Dopamine Modulates Synaptic Transmission Between Rat Olfactory Bulb Neurons in Culture

Nestor G. Davila, Laura J. Blakemore, and Paul Q. Trombley

Department of Biological Science, Program in Neuroscience, Florida State University, Tallahassee, Florida 32306-4340

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INTRODUCTION

Although many aspects of dopamine in the olfactory bulb (OB) have been characterized over the past few decades, much remains to be understood about the role(s) of dopamine in olfactory information processing. It has been known for some time that subpopulations of both periglomerular neurons in the glomerular layer and tufted cells in the superficial external plexiform layers of the OB express tyrosine hydroxylase (TH), the rate-limiting enzyme for the synthesis of dopamine (Halsasz et al. 1981). Other investigators have characterized the laminar and cellular expression of both D1 and D2 dopamine receptor subtypes in the OB (Coronas et al. 1997; Koster et al. 1999; Levey et al. 1993).

Olfactory experience has a significant effect on the expression of TH, OB dopamine content, and D2 receptors. After unilateral naris occlusion, TH and dopamine content decreases sharply (Baker et al. 1983) and D2 receptor expression dramatically increases (Guthrie et al. 1991). Behavioral experiments have shown that D1 receptor activation improved odor detection performance (Doty et al. 1998). Conversely, D2 receptor activation diminished odor detection performance in another series of experiments (Doty and Risser 1989).

Using electrophysiological techniques, other investigators have shown that dopamine reduces evoked potentials in mitral cells from the turtle OB (Nowycky et al. 1983) and the rat OB (Ennis et al. 2001). Furthermore, the reduction in endogenous dopamine caused by naris closure augments both the number of mitral cells that respond to an individual odorant and the number of odorants to which an individual mitral cell will respond upon odor presentation to the reopened naris (Wilson and Sullivan 1995). Recent electrophysiological studies have shed some light on the synaptic mechanisms underlying these effects. D2 receptor activation on the presynaptic terminal of olfactory receptor neurons (ORNs) decreases the probability of glutamate release, and hence, excitability of mitral/tufted (M/T) cells is diminished (Berkowicz and Trombley 2000; Ennis et al. 2001; Hsia et al. 1999). Also, activation of different dopamine receptor subtypes on M/T cells (D2) and interneurons (D1) mediates differential yet complementary transduction cascades with the overall effect of increasing GABAergic inhibition of M/T cells (Brunig et al. 1999).

In this study, we combined patch-clamp electrophysiological recording with primary culture techniques to further identify the synaptic effects of dopamine. Our results suggest that dopamine may contribute to odor information processing through presynaptic inhibition of excitatory synaptic transmission between M/T cells and interneurons.

METHODS

Tissue culture

The procedure for preparing primary cultures of OB neurons is described in detail elsewhere (Trombley and Blakemore 1999).

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Briefly, P1–P5 Sprague-Dawley rat pups were decapitated in accordance with the institutional guidelines of The Care and Use of Laboratory Animals approved by the National Institutes of Health and The Florida State University’s Animal Care and Use Committee. OBs were removed, cut into 1-mm cubes, and enzymatically treated in a calcium-buffered papain solution for 1 h at 37°C. The OBs were triturated with a fire-polished pipette until a single-cell suspension was achieved. The cells (250,000 cells/dish) were plated in 35-mm culture dishes on a confluent monolayer of previously prepared OB astrocytes. The neuronal media was comprised of 95% minimal essential medium (MEM, Gibco), 5% horse serum (Gibco), 6 g/l glucose, and a nutrient supplement (Serum Extender, Collaborative Research). Astrocyte layers were obtained by plating a suspension of OB cells in a 75-cm² flask containing 90% MEM, 10% fetal calf serum, and 6 g/l glucose. Once confluent, the cells were resuspended enzymatically with 0.125% trypsin and plated onto 35-mm dishes coated with poly-L-lysine (30,000 M Da/l). Addition of 10⁻⁵ M cytosine-β-d-arabinofuranoside (Sigma) to the media 1 day after plating the neurons prevented the overgrowth of astrocytes.

Neuronal Identity

Presumptive M/T cells and interneurons were identified based on the morphological, physiological, and immunohistochemical criteria established by Trombley and Westbrook (1990). Briefly, the cultures contained morphologically distinct populations of neurons: a small number of large-diameter pyramidal-shaped neurons (20–40 μm soma) and a much larger population of small-diameter bipolar neurons (5–10 μm soma). These characteristics correlate with M/T and granule/periglomerular cells, respectively. Electrophysiological analyses of these two morphologically distinct populations further support the notion that they reflect M/T cells and interneurons. Intracellular stimulation of neurons with morphology reflecting M/T cells in vivo invariably evoked glutamate-mediated EPSPs in monosynaptically coupled interneurons. In contrast, intracellular stimulation of the small bipolar neurons evoked GABA-mediated IPSPs, consistent with their identity as interneurons. We have previously shown that these morphologically and physiologically distinct populations can also be identified by immunohistochemical markers. The large pyramidal neurons, the presumptive M/T cells, were N-acetylaspartylglutamate immunoreactive; in contrast, the small bipolar neurons, presumptive granule/periglomerular neurons, were glutamic acid decarboxylase immunoreactive (Trombley and Westbrook 1990).

Electrophysiology

Electrophysiological recordings were obtained at room temperature from OB neurons after 6–14 days in culture. The acquisition software (AxoData and AxoGraph, Axon Instruments) was run on a Macintosh G3 computer and used to control an AxoClamp 2B amplifier (Axon Instruments). The 35-mm culture dishes functioned as the recording chambers and were perfused at 0.5–2.0 ml/min with a bath solution consisting of (in mM) 162.5 NaCl, 2.5 KCl, 2 CaCl₂, 10 HEPES, 10 glucose, 0 MgCl₂, and 1 μM glycine (pH 7.3, osmolality 325 mM/mole). Patch electrodes were pulled from borosilicate glass to a final tip resistance of 4–6 MOhm and filled with a solution containing (in mM) 145 KMeSO₄ or CsCl, 1 MgCl₂, 10 HEPES, 5 Mg-ATP, 0.5 Mg-GTP, and 1.1 EGTA (pH 7.2, osmolality 310 mM/mole).

Drugs were diluted in bath solution and applied via a gravity-fed flow-pipe perfusion system, comprised of a row of 600 μm OD, square glass barrels. An electronic manipulator (Warner Instruments) controlled the position of the flow pipes, and pinch clamps regulated drug delivery. The solution delivery system produced complete drug exchange within 100 ms. In the text and figures, “control” data represent cells perfused with bath solution. The drugs used in these experiments were dopamine, glutamate, and tetrodotoxin (TTX), SKF38393 (all from Sigma), and bromocriptine mesylate (from Tocris).

Spontaneous synaptic activity was recorded from interneurons in current-clamp mode. Quantitative analyses of the effects of dopamine and dopamine receptor agonists were done by comparing the number of excitatory postsynaptic potentials (EPSPs) during a 20-s interval that exceeded 5 mV before, during, and after drug application. Evoked potentials were obtained by simultaneous whole cell recording from monosynaptically coupled M/T cells and interneurons. Electrical stimulation of a presynaptic M/T cell evoked an action potential, which was recorded as an excitatory postsynaptic potential in the postsynaptic interneuron. Membrane input resistance was determined in current-clamp mode as well, using a current injection varying from 30–55 pA. To examine membrane currents evoked by glutamate-receptor agonists, whole cell recordings were made from interneurons in discontinuous, single-electrode, voltage-clamp mode at a switch frequency of 8–12 kHz. Membrane currents were filtered at 1–3 kHz and digitized at 5–10 kHz. Calcium current data were gathered in continuous, single-electrode, voltage-clamp mode to help reduce electrical noise. To more easily identify and quantify an effect on calcium channels, 10 mM BaCl₂ was substituted for CaCl₂ in the experimental drug solutions. Tetrodotoxin (1 μM) was added to block sodium currents, and Cs-based intracellular solutions were used to block potassium currents.

Immunocytochemistry

Our immunocytochemical procedures were modified from previous protocols (Trombley and Westbrook 1990). Primary neuronal cultures were rinsed three times for 10 min with phosphate-buffered saline (PBS) and fixed for 30 min in 4% buffered formaldehyde, which was osmotically adjusted to 320 mOsm with sucrose. The cultures were then rinsed three times for 10 min with PBS. To prevent nonspecific antibody binding, a blocking solution containing 10% normal donkey serum (NDS) or normal rabbit serum in PBS was added to each fixed culture for 10 min. The blocking solution was removed by rinsing three times for 10 min with PBS. The primary antibody was diluted in PBS then added to the cultures to incubate for 24 h at room temperature. The next day, the cultures were rinsed three times for 10 min with PBS to remove the primary antibody. The secondary antibody was also diluted in PBS and added to the cultures for 1 h. After incubation, the secondary antibody was removed by washing three times for 10 min with PBS. All PBS was then removed, and coverslips were applied inside the culture dish using Aquamount (Fisher Scientific, Fairlawn, NJ). The primaries used were a mouse anti-tyrosine hydroxylase monoclonal antibody (Chemicon International, Temecula, CA) at a 1:10,000 dilution and a goat anti-D2 receptor polyclonal antibody (Santa Cruz Biotech, Santa Cruz, CA) at a 1:1,000 dilution. The corresponding secondaries were a goat anti-mouse IgG conjugated to Cy3 (Jackson Immunoresearch, West Grove, PA) used at a 1:200 dilution for TH and a rabbit anti-goat IgG conjugated to AlexaFluor 568 (Molecular Probes, Eugene, OR) at a 1:3,000 dilution for D2 receptors. The fluorescent images were acquired on a Leica DM-LFS microscope using OpenLab 3.0 software.

Statistics

All statistics were performed using GraphPad Prism version 3.00 (San Diego, CA). Data were analyzed using a one-way ANOVA with Newman-Keuls multiple comparison post-test. All data are presented as the mean ± SE.
RESULTS

Tyrosine hydroxylase and D2 receptor expression in primary culture in the absence of olfactory sensory neuron innervation

Several studies have shown that innervation by olfactory sensory neurons is necessary to maintain both dopamine content (Kawano and Margolis 1982) and TH expression in OB juxtaglomerular cells (Baker et al. 1983; Brunjes et al. 1985; Cho et al. 1996). To determine whether dopamine could be synthesized in our cultures in the absence of olfactory sensory neurons, immunocytochemical techniques were used to examine TH expression. TH was expressed in every culture dish examined (ine TH expression. TH was expressed in every culture dish examined (n = 7; Fig. 1A), independent of the age of the animal at dissection (P1–P5) or time in vitro (7–12 days). Approximately 1% of the neurons were immunoreactive for TH. Immunocytochemical controls were prepared by omitting addition of the primary antibody in some cultures and by substituting NDS for primary antibody in others. No significant amount of secondary binding occurred in either situation. These results suggest that dopamine is likely synthesized in primary culture without innervation from the olfactory epithelium.

Although it has been suggested that the majority of OB dopamine D2 receptors are expressed in olfactory sensory terminals (Guthrie et al. 1991; Koster et al. 1999), other studies support the notion that D2 receptors are expressed by OB neurons (Coronas et al. 1997; Levey et al. 1993; Mansour et al. 1990) and are functional (Brunig et al. 1999; Coronas et al. 1999). To address this issue further, we used immunocytochemical techniques to determine whether D2 receptors are expressed by the cultures we examined electrophysiologically. The majority of OB neurons, including M/T cells (Fig. 1B), were immunoreactive for D2 receptors. Neurons from control cultures, which were exposed to the secondary antibody but not the primary, were not immunoreactive. These results are consistent with our electrophysiological results in which the majority of OB neurons were sensitive to dopamine or the D2 receptor agonist, bromocriptine.

Dopamine inhibits spontaneous excitatory synaptic activity

To determine whether dopamine has any effect on excitatory synaptic transmission between OB neurons, whole cell current-clamp recordings were obtained from interneurons before, during, and after dopamine (30 μM) application (Fig. 2). In 13 of the 14 cells tested, spontaneous excitatory activity in the interneuron was substantially inhibited during dopamine application. Dopamine reduced the number of excitatory synaptic events >5 mV during a 20-s interval to 24.2 ± 7.2% of control. The latency of recovery to baseline activity ranged from 10 s to 3 min. Figure 2 shows an example of the recovery latency. That the recovery was not instantaneous was likely due to the metabotropic nature of dopamine receptors and the duration necessary to inactivate the effects of second messengers.

To determine which dopamine receptor subtype was mediating the inhibitory effect of dopamine, spontaneous activity in interneurons was examined in the presence of dopamine receptor subtype-selective agonists. Application of the D2 receptor agonist bromocriptine mesylate (1 μM) dramatically inhibited spontaneous excitatory activity in 9 of 13 neurons (Fig. 3, 2nd trace). Bromocriptine reduced the number of excitatory synaptic events >5 mV in a 20-s interval to 21.8 ± 6.3% of control. Application of 30 μM SKF38393, a D1 receptor agonist, inhibited spontaneous excitatory transmission in 12 of 14 neurons tested (Fig. 3, 4th trace). SKF38393 reduced the number of excitatory synaptic events >5 mV during a 20-s interval to 36.1 ± 8.8% of control. The effects of dopamine, bromocriptine, and SKF38393 on the excitatory synaptic event number were not statistically different from one another (P > 0.05). Like dopamine, both SKF38393 and bromocriptine exerted effects on both individual and compound EPSPs.

Dopamine attenuates evoked EPSPs in interneurons

Dopamine could reduce spontaneous excitatory transmission as an indirect consequence of increasing spontaneous inhibitory transmission. That is, increasing inhibition onto excitatory neurons could reduce the probability of glutamate release from excitatory neurons. To eliminate this type of network effect as
the very high binding affinity of bromocriptine for the D2 receptor (Coronas et al. 1999). In contrast to the effects of D2 receptor activation, D1 receptor agonist SKF38393 (30 μM) did not significantly affect monosynaptic transmission ($n = 8$, $P = 0.67$), as shown in Fig. 4C. SKF38393 also did not alter the 10–90% rise time of the EPSP ($P > 0.05$).

**Dopamine exerts no direct effect on the postsynaptic interneuron**

We also wanted to determine whether the effects of dopamine on excitatory transmission were presynaptic, postsynaptic, or both. First, we examined the effects of dopamine on membrane resistance in the interneuron, since a decrease in postsynaptic membrane resistance would reduce the EPSP amplitude. Hyperpolarizing current injections (250 ms, 30–55 pA) were made in interneurons in current-clamp mode from a membrane potential of $-60$ mV. Flow-pipe application of 30 μM dopamine had no effect on the amplitude or kinetics of the resulting voltage deflection ($n = 4$, Fig. 5A). This result suggests that dopamine neither directly opens nor closes ion channels in the postsynaptic cell membrane sufficiently to cause a measurable change in resistance.

**Dopamine has no direct effects on glutamate receptors**

A second hypothesis is that dopamine reduces EPSP amplitude via inhibition of postsynaptic glutamate receptors. To test this hypothesis, interneurons were whole cell voltage-clamped at $-65$ mV, and a glutamate receptor-mediated current was evoked by flow-pipe application of 500 μM glutamate, a concentration that activates all subtypes of ionotropic glutamate receptors. Dopamine (30 μM) was then coapplied during the glutamate-evoked current. Dopamine had no effect on either the kinetics or amplitude of the glutamate-mediated current ($n = 8$, Fig. 5B). Potential effects of dopamine on
glutamate receptors were further examined by comparing currents evoked by glutamate alone with currents evoked by the coapplication of glutamate and dopamine (Fig. 5C). (These traces are in contrast to the trace in 5B, in which dopamine was coapplied during the middle of a glutamate-evoked current). Under these conditions too, dopamine did not alter the glutamate-evoked current (Fig. 5C, n = 6).

The possibility that prolonged exposure is required for dopamine to exert its effects was also tested. The initial current evoked by 500 μM glutamate served as a control. The neurons were incubated in 100 μM dopamine for 15–20 min, and glutamate was reapplied. Preincubation with dopamine did not affect the amplitude of the current evoked by glutamate (data not shown). This lack of evidence for postsynaptic modulation of glutamate-mediated excitation by dopamine suggests that the presynaptic M/T cell is the likely target of dopamine’s action.

**Dopamine inhibits calcium channel currents in M/T cells**

Because calcium channel modulation is a common mechanism of action for presynaptic inhibition, the effect of dopamine on calcium channel currents in M/T cells was examined. M/T cells were voltage clamped at −60 mV and stepped to 0 mV for 50 ms. For ease of analysis, 10 mM Ba was substituted for Ca to enhance the amplitude of the current. Sodium currents were blocked with 1 μM TTX, and potassium currents were blocked with intracellular Cs. As shown in Fig. 6A, calcium channel currents were diminished to 30 ± 7% of control amplitudes (n = 8 of 11, P < 0.001) in the presence of 30 μM dopamine. Due to a combination of the rundown of calcium currents over time (which normally occurs with whole cell recording) and possible lingering dopaminergic effects, the currents in the cells tested only recovered to 67% of their original amplitudes. Application of 1 μM bromocriptine also attenuated calcium currents in M/T cells (Fig. 6B). Bromocriptine diminished calcium currents to 66 ± 5% of the original amplitude, with subsequent recovery to 83% of the original current amplitude (n = 8 of 12, P < 0.001). Although dopamine and bromocriptine had similar effects on evoked EPSPs, dopamine had a greater effect on calcium channel currents. A potential explanation for this discrepancy is that dopamine activates additional dopamine receptors that also attenuate calcium channel currents. In contrast to dopamine and bromocriptine, the reduction in calcium channel currents in response to application of 30 μM SKF38393 was no greater than that expected from normal current rundown (n = 7; Fig. 6C).

To further identify the type of calcium channels affected by dopamine, 30 μM dopamine was applied after the L-type channels had been blocked by nifedipine (10–100 μM). Nifedipine alone reduced the calcium channel current to 64 ± 5% of control (n = 9, P < 0.01). Application of dopamine blocked the nifedipine-resistant calcium channel currents by 29 ± 7% (Fig. 6D, n = 9, P < 0.001). These results suggest that dopamine can inhibit the dihydropyridine-resistant high-voltage calcium channels (N- and P/Q-type) previously implicated in transmitter release in the OB (Isaacson 2001; Isaacson and Strowbridge 1998). The effects of nifedipine at 10 μM were near saturating and not significantly different those at 100 μM (data not shown). The degree of calcium channel current block at either 10 or 100 μM nifedipine was similar to the 40% block with 10 μM nifedipine previously observed in these neurons (Trombley 1992).

**Discussion**

It has been recently reported that dopamine can modulate glutamate release from olfactory receptor neuron nerve terminals via a presynaptic D2 receptor-mediated mechanism (Berkowicz and Trombley 2000; Ennis et al. 2001; Hsia et al.
Dopamine modulates neurotransmission

Previous reports have shown that dopamine can modulate neurotransmission in the OB. In the turtle OB, exogenous dopamine application both delayed the onset and decreased the amplitude of field potentials recorded from mitral cells (Nourney et al. 1983). More recently, in the rat and turtle OB, olfactory sensory neuron activation of mitral cells was reduced in the presence of dopamine or D2 receptor agonists (Berkowicz and Trombley 2000; Ennis et al. 2001: Hsia et al. 1999). Ennis et al. (2001) also reported that dopamine inhibits olfactory sensory neuron activation of M/T cells in both rat and mouse and further demonstrated that dopamine inhibits olfactory sensory neuron activation of juxtaglomerular cells. Significantly, these effects were eliminated in D2 receptor knockout mice.

The present results are consistent with these previous reports. They also provide additional understanding of the role of

Glomeruli mediate feedback inhibition within glomeruli and lateral (feedforward) inhibition between glomeruli (Shepherd 1972). A large subset of PG cells is also dopaminergic (Gall et al. 1987; Kosaka et al. 1985), as is a subpopulation of superficial tufted cells (Halasz et al. 1981). The latter neurons make reciprocal dendrodendritic synapses with PG cells and often project their axon collaterals to synapse on PG and short axon cell bodies (Pinching and Powell 1971). Their axons and axon collaterals may not project outside the bulb, and the function of these neurons is not yet understood.

Although it has been reported that olfactory nerve transection reduces TH and dopamine expression in the OB (Brunjes et al. 1985), the present study provides immunocytochemical evidence that there is significant expression of TH (and perhaps dopaminergic transmission) in cultured neurons in the absence of olfactory sensory neuron input. The proportion of TH-immunoreactive neurons in our cultures is consistent with recent findings in cultured neurons (Cigola et al. 1998; Puche et al. 1999) and the intact bulb (McLean and Shipley 1998). Cigola and colleagues reported that a 48-h treatment with high external potassium concentrations, used to maintain membrane depolarization, increased baseline TH expression in mouse OB cultures 2.4-fold. Application of an L-type calcium channel antagonist blocked this increase in expression (Cigola et al. 1998). Puche and colleagues reported that co-culturing rat OB cells with olfactory epithelium also caused a 2.4-fold increase in TH expression. Interestingly, they found that application of an N-methyl-D-aspartate (NMDA) receptor antagonist could block this effect. These data suggest that calcium influx is an important modulator of TH expression and that the route of calcium entry may be either through NMDA receptors or voltage-gated calcium channels.

Our use of astrocyte feeder layers promotes high neuronal survival and rapid formation of synaptic contacts. Given this, our data may suggest that ongoing spontaneous synaptic activation of NMDA receptors and/or depolarization sufficient to activate voltage-gated calcium channels are adequate to maintain levels of TH expression in culture similar to those observed in the intact bulb (McLean and Shipley 1998). Alternatively, some expression of TH may be independent of these mechanisms. Differentiation between these hypotheses awaits further experimentation.

Synaptic organization and dopamine neurons in the OB

The axons of the olfactory sensory neurons enter the OB and innervate periglomerular (PG) neurons, superficial tufted cells, and the primary dendrites of mitral cells within glomeruli. Each glomerulus is thought to operate as a functional unit, and each responds best to a specific group of odors (Mombaerts 1999). The GABAergic PG neurons that circumscribe the glomeruli mediate feedback inhibition within glomeruli and lateral (feedforward) inhibition between glomeruli (Shepherd 1972). A large subset of PG cells is also dopaminergic (Gall et al. 1987; Kosaka et al. 1985), as is a subpopulation of superficial tufted cells (Halasz et al. 1981). The latter neurons make reciprocal dendrodendritic synapses with PG cells and often project their axon collaterals to synapse on PG and short axon cell bodies (Pinching and Powell 1971). Their axons and axon collaterals may not project outside the bulb, and the function of these neurons is not yet understood.

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Dopamine by demonstrating that dopamine can modulate excitatory transmission between OB M/T cells and interneurons. Our results, as well as the results of Brunig et al. (1999) described in the next paragraph, support the notion of functional D2 receptors on M/T cells. However, in contrast to our results, Ennis et al. (2001) reported that dopamine had no effect on mitral cell-evoked excitatory postsynaptic currents (EPSCs) in juxtaglomerular cells. Although the reason for this discrepancy is unclear, there are at least several possible explanations. The experiments by Ennis and colleagues were conducted in rats, whereas ours were in mice; there may be species differences (explain the different findings). In addition, their results by Brunig and colleagues may explain why the D1 receptor agonist SKF38393 did not reduce calcium channel currents beyond what could be attributed to current rundown (n = 7). In some of our experiments, we used synaptically coupled pairs of neurons; this allowed us to focus the effects of dopamine on the initial monosynaptic component of transmission between M/T cells and interneurons, while also eliminating polysynaptic or network effects. It is possible that these initial monosynaptic events are more sensitive to the effects of dopamine than are the mechanisms that underlie bursting behavior. Another possible explanation for these disparate findings is that mitral and tufted cells may differ in their sensitivity to dopamine. It is possible that many of our experiments included tufted cell-to-interneuron transmission. In contrast, the experiments by Ennis and colleagues may have included only mitral cell transmission, since five of the six cells examined involved direct stimulation of the mitral cell layer.

Collectively, these results are consistent with observations in other brain regions in the rat, including the parabrachial region (Chen et al. 1999), nucleus accumbens (Kalivas and Duffy 1997), and ventral tegmental area (Koga and Momiyama 2000), where dopamine also decreases glutamatergic neurotransmission. However, the effects of dopamine on neurotransmission are not limited to excitatory transmission. For example, GABAergic inputs to the rat striatum are also sensitive to dopamine receptor-mediated inhibition (Delgado et al. 2000).

Dopamine also modulates inhibitory transmission of OB neurons in culture. Brunig et al. (1999) reported that D1 receptor activation attenuates GABA-gated chloride currents in cultured interneurons and that stimulation of D2 receptors on cultured M/T cells potentiates GABA-gated chloride currents. These findings by Brunig and colleagues may explain why the D1 receptor agonist SKF38393 appeared to affect spontaneous activity in our experiments while not affecting other aspects of excitatory neurotransmission (e.g., evoked monosynaptic potentials, voltage-gated calcium currents, membrane resistance). That is, a SKF38393-mediated reduction in inhibition of GABAergic interneurons, which could potentiate inhibition of
M/T cells, could secondarily reduce spontaneous excitatory transmission.

Dopamine’s effects may be mediated via inhibition of calcium channel currents

In the present experiments, as in most of the cited examples, the effects of dopamine appear to be presynaptic. This interpretation is based on our finding that dopamine did not directly affect postsynaptic membrane resistance nor directly inhibit postsynaptic glutamate-mediated currents. It is also supported by previous OB studies demonstrating that dopamine has no effect on M/T cell NMDA, AMPA, or kainate receptors (Berkowicz and Trombley 2000), juxtaglomerular cell input resistance, or the amplitude of miniature excitatory postsynaptic currents (Ennis et al. 2001; Hsia et al. 1999). Furthermore, the possibility that postsynaptic effects on network behavior were indirectly mediating dopamine’s effects on excitatory transmission (as might occur with recording of spontaneous activity) was eliminated by recording from monosynaptically coupled pairs of identified M/T cells and interneurons.

It has been demonstrated in the turtle that dopamine reduces calcium influx in olfactory sensory neuron terminals (Wachowiak and Cohen 1999) and that presynaptic D2 receptor activation inhibits glutamate release from these terminals (Berkowicz and Trombley 2000). The fact that dopamine, or D2 receptor agonists, can reduce calcium channel currents evoked from presynaptic M/T cells lends further support to the notion that dopamine reduces excitatory transmission via reductions in glutamate release. Dopamine may have had a greater effect on calcium channel currents than bromocriptine because dopamine activates all dopamine receptors (more than 1 of which may affect calcium channels), whereas bromocriptine is selective for D2 receptors. That dopamine and bromocriptine had similar effects on monosynaptic transmission suggests that dopamine’s D2 effects on calcium channels is all that is necessary to account for dopamine’s effects on EPSPs. Our results on the effects of dopamine and D2 receptor agonists on calcium currents and glutamate release are consistent with recent results from experiments on ventral tegmental neurons (Koga and Momiyama 2000).

This is not the first examination of calcium channel currents in cultured OB neurons. From a holding potential of −60 mV, we previously observed calcium currents in cultured M/T cells that activated near −40 mV, peaked at 0 mV, and reversed near +55 (Trombley 1992; Trombley and Westbrook 1992). Transient or T-type currents were small and rarely observed, even from a holding potential of −100 mV where most T-type channels would be available for activation (Trombley 1992; Trombley and Westbrook 1992).

Dopamine receptors

The recently cloned dopamine receptors are typically grouped into either the D1 or D2 subfamily according to sequence homology and pharmacological attributes (Neve and Neve 1997; Sidhu and Niznik 2000). These receptors have a distinct laminar and cellular distribution throughout the OB. In the rat, D1 receptors are found in the glomerular, external plexiform, mitral cell, internal plexiform, and granule cell layers, whereas D2 receptors are located in the olfactory nerve, glomerular, and external plexiform layers (Coronas et al. 1997; Levey et al. 1993; Mansour et al. 1990; Nickell et al. 1991).

Although there is some controversy, several studies support the notion that M/T cells express D2 receptors. Although not specifically addressed in the text, autoradiographic in situ hybridization data presented by Mansour et al. (1990) suggest that D2 receptor mRNA is expressed in the mitral cell layer. Levey et al. (1993) reported D2 receptor immunoreactivity in the external plexiform layer as well as the glomerular and olfactory nerve layers. In a functional study, Brunig et al. (1999) demonstrated a D2 receptor-mediated action on GABA_A receptor-mediated currents in M/T cells. In the present study, we provide immunocytochemical evidence of D2 receptors on M/T cells. Furthermore, our electrophysiological data showing that dopamine and the D2 receptor agonist bromocriptine modulate calcium channels and excitatory transmission between M/T cells and interneurons indicate that these receptors are functional.

Dopamine receptors are metabotropic. Therefore each receptor subtype may interact with G-proteins specific to a certain cell type or location (Sidhu and Niznik 2000), resulting in cell type-specific effects. Brunig et al. (1999) have reported that dopamine modulates GABA_A receptors in rat OB in a cell-specific manner that involves differential effects at D1 and D2 receptors. Dopamine reduced currents through GABA-gated Cl− channels in interneurons via activation of D1 receptors and subsequent phosphorylation of GABA_A receptors by protein kinase A. In contrast, dopamine enhanced GABA-mediated responses in M/T cells via activation of D2 receptors and phosphorylation of GABA_A receptors by protein kinase C.

The two receptor subtypes also tend to have a specific synaptic deposition (Brunig et al. 1999; Nickell et al. 1991), which may allow dopamine to differentially modulate synaptic circuits. Results from the OB and other brain regions indicate that D2 receptors tend to play a presynaptic role and D1 receptors, a postsynaptic role, in neurotransmission (Hsu et al. 1995). Our results are consistent with the general notion that D2 receptor activation can reduce transmitter release. In contrast to the effects of the D1 selective agonist SKF38393, the D2 selective agonist bromocriptine mimicked dopamine’s inhibition of calcium channel currents and evoked EPSPs.

Significance to olfactory function

Olfactory experience has a significant effect on the concentration of both TH and dopamine within the OB (Baker et al. 1983). Olfactory deprivation, from either unilateral olfactory nerve transection or unilateral naris occlusion, reduces OB dopamine content in the ipsilateral bulb by as much as 75% (Baker et al. 1983) and enhances bulb responsiveness to odors, as measured by single-unit recordings and 2-deoxyglucose autoradiography (Guthrie et al. 1990; Wilson and Sullivan 1995). This enhanced responsiveness, as well as a decrease in odor discrimination, is mimicked by application of the D2 receptor antagonist, spiperone (Wilson and Sullivan 1995). Other behavioral experiments have shown that activation of D1 or D2 receptors can increase or decrease the threshold for odor detection, respectively (Doty and Risser 1989; Doty et al. 1998). These results suggest that dopamine may contribute to both odor detection sensitivity and discrimination. Previous electrophysiological studies, along with the present study, may
Dopamine may in some way affect glutamatergic transmission in the olfactory bulb (OB). However, a complete understanding of how dopamine may attenuate glutamatergic transmission by M/T cells requires further knowledge of what patterns of activity evoke dopamine release (e.g., olfactory sensory neuron and/or M/T cell stimulation of dopaminergic neurons) and whether the conditions of dopamine release can differentially affect circuits (e.g., synaptic transmission from olfactory sensory neuron to M/T cell, olfactory sensory neuron to juxtaglomerular cell, or M/T to interneuron).

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