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Dopamine Depresses Synaptic Inputs Into the Olfactory Bulb

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Hsia, Albert Y., Jean-Didier Vincent, and Pierre-Marie Lledo.

Dopamine depresses synaptic inputs into the olfactory bulb. *J. Neurophysiol.* 82: 1082–1085, 1999. Both observations in humans with disorders of dopaminergic transmission and molecular studies point to an important role for dopamine in olfaction. In this study we found that dopamine receptor activation in the olfactory bulb causes a significant depression of synaptic transmission at the first relay between olfactory receptor neurons and mitral cells. This depression was found to be caused by activation of the D2 subtype of dopamine receptor and was reversible by a specific D2 receptor antagonist. A change in paired-pulse modulation during the depression suggests a presynaptic locus of action. The depression was found to occur independent of synaptic activity. These results provide the first evidence for dopaminergic control of inputs to the main olfactory bulb. The magnitude and locus of dopamine's modulatory capabilities in the bulb suggest important roles for dopamine in odorant processing.

INTRODUCTION

Both behavioral and molecular studies point to a potentially important role for dopamine in olfaction. Parkinson's patients, for instance, have been found to have impaired odor recognition (Hawkes and Shephard 1998). In addition, systemic injection of dopamine analogues has been shown to result in impaired odor detection (Doty and Risser 1989). On the molecular level, dopamine receptor expression has been found to be high in the main olfactory bulb, as are levels of the rate-limiting synthetic enzyme of dopamine, tyrosine hydroxylase (TH) (Coronas et al. 1997, Halasz et al. 1977).

Together these observations motivated us to study at the cellular level a possible modulatory role of dopamine in the mammalian olfactory bulb. The bulb receives inputs from the olfactory epithelium via the olfactory nerve, which forms excitatory, glutamatergic synapses in regions of the bulb known as glomeruli. There, synapses are made onto the dendrites of mitral cells, the primary output neurons of the bulb. Glomerular borders are defined in part by periglomerular cells, which are both GABAergic and dopaminergic. These periglomerular cells are excited by dendrodendritic synapses from mitral cells, and possibly also by axodendritic synapses from the olfactory nerve (Pinching and Powell 1971). Because TH levels are highest in the glomerular layer (Halasz et al. 1977), and because dopamine receptors have been localized to the olfactory nerve and glomerular layers (Coronas et al. 1997), we hypothesized that it is at the first synapse between the olfactory nerve and mitral cells where dopamine might play a modulatory role.

Dopamine receptors are classified into two broad families: D1 and D2 (Missale et al. 1998). D1 receptors are only sparsely

expressed in the bulb and are absent in the olfactory nerve layer; in fact, their presence had been doubtful until recently (Coronas et al. 1997). It is the D2 receptors that show prominent expression in the bulb, specifically in the olfactory nerve and glomerular layers (Coronas et al. 1997), and hence in this study we used a specific D2 receptor agonist to probe for a possible role of dopamine in modulating olfactory nerve input to the bulb.

METHODS

Experiments were performed on olfactory bulb slices obtained from 1- to 6-wk-old Wistar rats. Slices (300–400 μm) were prepared as described (Chen and Shepherd 1997). After at least a 1-h recovery period at 30°C, slices were transferred to a submersion chamber, where they were continuously superfused (~ 2 ml/min) with a 22–25°C ACSF solution saturated with 95% O_2 –5% CO_2 . The external ACSF solution was composed of (in mM) 119 NaCl, 2.5 KCl, 2.5 CaCl_2 , 1.3 Mg_2SO_4 , 1.0 NaH_2PO_4 , 26.2 NaHCO_3 , and 10 D-glucose.

For field recording, bipolar, stainless-steel stimulating electrodes were placed in the olfactory nerve layer, and recording pipettes were placed in individual glomeruli (see Fig. 1A).

Whole-cell recordings were performed under visual control with an upright Zeiss Axioskop microscope and infrared differential interference contrast (IR-DIC) videomicroscopy. Mitral cells were easily identified by their location and morphology (see Fig. 2A) (Shepherd and Greer 1998). Microelectrodes had a resistance of ~ 8 M Ω . The whole-cell pipette solution was composed of (in mM) 123 Cs-glucuronate, 10 CsCl, 1 CaCl_2 , 10 Cs-EGTA, 10 HEPES-Na, 8 NaCP, 10 D-glucose, 0.3 GTP, 2 Mg-ATP, and 0.2 AMPc, pH 7.3. Cells were voltage clamped at -80 mV.

Evoked synaptic responses were elicited at 0.05 Hz. For paired-pulse modulation experiments, paired pulses were delivered 40 ms apart, and the peak amplitude of the second response was divided by the first. On- and off-line data analysis was carried out with Acquisil (G. Sadoc, CNRS-ANVAR, France).

Fast inhibitory transmission was blocked with picrotoxin (100 μM). Excitatory responses were blocked through the addition of the ionotropic glutamate receptor antagonist, kynurenic acid (10 mM). The *N*-methyl-D-aspartate NMDA receptor antagonist, D,L-2-amino-5-phosphonopentanoic acid (D,L-APV, 100 μM), was added in some experiments to isolate responses mediated by α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors.

D,L-APV, quinpirole, and sulpiride were obtained from Tocris (Illkirch, France) and all other drugs and salts were purchased from Sigma (Strasbourg, France).

Results are presented as means \pm SE. Data were compared statistically by the Student's *t*-test, and significance was defined as $P < 0.05$.

RESULTS

D2 receptor agonist quinpirole inhibits transmission at the olfactory nerve-mitral cell synapse

We first performed extracellular field recordings in individual glomeruli (see Fig. 1A) and assessed the effect of D2

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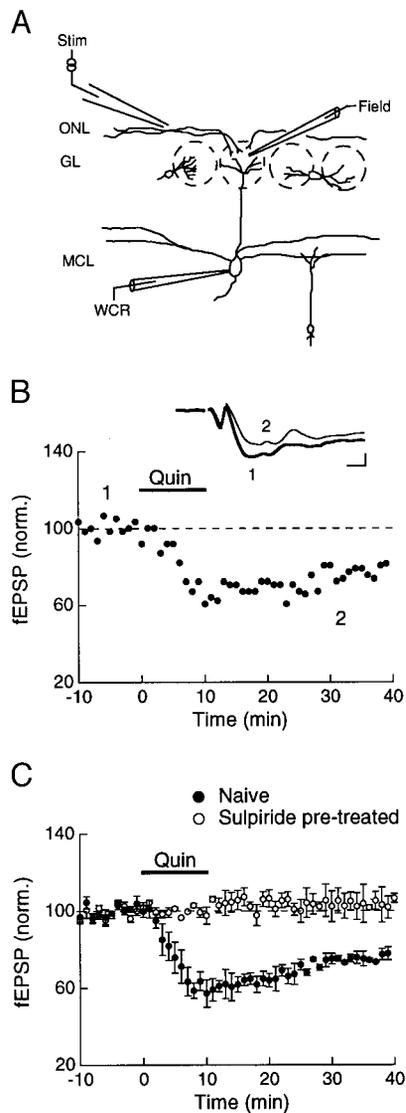


FIG. 1. D2 receptor activation inhibits olfactory nerve inputs to olfactory bulb glomeruli. *A*: schema illustrating recording configurations for extracellular field recording in individual glomeruli and whole-cell recording from mitral cells during stimulation of the olfactory nerve. ONL, olfactory nerve layer; GL, glomerular layer; MCL, mitral cell layer. *B*: representative experiment in which the effects of the D2 receptor agonist, quinpirole (100 μ M), on pharmacologically-isolated excitatory field postsynaptic potentials (fEPSPs) were assessed. Superimposed average sweeps before (1) and after (2) quinpirole administration are shown above the graph (scale: 0.1 mV, 2 ms). Smaller but significant levels of depression were observed with 10 μ M quinpirole ($32 \pm 2\%$; $P = 0.002$, $n = 4$, data not shown). *C*: summary graph illustrating that quinpirole significantly depressed glomerular fEPSPs induced by olfactory nerve stimulation (\bullet). When slices were pretreated with the specific D2 receptor antagonist, sulpiride (100 μ M), no effect of quinpirole was observed (\circ).

receptor activation on the strength of field excitatory postsynaptic potentials (fEPSPs) in response to olfactory nerve stimulation. We found that quinpirole, a specific D2 receptor agonist, significantly depressed fEPSP strength by $41 \pm 5\%$ (Fig. 1, *B* and *C*; $P = 0.0023$, $n = 5$).

Because a component of the glomerularly recorded fEPSP could be contributed by the depolarization of periglomerular cells in addition to mitral cells, we performed the analogous experiment in the whole-cell configuration, recording from visually identified mitral cells (see Fig. 2*A*). A depression of

similar magnitude ($47 \pm 15\%$) was observed (Fig. 2, *B–D*; $P = 0.047$, $n = 5$), suggesting that quinpirole's depressive effect does indeed occur at olfactory nerve synapses onto mitral cells.

Depressive effect of quinpirole is D2 receptor specific and reversible

Pretreatment of slices with the specific D2 receptor antagonist, sulpiride, completely prevented the depressive effect of quinpirole (Fig. 1*C*; $P = 0.0068$, $n = 4$). In addition, the quinpirole-induced response was completely reversed by subsequent application of sulpiride (Fig. 2, *C* and *D*; $n = 5$). Sulpiride alone had a very small ($+5 \pm 1\%$) effect on baseline synaptic transmission in naive slices (Fig. 2, *E* and *F*; $P = 0.012$, $n = 3$).

D2 receptor-mediated depression causes a change in paired-pulse modulation and is activity independent

To determine the locus of D2 receptor-mediated depression, we first tested whether quinpirole application changed the degree of paired-pulse modulation, measured as the ratio of the strengths of two closely-spaced EPSPs. The degree of paired-

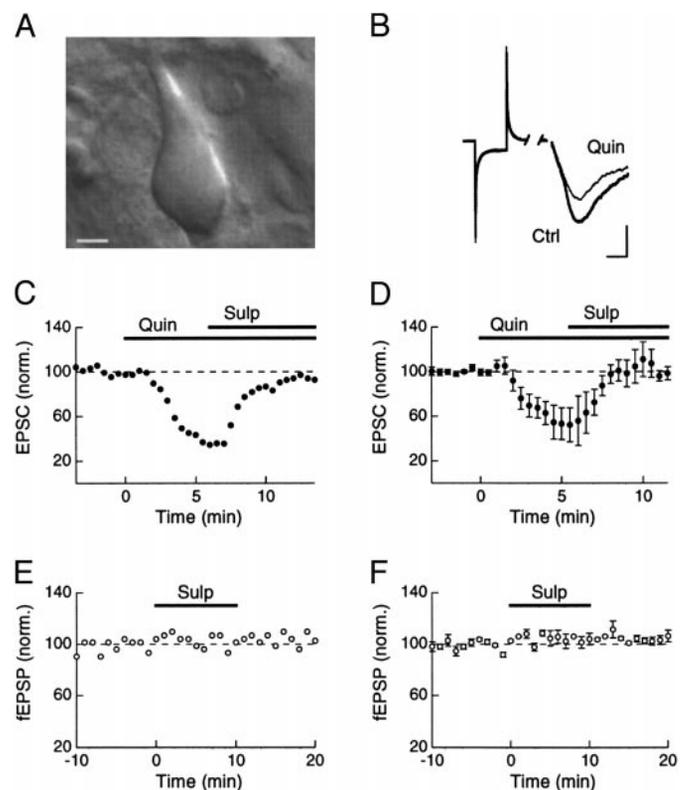


FIG. 2. D2 receptor activation depresses transmission onto mitral cells in a reversible manner. *A*: infrared differential interference contrast (IR-DIC) image of a mitral cell body in the olfactory bulb of a 2-wk-old rat. Scale bar: 5 μ m. *B*: superimposed average excitatory postsynaptic currents (EPSCs) from a representative experiment illustrating the depressive effect of quinpirole (100 μ M) on mitral cell responses to olfactory nerve stimulation (scale: 20 pA, 10 ms). Superimposed calibration pulses demonstrate the stability of the recording (scale: 150 pA, 12 ms). *C* and *D*: example experiment and summary graph showing a depression of EPSC amplitude by quinpirole (100 μ M), which is then reversed by sulpiride (100 μ M). *E* and *F*: representative experiment and summary graph demonstrating the slight effect of sulpiride alone (100 μ M) on naive slices.

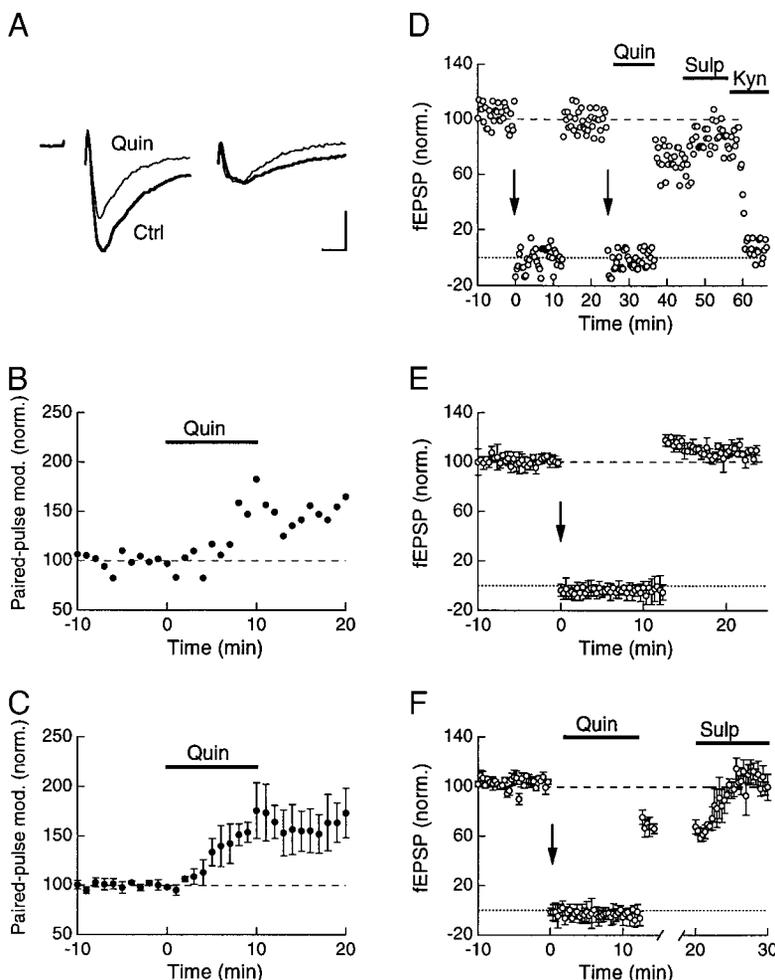


FIG. 3. D2 receptor-mediated depression significantly affects paired-pulse modulation and is activity independent. *A–C*: representative experiment and summary graph illustrating that quinpirole (100 μ M) causes a significant change in the degree of paired-pulse modulation. Average responses before and after quinpirole application are shown in *A* (scale: 0.1 mV, 5 ms). *D–F*: the dependence of the quinpirole-induced depression on olfactory nerve activity was tested by applying quinpirole in the absence of olfactory nerve stimulation. A representative experiment is depicted in *D*. Arrows indicate when stimulation was suspended: 1st in the absence of quinpirole, and then in the presence of quinpirole (100 μ M). A depression of fEPSP slope by quinpirole was observed from the very 1st response following nonstimulation. Sulpiride (100 μ M) was added to demonstrate the D2 receptor specificity of the depression, and kynurenic acid (10 mM) was added to confirm measurement of excitatory responses. In *E*, a summary graph shows that there is a small ($18 \pm 1\%$), transient potentiation following nonstimulation in the absence of quinpirole. In *F*, the summary graph shows that despite nonstimulation, quinpirole still induces a depression of similar magnitude as during experiments with continual stimulation (compare with Fig. 1C).

pulse modulation has been found to correlate with the probability of transmitter release from presynaptic terminals (Markram and Tsodyks 1996). Indeed, quinpirole caused a $71 \pm 24\%$ change in the degree of paired-pulse modulation (Fig. 3, *A–C*; $P = 0.042$, $n = 5$), suggesting a presynaptic locus of inhibition.

We next assessed whether the D2 receptor-mediated depression required olfactory nerve activity. Figure 3*D* illustrates a typical experiment. First, as a control, we stopped stimulation of the olfactory nerve for 10 min, and then resumed stimulation. Then, after reestablishing a baseline, we again stopped stimulation for 10 min, although this time applying quinpirole just after halting stimulation. On resuming stimulation, we found that from the very first response in the presence of quinpirole, synaptic transmission was depressed to a similar degree as during experiments with continual stimulation ($32 \pm 6\%$ for 1st response after nonstimulation; Fig. 3, *D* and *F*; $P = 0.023$, $n = 3$). Figure 3*E* summarizes the effect of a period of nonstimulation alone and shows that, in contrast to results obtained with quinpirole, there is a small ($18 \pm 1\%$), transient potentiation following nonstimulation ($P = 0.0019$, $n = 3$).

DISCUSSION

Dopamine has been shown to act as a neuromodulator in a variety of systems, including the retina (Djamgoz and Wagner 1992), nucleus accumbens (Nicola and Malenka 1998), and

hippocampus (Otmakhova and Lisman 1999). Our demonstration of a strong synaptic depression induced by dopamine receptor activation at the first synaptic relay between the olfactory epithelium and the olfactory bulb suggests an important role for dopamine in mediating the entry of olfactory information into the brain.

D2 receptor activation most likely depresses transmitter release from olfactory nerve terminals

D2 receptors are the most abundant subtype of dopamine receptor in the olfactory bulb (Coronas et al. 1997). We found that one functional effect of D2 receptor activation in the bulb is a significant depression of synaptic transmission between olfactory receptor neurons and mitral cells. The finding of a concomitant change in paired-pulse modulation suggests that this depression is due at least in part to a presynaptic mechanism. To determine whether there may also be a postsynaptic contribution to the depression (e.g., downregulation of glutamate receptors at individual synapses) would require the analysis of miniature EPSCs (mEPSCs). However, we found that mEPSC frequency was too low to permit analysis (<0.1 Hz). In addition, attempts to stimulate asynchronous release through the replacement of Ca^{2+} by Sr^{2+} were also unsuccessful, perhaps due to significant cable filtering between olfactory nerve-mitral cell synapses and the mitral cell soma (~ 400 μ m apart). Although we cannot rule out such additional depression

postsynaptically, molecular evidence points to D2 receptors being expressed in olfactory receptor neurons. D2 mRNA transcripts are abundant in olfactory receptor neurons (Shipley et al. 1991), although undetectable in the mitral cell layer (Coronas et al. 1997). Certainly our inclusion of Cs-gluconate in the whole-cell pipette solution does allow us to rule out postsynaptic modification of K⁺ conductances.

Possible mechanisms leading to synaptic depression by D2 receptors

The fact that experiments were carried out in the presence of a blocker of fast inhibitory transmission rules out the possibility that D2 receptor activation led to inhibition via GABAergic transmission. We therefore suggest that D2 receptors have a direct depressive effect at presynaptic terminals.

We found that the D2 receptor-induced depression is independent of activity (Fig. 3, D–F), ruling out a necessity for concurrent calcium signaling. One possibility is that D2 receptor activation leads to inhibition of adenylyl cyclase (AC); decreased AC activity has been shown in numerous systems to inhibit transmitter release (e.g., Tzounopoulos et al. 1998). In the bulb, D2 receptor activation both decreases cAMP levels (Mania-Farnell et al. 1993) and reduces AC activity (Coronas et al. 1999). Another possible mechanism is a direct effect of D2 receptor activation on voltage-gated ionic channels (Misale et al. 1998).

Potential role of dopamine as a neuromodulator in the bulb

The fact that TH is found exclusively in periglomerular cells (Halasz et al. 1977) suggests that endogenous dopamine is released from these cells. Electron microscopic studies have not found synapses between periglomerular cells and the presynaptic terminals or axons of olfactory neurons (Pinching and Powell 1971), necessitating that this dopamine activate receptors extrinsic to synaptic specializations, perhaps in a diffuse fashion. One possibility is that dopamine may set the olfactory detection threshold by tonically suppressing all inputs into the bulb. The circuit could then adapt to decreasing odorant concentrations by decreasing dopamine release. Consistent with this model is the observation that in response to inactivity resulting from unilateral odor deprivation, TH activity in the glomerular layer markedly decreases (Nadi et al. 1981).

Dopamine might also act as a mediator of heterosynaptic depression, fine-tuning the glomerular activation pattern in response to odors. Dopamine levels have also been found to increase during odor learning (Coopersmith et al. 1991), suggesting that dopamine modulation may have important roles in synaptic plasticity in the bulb.

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