that contribute to the mEPSC distribution remain unknown. To exclude distinct pathway-dependent mechanisms for the DA-induced depression, we tested the effect of DA (100 µM) on alveus and perforant path–evoked paired-pulse facilitation (PPF), which is considered to depend on a presynaptic mechanism (Zucker 1989) (Fig. 5). Surprisingly, DA inconsistently altered the paired-pulse ratio (PPR; 50-ms interevent interval, 2nd response relative to the 1st one) of alveus and perforant path–evoked AMPAR-mediated EPSCs. In the majority of cells (12 of 16), the PPR declined significantly during application of DA. The PPR of alveus-evoked responses decreased from 1.54 ± 0.07 to 1.30 ± 0.07 (n = 12); in five of these cells the PPR following perforant path stimulation declined from 1.53 ± 0.11 to 1.27 ± 0.06. In these five cells there was no pathway-dependent difference in the change of the PPRs. In four cells, however, we observed either an increase of both alveus- and perforant path-evoked PPF (n = 1) or coinciding increases and decreases of the PPR (n = 3).

**DISCUSSION**

An increasing number of studies suggest that the subiculum, the major output region of the hippocampal formation, seems to be relevant to schizophrenia (Gray 1998; Gray et al. 1995a; Greene 1999; Joyce 1993; Mogenson et al. 1993). Morphological studies showed neuropathological alterations of the subiculum in schizophrenic patients (Arnold et al. 1991, 1995; Eastwood et al. 1995; Gray et al. 1991; Nicola et al. 1996). In an effort to clarify how the subiculum might be affected by dopaminergic
 consider the recently described pronounced and selective hyperfunction, the present study investigated the effect of DA on excitatory synaptic transmission in this region. Our study demonstrates that DA strongly depresses the hippocampal and the entorhinal excitatory input onto subicular cells by decreasing the glutamate release following activation of presynaptic D1-like DA receptors. While DA depressed alveus-elicited AMPA receptor-mediated EPSCs in subicular neurons to 56 ± 8% of control, perforant path-evoked EPSCs, however, were attenuated to only 76 ± 7% of control. The presynaptic mechanism for the observed reduction of excitatory synaptic transmission was confirmed by employing paired-pulse protocols, recordings of spontaneous miniature EPSCs, wash out of postsynaptic G-proteins, the use of AMPA-pressure application, and the analysis of EPSC kinetics before and after application of DA.

The subiculum receives strong glutamatergic input from area CA1 (Behr et al. 1998; Taube 1993) and is thus part of the polysynaptic loop from the entorhinal cortex layer II through the dentate gyrus, area CA3, area CA1 and the subiculum back to the entorhinal cortex. In addition, the subiculum receives a monosynaptic excitatory perforant path projection from layer III of the entorhinal cortex, which bypasses the classic trisynaptic hippocampal loop and terminates exclusively in area CA1 and the subiculum (Steward and Scoville 1976; Witter 1993). The functional interaction of both inputs balances the activity of the subiculum and hence controls the hippocampal output. Considering the recently described pronounced and selective DA-induced inhibition of the excitatory perforant path input onto CA1 pyramidal cells (Otmakhova and Lisman 1999), we examined the effect of DA on the entorhinal input and compared it with that originating in area CA1. As previously described, both synaptic inputs consist of AMPAR- and NMDAR-mediated components (Behr et al. 1998). Neither the AMPAR- nor the NMDAR-mediated components of each input differed in their kinetics, suggesting similar pathway-independent postsynaptic receptor sites. Interestingly, application of DA affected both pathways selectively: while the CA1 input was attenuated to 56% of control, the entorhinal input was diminished to only 76% of control.

Our data indicate that the D1-like DA receptor most likely mediates the decrease in transmitter release onto subicular cells. The D1 receptor agonist SFK 38393, but not the D2 receptor agonist quinolone, mimicked the action of DA. Consistent with these results, the D2 antagonist sulphuride failed to inhibit the depressive effect of DA. However, preapplication of the D1 receptor antagonist SCH 23390 could not completely block the DA effect. Considering previous studies that had also difficulties to mimic or antagonize DA effects (Nicola et al. 1996; O’Donnell and Grace 1994; Otmakhova and Lisman 1999; Pralong and Jones 1993), the present result appears likely to result from either a decreased sensitivity of subicular D1-like DA receptors to SCH 23390 or a subset of atypical DA receptors that are not blocked by this antagonist. As the D1 receptor agonist SFK 38393 was less effective than DA in depressing evoked EPSCs, we favor the latter explanation. Alternatively, DA exerts its action by activating other monoamine receptors (Aguayo and Grosse 1994; Malenka and Nicoll 1986); however, the concentrations to be needed were reported to be at least 10-fold higher than those we employed (Haas and Konnerth 1983). Several studies have demonstrated that D1-like DA receptors control glutamate release in different regions of the brain including the nucleus accumbens (Nicola et al. 1996; Pennartz et al. 1992), the entorhinal cortex (Pralong and Jones 1993), the prefrontal cortex (Law-Tho et al. 1994; Williams and Goldman-Rakic 1999), and the basal forebrain (Momiya et al. 1996). As each of these structures receive dopaminergic projections from the VTA, one may conclude that the dopaminergic system controls excitatory neurotransmission by D1-like DA receptors. These receptors regulate the activity of cyclic AMP (cAMP)-dependent protein kinase (PKA) (Kebabian and Calne 1979) and were shown to reduce N-type Ca$^{2+}$ currents (Surmeier et al. 1995), an effect that might account for the reduction of evoked EPSCs. However, it is noteworthy that recent studies in the hippocampal area CA1 (Hsu 1996) as well as in layer III of the entorhinal cortex (Stenkamp et al. 1998) indicated a D2-like DA receptor-mediated presynaptic inhibition of glutamate release.

As the decreases in the AMPAR- and NMDAR-mediated alveus-evoked EPSC components were similar and DA had no effect on the kinetics of AMPAR- and NMDAR-mediated EPSCs, we assumed a presynaptic mechanism for the DA-induced depression. It is conceivable that postsynaptic ion channels are modulated indirectly via the activation of G-proteins and second-messenger pathways. However, altering the cellular metabolism by employing intracellular perfusions with a patch pipette could not prevent the DA-induced depression of EPSCs. Therefore a postsynaptic G-protein-mediated cellular pathway appears unlikely to underlie the observed depression. In contrast, DA had no effect on the membrane inward currents induced by pressure application of AMPA indicating that the depression of EPSC by DA is not mediated by a postsynaptic action on glutamate receptors. To support the hypothesis of a presynaptic mechanism, we examined the

FIG. 3. Effect of D1 and D2 agonists and antagonist on alveus-evoked AMPA receptor-mediated EPSCs. A and B: like DA, the D1 receptor agonist SFK 38393 (20 μM) decreased the EPSC amplitude, while the D2 receptor agonist quinolone (20 μM) failed to suppress EPSCs. C: consistent with this result, the D2 receptor antagonist sulpiride (20 μM) did not prevent the DA-induced synaptic depression. D: however, the D1 receptor antagonist SCH 23390 (20 μM) did not completely prevent the significant depressive action of DA.
effect of DA on mEPSCs. Dopamine significantly reduced the frequency of mEPSCs without affecting their amplitude, thereby excluding an interaction of DA with postsynaptic glutamate receptors and giving strong evidence for a suppression of glutamate release from synaptic terminals. As the DA-induced suppression was recorded in the presence of the GABAB receptor antagonist CGP 55845A, we can exclude an interaction of DA with presynaptic GABA_B receptors.

To elucidate pathway-specific mechanisms for the depression of EPSCs, we finally investigated the effect of DA on alveus and perforant path–evoked PPF, a phenomenon sensitive to changes in release probability (Zucker 1989). While PPF usually increases under conditions where transmitter release is decreased in our study, the majority of cells showed a decreased PPF in the presence of DA independent of the input stimulated. Presently, we do not have a conclusive explanation for the discrepancy. Even more intriguing was the observation that in some cells increases and decreases of PPF coincided within one cell, suggesting that DA does not alter the release machinery only by reducing Ca^{2+} influx.

The moderate pathway-dependent difference in DA-induced EPSC depression stands in sharp contrast to the findings in area CA1 where perforant path–elicited field EPSPs were strongly depressed by DA while the Schaffer collateral-mediated input was rather unaffected (Otmakhova and Lisman 1999). Interestingly, the DA-induced depression of the excitatory perforant path input in area CA1 seems to be mediated by a presynaptic mechanism as well and shows a similar dose effect like in the subiculum. Hence, it appears conceivable that the perforant path projection from layer III of the entorhinal cortex to area CA1 and the subiculum is similarly modulated by DA receptors located on perforant path terminals. While in the present study the hippocampal input to the subiculum was strongly attenuated by DA, the Schaffer collateral mediated synaptic

FIG. 4. Effect of DA on mEPSCs. Consecutive whole cell voltage-clamp recording of mEPSCs from subicular neurons in the presence of the Na^+ channel blocker tetrodotoxin (1 μM) before and after bath application of DA (100 μM) and corresponding cumulative probability plots and histograms of mEPSC amplitude and frequency, respectively. The amplitude distribution of mEPSCs revealed a small but not significant shift to the left (smaller amplitudes) following application of DA (9.61 ± 0.64 pA vs. 9.12 ± 0.86 pA). However, application of DA significantly prolonged the mean inter-event interval from 0.198 ± 0.057 s to 1.149 ± 0.249 s (n = 4; paired t-test analysis, P < 0.05), indicating a presynaptic action of DA on glutamate release onto subicular neurons. Left: superimposed cumulative probability plots of amplitude and inter-event interval before and after application of DA.
transmission to area CA1 was reported to be unaffected by DA (Marciani et al. 1984; Otomakova and Lisman 1999; but see Hsu 1996). This different pathway-dependent depression of excitation is consistent with the distinct distribution of DA receptors in area CA1 and the subiculum: while area CA1 contains a pathway-dependent laminar distribution of DA receptors (Goldsmith and Joyce 1994; Swanson et al. 1987), in the subiculum D1-like DA receptors are rather homogeneously (Köhler et al. 1991) distributed throughout its molecular layer, the target site for both, the perforant path and the alvear projection, respectively. Nonetheless, with respect to the stronger DA effect on alveus-evoked responses, alvear terminals seem to have a slightly higher density of D1-like DA receptors or a distinct DA receptor subunit expression.

The concentration of DA used in this study must be related to the in vitro conditions of the experiment. Full equilibrium of applied drugs within slices takes at least 1 h within the interface type of recording chamber (Müller et al. 1988). The demonstrated effects, however, were recorded after 10 min of DA perfusion. In addition, rapid oxidation (Sutor and ten Bruggencate 1990) as well as uptake of DA will reduce the DA perfusion. In addition, rapid oxidation (Sutor and ten Bruggencate 1990) as well as uptake of DA will reduce the final concentration of DA. Hence, the nominal applied concentrations of DA were considerably higher than those that actually induced the observed effects. This is in agreement with studies in the hippocampus (Gribkoff and Ashe 1984), the entorhinal cortex (Pralong and Jones 1993; Stenkamp et al. 1998), the nucleus accumbens (Nicola et al. 1996), and the basal forebrain (Momiyama et al. 1996), which reported that alterations in DA concentration and the prefrontal cortex (Weinberger et al. 1992). As schizophrenia is associated with a hyperfunction of the dopaminergic system (Gray et al. 1995b; Joyce 1993; Joyce and Meador-Woodruff 1997), an enhanced DA-induced depression of the excitatory drive onto subicular cells may cause abnormalities in the hippocampal output. Considering that antagonists at DA receptors have a high antipsychotic potency, the subiculum may represent a potential site of action. A reduction of the DA-induced inhibition of glutamate release by D1 antagonists may partially restore the diminished hippocampal information transfer.

We are indebted to A. Piechotta and A. Duerkop for excellent technical assistance and editorial help.

This work was supported by a grant from the Bundesministerium für Bildung und Forschung and Deutsche Forschungsgemeinschaft Grant 2022-2-1, 2-2 to J. Behr.

REFERENCES


Brumink A and Bischof S. Dopamine D2 receptors are unevenly distributed in the rat hippocampus and are modulated differently than in striatum. Eur J Pharmacol 245: 157–164, 1993.


Fremaut RT Jr, Duncan GE, Fornaretto MG, Deary A, Ginsrich JA, Breese GR, and Caron MG. Localization of D1 dopamine receptor mRNA in brain supports a role in cognitive, affective, and neuroendocrine aspects.


