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Dopamine Reduces Odor- and Elevated-K⁺-Induced Calcium Responses in Mouse Olfactory Receptor Neurons In Situ

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Abstract

Although D2 dopamine receptors have been localized to olfactory receptor neurons (ORNs) and dopamine has been shown to modulate voltage-gated ion channels in ORNs, dopaminergic modulation of either odor responses or excitability in mammalian ORNs has not previously been demonstrated. We found that <50 μ M dopamine reversibly suppresses odor-induced Ca²⁺ transients in ORNs. Confocal laser imaging of 300- μ m-thick slices of neonatal mouse olfactory epithelium loaded with the Ca²⁺-indicator dye fluo-4 AM revealed that dopaminergic suppression of odor responses could be blocked by the D2 dopamine receptor antagonist sulpiride (<500 μ M). The dopamine-induced suppression of odor responses was completely reversed by 100 μ M nifedipine, suggesting that D2 receptor activation leads to an inhibition of L-type Ca²⁺ channels in ORNs. In addition, dopamine reversibly reduced ORN excitability as evidenced by reduced amplitude and frequency of Ca²⁺ transients in response to elevated K⁺, which activates voltage-gated Ca²⁺ channels in ORNs. As with the suppression of odor responses, the effects of dopamine on ORN excitability were blocked by the D2 dopamine receptor antagonist sulpiride (<500 μ M). The observation of dopaminergic modulation of odor-induced Ca²⁺ transients in ORNs adds to the growing body of work showing that olfactory receptor neurons can be modulated at the periphery. Dopamine concentrations in nasal mucus increase in response to noxious stimuli, and thus D2 receptor-mediated suppression of voltage-gated Ca²⁺ channels may be a novel neuroprotective mechanism for ORNs.

INTRODUCTION

The vertebrate olfactory mucosa is composed of a sensory neuroepithelium resting on a lamina propria containing blood vessels, Bowman's glands, and nerve fibers. The olfactory mucosa receives extrinsic innervation from nervus terminalis luteinizing hormone-releasing hormone (LHRH)-containing fibers (Wirsig-Wiechmann 1993) and from a variety of trigeminal afferents that penetrate the basement membrane and extend to the apical portion of the neuroepithelium (Schaefer et al. 2002). Sympathetic fibers from the superior cervical ganglion (SCG) containing norepinephrine and dopamine (Kawano and Margolis 1985) innervate blood vessels as well as mucus producing Bowmans' glands (Chen et al. 1993; Getchell and Getchell 1992). Noxious stimuli induce a transient increase in dopamine concentration in mucus collected from the olfactory region of the nasal cavity (Lucero and Squires 1998). This suggests that dopamine, released from SCG fibers during noxious stimulation, would have access to olfactory receptor neurons (ORNs) not only via the mucus but also potentially via diffusion from the lamina propria. Thus as previously described for dopamine release onto ORN terminals in the olfactory bulb (Koster et al. 1999), dopamine released in the olfactory mucosa diffuses to receptors on ORNs rather than having specialized dopaminergic synapses.

Although D1, D3, or D4 dopamine receptors were not detected in olfactory mucosa using RT-PCR, in situ hybridization or ligand binding autoradiography (Coronas et al. 1997b), D2 dopamine receptor mRNA has been identified in olfactory mucosa and in ORNs (Coronas et al. 1997b; Koster et al. 1999). While D2-mediated modulation of olfactory signals is generally considered to occur at receptors on olfactory nerve terminals (Berkowicz and Trombley 2000; Ennis et al. 2001; Hsia et al. 1999; Wachowiak and Cohen 1999), there is evidence from studies of olfactory neuron primary cell cultures and immortalized cell lines that functional D2 dopamine receptors are present on ORN somata (Coronas et al. 1997a; Murrell and Hunter 1999; Vargas and Lucero 1999). One of the caveats of primary cell cultures and cell lines is that receptors or ion channels normally expressed in axons or nerve terminals will become inappropriately expressed when the axonal target is eliminated (Gilly et al. 1990). Therefore we used confocal Ca^{2+} imaging of acutely prepared slices of mouse olfactory epithelium to determine the functionality and role of D2 dopamine receptors in ORNs in situ. We found that dopamine decreased the amplitude of both odor- and elevated- K^{+} -evoked calcium transients, effects reversed by the D2 receptor antagonist sulpiride and the L-type Ca^{2+} channel blocker nifedipine. These data suggest that functional D2 dopamine receptors are present on ORN somata in vivo and decrease the odor sensitivity and excitability of ORNs on activation. Reduced excitability was confirmed by the observation that dopamine decreased the probability of obtaining a response to elevated K^{+} .

METHODS

Olfactory epithelium slice preparation

Neonatal mice (postnatal day 0–5) were quickly killed by decapitation, and their skin was removed from the skull. The lower jaw was removed. Tissue was mounted onto a vibratome-cutting block, supported by carrots, and 250- to 300- μm slices were cut (Fig. 1A). Ice-cold slices were transferred to oxygenated Ringer solution until ready to load with dye. Ringer solution contained (in mM) 140 NaCl, 5 KCl, 1 MgCl_2 , 2 CaCl_2 , 10 HEPES, 10 glucose, and 0.5 probenidol (to prevent dye loss). Elevated- K^{+} Ringer (high K^{+}) was the same as Ringers except that 140 mM KCl was substituted for 140 mM NaCl and mixed with normal Ringer to obtain the desired K^{+} concentration (50 mM to 100 mM K^{+}). The dopamine solution was made fresh daily in equal molar ascorbic acid to prevent oxidation. Control application of ascorbic acid did not elicit Ca^{2+} responses in cells from six different slices. Slices were loaded with 18 μM fluo-4 AM (Molecular Probes, Eugene, OR) for 90 min at 25°C. A stock solution of fluo-4 AM was prepared in DMSO containing 20% pluronic F-127. Ringer solution was added to this stock solution for a final concentration of 18 μM fluo-4 AM, 0.08% pluronic F-127, and 0.4% DMSO.

Intracellular calcium measurements

We measured intracellular calcium using laser scanning confocal imaging of fluo-4-loaded olfactory epithelium slices attached to glass coverslips with 5% agarose (Sigma type VII) and placed in a laminar flow chamber (Warner Instruments, Hamden, CT). Dye-free Ringer solution at a flow rate of 1.5–2.0 ml/min was continuously perfused over the slice for 15 min prior to starting the experiment. Recordings were made between 50 and 100 μm below the surface of the slice to avoid damaged cells. Test solutions were applied using bath exchange and a 200- μl -volume loop injector ($\blacktriangle$ in Figs. 2–7). The concentrations of test solutions that actually reach the cells at the recording depth in the slice are unknown; however, measurements of fluorescein application indicate a 90% reduction in fluorescence intensity in the tissue compared with bath, suggesting that applied test solutions are also reduced by 90%. In RESULTS, we provide the initial test solution concentration rather than the ~10% concentration that reaches the cell. An odor mixture of 10 μM *r*-carvone and 10 μM amyl acetate was made fresh daily and is referred to as odor throughout. Because odor responses did not significantly differ

in slices obtained from neonates of different ages, the data from P0 to P5 mice were grouped. Dopamine, the D2 receptor antagonist sulpiride, the L-type Ca^{2+} channel inhibitor nifedipine, or the selective inhibitor of I_h channels ZD7288 (Tocris) (Sciancalepore and Constanti 1998) were continuously perfused for 5 min prior to odor or elevated- K^+ application ($\square$ in Figs. 2–7). Dopamine, sulpiride, nifedipine, or ZD7288 did not elicit Ca^{2+} responses when applied alone (data not shown). A pilot study showed that inclusion of 500 nM tetrodotoxin (TTX) had no effect on elevated- K^+ responses, and so TTX was not included in bath or test solutions.

A Zeiss LSM 510 confocal laser scanning system attached to a Zeiss upright Axioskop 2 FS motorized microscope was used, and data analysis was performed using Zeiss software. The system consists of a krypton-argon ion laser used for fluorescence excitation. The total laser power was 25 mW. To reduce dye bleaching and protect slices from possible photo damage, the excitation wavelength transmission was $<5\%$ of the total power. The laser intensity and scale was adjusted so that basal fluorescence intensity in arbitrary units was 35–50 on a scale with a maximum of 255 to avoid saturation during responses. Increases in fluorescence $>10\%$ above baseline fluorescence fluctuations were considered responses. All traces were baseline corrected by subtracting a linear fit to the first 20 s of recording and normalized as the change in fluorescence intensity/the maximum fluorescence intensity of 255 and multiplied by 100 ($\Delta F/F$). Fluorescence emissions were long pass filtered at 510 nm. Time series experiments were performed collecting 1024×1024 pixel images at 0.5 Hz.

Data analysis

In general, each slice was exposed to only three applications of test compounds and a Ringer control given at 5- to 10-min intervals. Occasionally, if the initially selected region showed low activity after the first application, a new region was selected. Thus a maximum of four test applications, a Ringer control, and the fluorescein application were performed on a given slice. Only ORNs that responded to the final test application were included in the data analysis. Data for effects of dopamine, sulpiride, nifedipine, or ZD7288 on odor responses were collected from a total of 78 slices (3–11 slices/treatment; $n = 67$ animals from 28 litters) while high- K^+ data were collected from a total of 11 slices (2–4 slices/treatment; $n = 16$ animals from 6 litters). Linear rundown occurred during multiple applications of odor or high K^+ (see Fig. 2A). To determine if there were treatment effects, we performed a linear regression on the peak amplitude of the first and last Ca^{2+} responses of every ORN and calculated predicted peak amplitude for the second application from the regression. Paired Student's t -test was used to determine significant differences ($P < 0.05$) between the predicted peak amplitude and the observed peak amplitude from each cell. The actual P values are given in the text. All averages are presented as means $\pm$ SE.

RESULTS

Odorant binding to G-protein-coupled receptors on the cilia of ORNs activate an adenylyl-cyclase-III-mediated cascade and open cyclic nucleotide-gated cation (CNG) channels. Nonselective CNG channels allow Ca^{2+} entry, which in turn activates Ca-dependent Cl^- channels. Because of high intracellular Cl^- in mammalian ORNs, opening Cl^- channels provides the majority of the depolarizing receptor potential (Lowe and Gold 1993). ORNs also contain voltage-gated Ca^{2+} channels (Trombley and Westbrook 1991) and release Ca^{2+} from intracellular stores (Zufall et al. 2000). Thus odor activation of ORNs results in both a depolarizing receptor potential and robust transient changes in intracellular Ca^{2+} in the dendritic knob and soma (Fig. 1, B–E). In addition, depolarizing ORNs with elevated extracellular K^+ directly activates voltage-gated Na^+ and Ca^{2+} channels and increases intracellular Ca^{2+} concentrations ($[\text{Ca}^{2+}]_i$). Thus Ca^{2+} increases associated with elevated K^+ are routinely used as a measure of neuronal excitability.

Dopamine reduces odor-induced Ca^{2+} responses in ORNs

In the present study, we initially hypothesized that by inhibiting adenylyl cyclase activity, dopamine may reduce excitability and odor responsiveness of ORNs. We used confocal Ca^{2+} imaging of slices of olfactory epithelium to visualize intracellular Ca^{2+} changes in ORNs in response to odor and elevated K^+ (Fig. 1). Initial control experiments in which we applied odor at 10-min intervals revealed a $24 \pm 3\%$ ($n = 158$ cells) linear rundown in the peak amplitude of the odor-induced Ca^{2+} transient as determined by fitting a linear regression between the first and last odor application (i.e., see Fig. 2A). Based on linear regression, the predicted amplitude of the middle odor application was not different from the actual amplitude both in the single cell shown in Fig. 2A and in the average of 48 cells in Fig. 2B (paired Student's t -test, $P = 0.19$). In contrast, when the middle odor application was preceded by and concomitant with perfusion of $50 \mu\text{M}$ dopamine, we observed two types of responses; in 29% of the cells, the $8 \pm 5\%$ reduction in the middle response was neither significantly different from control nor predicted ($n = 6/21$; paired Student's t -test, $P > 0.3$). However, in 71% of the odor responsive cells, there was a significant $42 \pm 4\%$ reduction in the Ca^{2+} transient amplitude ($n = 15/21$; paired Student's t -test, $P = 0.002$). Figure 2C shows Ca^{2+} transients from a single cell, whereas Fig. 2D shows the average percentage change in fluorescence for all 21 cells that were exposed to $50 \mu\text{M}$ dopamine. Dopamine induced a significant reduction in the mean observed Ca^{2+} transient compared with predicted ($n = 21$; paired Student's t -test, $P < 0.04$), even when including the six cells that did not show a significant response to dopamine.

Dopamine suppresses odor-induced Ca^{2+} responses through D2 dopamine receptors

Although only D2 dopamine receptors are known to occur in olfactory mucosa, we confirmed that dopamine acted through the D2 pathway by application of sulpiride, a D2 receptor antagonist. Sulpiride should bring odor responses back up to control levels or higher depending on basal dopamine concentrations. Figure 3, A and B, shows that the maximum sulpiride dose ($500 \mu\text{M}$) resulted in a slight increase in Ca^{2+} responses (Fig. 3A, *middle*), such that the mean was slightly larger than predicted (Fig. 3B, ■). The possibility that sulpiride was blocking endogenous dopamine activity was investigated further in the elevated- K^+ experiments in the following text.

In a two-dose study, exposure to $50 \mu\text{M}$ sulpiride did not prevent the dopamine-induced reduction in odor responses, and the mean response was significantly smaller than predicted (see Fig. 3, C and D, $n = 30$; paired Student's t -test, $P = 0.03$). In contrast, $500 \mu\text{M}$ sulpiride completely inhibited the effect of dopamine such that the predicted amplitude was not different from the observed (Fig. 3, E and F, $n = 20$; paired Student's t -test, $P = 0.57$). Collectively, our data show that functional D2 dopamine receptors are present in acutely prepared slices of mouse olfactory epithelium and that dopamine reduces odor-activated Ca^{2+} responses at the level of the primary sensory neurons. However, it is unclear whether dopamine reduces odor responsiveness directly, by inhibiting the olfactory specific adenylyl cyclase III, or indirectly, by second-messenger-mediated modulation of voltage-gated ion channels.

Our previous study using cultured rat ORNs showed that dopamine reversibly inhibited the I_h channel via the D2 dopamine receptor, which acts through the G_i protein to reduce adenylyl cyclase activity (Vargas and Lucero 1999). We tested whether the dopamine-induced reduction in odor responses was due to reduced I_h by recording odor responses in the presence and absence of the I_h channel-specific inhibitor ZD7288. We found that application of $100 \mu\text{M}$ ZD7288, using the same protocol as in the dopamine applications in Fig. 2C, had no effect on the amplitude of odor responses (data not shown; $P = 0.92$; $n = 12$ cells from 4 slices from 2 litters). These data indicate that under our experimental conditions (25°C , 5 mM KCl bath), there was no effect of I_h on the odor-induced Ca^{2+} transient. Therefore we investigated the

effects of dopamine on excitability by measuring Ca^{2+} responses to elevated extracellular K^+ .

Dopamine reduces excitability through D2 dopamine receptors

Elevated K^+ (100 mM) was used to depolarize ORNs and activate voltage-gated Ca^{2+} channels. Figure 4, A and B, shows Ca^{2+} transients in a single cell and averaged responses from 12 cells. The predicted amplitude of the middle response obtained by linear regression from the first and third responses was not different from the observed (paired Student's *t*-test, $P = 0.31$). As with odor application, the elevated- K^+ responses in the presence of 50 μM dopamine fell into two groups: 33% of the cells (7/21) were not significantly inhibited by dopamine ($P = 0.6$), whereas in 66% of the cells (14/21), dopamine reversibly reduced the observed elevated- K^+ response from the predicted by $64 \pm 8\%$ (paired Student's *t*-test, $P < 0.01$). Figure 4C shows K^+ -induced Ca^{2+} transients from a single cell, whereas D shows the average $\% \Delta F/F$ for all 21 cells that were exposed to 50 μM dopamine. Dopamine induced a significant $32 \pm 12\%$ reduction in the mean observed Ca^{2+} transient compared with expected ($n = 21$; paired Student's *t*-test, $P < 0.01$) even when including the seven cells that did not show a significant response to dopamine. These data indicate that 50 μM dopamine reduces excitability in the majority of ORNs. A higher concentration of dopamine (500 μM) increased the percentage of cells that were reversibly suppressed by dopamine to 90% (45/50 cells) and reversibly reduced the elevated- K^+ response by $96 \pm 2\%$ (see following text and Fig. 6).

To confirm that dopamine was acting specifically through D2 dopamine receptors, we repeated these experiments in the presence of the D2 antagonist sulpiride. We found that elevated- K^+ responses were significantly enhanced by $13 \pm 6\%$ in the presence of 500 μM sulpiride as would be expected if endogenous dopamine in the slice tonically reduced ORN excitability (Figs. 5, A and B; $n = 19$; paired Student's *t*-test, $P = 0.01$). Consistent with this observation, we found that application of 500 μM sulpiride completely inhibited the dopamine-induced reduction in the amplitude of K^+ responses (Fig. 5, C and D; $n = 10$, paired Student's *t*-test, $P = 0.23$). Thus our data show that both endogenous and exogenous dopamine reversibly reduce the amplitude of elevated- K^+ responses through D2 receptors, suggesting that dopamine decreases ORN excitability.

In addition to reducing the amplitude of Ca^{2+} transients, we found that the number of cells responding to elevated K^+ (frequency of response) decreased with increasing dopamine concentrations. In Fig. 6A, we show only the second and third Ca^{2+} transients in response to K^+ application for clarity. In the absence of dopamine superfusion (0 μM dopamine), 100% of the cells that responded to the third K^+ application also responded to the second application. When 50 μM dopamine was included in the second K^+ application, 80% of the cells responded to K^+ , whereas only 20% responded in the presence of 500 μM dopamine, but on washout, the cells showed robust elevated- K^+ responses (Fig. 6, A and B). When the ratio of peak amplitudes of the second to the third responses of all of the cells are plotted, we see that in the absence of dopamine, the peak of the Gaussian fit centers near 1, but in the presence of dopamine, the ratio shifts toward 0 (Fig. 6C). Collectively, our experiments using elevated K^+ indicate that dopamine acts through D2 dopamine receptors to significantly reduce the excitability of ORNs in the absence of odor stimulation.

Dopamine reduces odor responses via Ca^{2+} channel inhibition

To determine whether the odor-suppressive effects of dopamine were mediated via voltage-gated channels alone or in concert with cAMP-gated channels, we blocked the voltage-gated Ca^{2+} channels with nifedipine and minimized I_h by recording at room temperature in low (5 mM) external K^+ . We found that 50 μM nifedipine (Fig. 7G) and 100 μM nifedipine (Fig. 7, A, B, and G) inhibit $35 \pm 8\%$ ($n = 20$; $P < 0.05$) and $34 \pm 7\%$ ($n = 8$, $P = 0.01$), respectively,

of the odor-induced Ca^{2+} transient. In subsequent experiments, we continuously superfused either 50 or 100 μM nifedipine over the slice and tested the effects of dopamine on odor-induced Ca^{2+} transients. In the presence of 50 μM nifedipine, dopamine still caused a significant $25 \pm 9\%$ decrease in the odor-induced Ca^{2+} transient (Fig. 7, C, D, and G; $n = 11$; $P < 0.01$). However, 100 μM nifedipine completely reversed the dopamine-mediated suppression of odor-induced Ca^{2+} transients (Fig. 7, E–G; $n = 9$, $P = 0.63$). These data indicate that the majority of the suppressive effect of dopamine on odor-induced Ca^{2+} transients is due to reduction of voltage-gated Ca^{2+} channels.

DISCUSSION

Dopamine reduces odor and elevated- K^+ responses

Ca^{2+} imaging of slice preparations of olfactory epithelium using confocal microscopy reveals that dopamine reversibly reduces odor-induced Ca^{2+} transients in the majority of responding ORNs from neonatal mice. The dopamine effects could be completely blocked by the specific D2 dopamine antagonist, sulpiride, suggesting that they are mediated through D2 dopamine receptor activation. Although inappropriate somata expression of D2 dopamine receptors may occur in cell lines or primary culture systems, it is unlikely, given the short time period between slice preparation and recording, that the D2 dopamine receptors were being inappropriately expressed in the soma during our *in situ* recordings.

To test whether dopamine affected excitability of ORNs in the absence of odors, we applied elevated K^+ to the slices. We found that dopamine reversibly reduced both the amplitude and frequency of Ca^{2+} responses to elevated K^+ in ORNs in a dose-dependent manner. As with the inhibition of the odor responses, dopamine's effects on excitability were completely blocked by the D2 antagonist sulpiride.

Mechanism for dopamine effects

The D2 dopamine receptors are coupled to the inhibitory G protein G_i , which generally inhibits adenylyl cyclase (Senogles 1994). ORNs contain an olfactory specific type III adenylyl cyclase that plays a key role in odor transduction (Bakalyar and Reed 1990). Previous studies show that D2 receptor agonists inhibit both basal- and odor-activated adenylyl cyclase activity in membranes from olfactory mucosa (Coronas et al. 1999; Mania-Farnell et al. 1993). Depending on the actual location of receptors, dopamine could reduce ciliary adenylyl cyclase activity to reduce odor responses or it could reduce somal cAMP levels and in turn affect voltage-gated ion channels. In rat, the olfactory I_h currents increase with internal perfusion of cAMP or PKA and dopamine acts through D2 receptors to reduce the I_h current (Vargas and Lucero 1999). In various other species, FMRFamide (Park et al. 2003), GnRH (Park and Eisthen 2003), dopamine and serotonin (Wetzel et al. 2001), and adrenalin (Kawai et al. 1999) also affect voltage-gated ion channels in ORNs. In the present study, we found that inclusion of 500 nM TTX had no effect on the elevated- K^+ responses, suggesting that at the frequency resolution of our system (0.5 Hz), voltage-gated Na^+ channels are too fast to significantly contribute to the elevated- K^+ responses. The remaining candidates are voltage-gated Ca^{2+} channels of which only the L-type has been positively identified in mammalian ORNs (Okada et al. 2003; Trombley and Westbrook 1991). D2 dopamine receptor activation has been recently shown to inhibit L-type Ca^{2+} currents in isolated ORNs (Okada et al. 2003). Therefore it is plausible that dopamine-induced reductions in odor and elevated- K^+ responses in ORNs are due to D2 receptor-mediated effects on Ca^{2+} channels. Our data showing that the L-type Ca^{2+} channel blocker nifedipine was sufficient to completely reverse dopamine effects on odor responses suggest that the major mechanism for reduction of odor or elevated- K^+ -induced Ca^{2+} transients in ORNs is via inhibition of L-type Ca^{2+} channels.

In heterologous expression systems, dopamine or D2 antagonists have been found to cause an open channel block of Ca^{2+} channels (Santi et al. 2002). If dopamine was directly blocking Ca^{2+} channels in our system, then co-application of dopamine and sulpiride should exacerbate the block. Because co-application leads to recovery of odor or K^{+} responses, we conclude that direct block of ion channels by dopamine is not a mechanism in this system.

Differential effects of sulpiride on odor and elevated- K^{+} responses

Control odor responses were not different from those measured in the absence of exogenous dopamine and presence of sulpiride, whereas sulpiride alone significantly increased elevated- K^{+} calcium transients. These observations are consistent with the idea that endogenous dopamine is present in the slice and is affecting cAMP levels in the same compartment as the voltage-gated Ca^{2+} channels.

Physiological significance

Our finding of dopaminergic suppression of odor-induced Ca^{2+} transients adds to the growing list of physiologically relevant molecules that modulate odor sensitivity at the level of the olfactory receptor neuron. Recent studies showing that in addition to dopamine, ATP (Hegg et al. 2003), GnRH (Park and Eisthen 2003) and FMRFamide (Park et al. 2003) reversibly alter odor responses and suggest that peripheral modulation of odor sensitivity may occur under a number of circumstances. Thus in addition to noxious odors that cause release of dopamine into nasal mucus, it is possible that trigeminal afferents able to release ATP, substance P, or CGRP, and terminal nerve fibers known to synthesize GnRH are all potential modulators of ORN sensitivity. D2 dopamine receptor-mediated reductions in Ca^{2+} signaling during trauma may be a neuroprotective mechanism to prevent Ca^{2+} overload and toxicity in the olfactory epithelium.

What effect would a dopamine-induced reduction of L-type Ca^{2+} channels have on ORN signaling? Blocking Ca^{2+} channels in ORNs has a dramatic effect on their ability to fire action potentials (Madrid et al. 2003). Specifically, the reduced Ca^{2+} influx prevents activation of Ca^{2+} -gated K^{+} channels, resulting in a shift from a cell that fires tonically in response to a receptor potential to a phasic cell capable of only firing one or two action potentials. Thus dopaminergic modulation is predicted to both decrease intracellular Ca^{2+} and change the signaling properties of the cell. Further work will be required to determine if neuropeptides or other neuromodulators released into the olfactory epithelium by hormonal changes, stress, or noxious odors have similar odor-suppressive effects.

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REFERENCES

- Bakalyar HA, Reed RR. Identification of a specialized adenylyl cyclase that may mediate odorant detection. *Science* 1990;250:1403–1406. [PubMed: 2255909]
- Berkowicz DA, Trombley PQ. Dopaminergic modulation at the olfactory nerve synapse. *Brain Res* 2000;855:90–99. [PubMed: 10650134]
- Chen Y, Getchell TV, Sparks DL, Getchell ML. Patterns of adrenergic and peptidergic innervation in human olfactory mucosa: age-related trends. *J Comp Neurol* 1993;334:104–116. [PubMed: 8104961]

- Coronas V, Feron F, Hen R, Sicard G, Jourdan F, Moysé E. In vitro induction of apoptosis or differentiation by dopamine in an immortalized olfactory neuronal cell line. *J Neurochem* 1997a; 69:1870–1881. [PubMed: 9349530]
- Coronas V, Krantic S, Jourdan F, Moysé E. Dopamine receptor coupling to adenylyl cyclase in rat olfactory pathway: a combined pharmacological-radioautographic approach. *Neuroscience* 1999;90:69–78. [PubMed: 10188934]
- Coronas V, Srivastava LK, Liang JJ, Jourdan F, Moysé E. Identification and localization of dopamine receptor subtypes in rat olfactory mucosa and bulb: a combined in situ hybridization and ligand binding radioautographic approach. *J Chem Neuroanat* 1997b;12:243–257. [PubMed: 9243344]
- Ennis M, Zhou FM, Ciombor KJ, Aroniadou-Anderjaska V, Hayar A, Borrelli E, Zimmer LA, Margolis F, Shipley MT. Dopamine D2 receptor-mediated presynaptic inhibition of olfactory nerve terminals. *J Neurophysiol* 2001;86:2986–2997. [PubMed: 11731555]
- Getchell ML, Getchell TV. Fine structural aspects of secretion and extrinsic innervation in the olfactory mucosa. *J Microsc Res Tech* 1992;23:111–127.
- Gilly WF, Lucero MT, Horrigan FT. Control of the spatial distribution of sodium channels in giant fiber lobe neurons of the squid. *Neuron* 1990;5:663–674. [PubMed: 2171590]
- Hegg CC, Greenwood D, Huang W, Han P, Lucero MT. Activation of purinergic receptor subtypes modulates odor sensitivity. *J Neurosci* 2003;23:8291–8301. [PubMed: 12967991]
- Hsia AY, Vincent JD, Lledo PM. Dopamine depresses synaptic inputs into the olfactory bulb. *J Neurophysiol* 1999;82:1082–1085. [PubMed: 10444702]
- Kawai F, Kurahashi T, Kaneko A. Adrenaline enhances odorant contrast by modulating signal encoding in olfactory receptor cells. *Nat Neurosci* 1999;2:133–138. [PubMed: 10195196]
- Kawano T, Margolis F. Catecholamines in olfactory mucosa decline following superior cervical ganglionectomy. *Chem Senses* 1985;10:353–356.
- Koster NL, Norman AB, Richtand NM, Nickell WT, Puche AC, Pixley SK, Shipley MT. Olfactory receptor neurons express D2 dopamine receptors. *J Comp Neurol* 1999;411:666–673. [PubMed: 10421875]
- Lowe G, Gold GH. Nonlinear amplification by calcium-dependent chloride channels in olfactory receptor cells. *Nature* 1993;366:283–286. [PubMed: 8232590]
- Lucero MT, Squires A. Catecholamine concentrations in rat nasal mucus are modulated by trigeminal stimulation of the nasal cavity. *Brain Res* 1998;807:234–236. [PubMed: 9757051]
- Madrid R, Sanhueza M, Alvarez O, Bacigalupo J. Tonic and phasic receptor neurons in the vertebrate olfactory epithelium. *Biophys J* 2003;84:4167–4181. [PubMed: 12770919]
- Mania-Farnell BL, Farbman AI, Bruch RC. Bromocriptine, a dopamine D2 receptor agonist, inhibits adenylyl cyclase activity in rat olfactory epithelium. *Neuroscience* 1993;57:173–180. [PubMed: 7904056]
- Murrell JR, Hunter DD. An olfactory sensory neuron line, *Odora*, properly targets olfactory proteins and responds to odorants. *J Neurosci* 1999;19:8260–8270. [PubMed: 10493727]
- Okada Y, Miyamoto T, Toda K. Dopamine modulates a voltage-gated calcium channel in rat olfactory receptor neurons. *Brain Res* 2003;968:248–255. [PubMed: 12663094]
- Park D, Eisthen HL. Gonadotropin releasing hormone (GnRH) modulates odorant responses in the peripheral olfactory system of axolotls. *J Neurophysiol* 2003;90:731–738. [PubMed: 12672784]
- Park D, Zawacki SR, Eisthen HL. Olfactory signal modulation by molluscan cardioexcitatory tetrapeptide (FMRFamide) in axolotls (*Ambystoma mexicanum*). *Chem Senses* 2003;28:339–348. [PubMed: 12771020]
- Santi CM, Cayabyab FS, Sutton KG, McRory JE, Mezeyova J, Hamming KS, Parker D, Stea A, Snutch TP. Differential inhibition of T-type calcium channels by neuroleptics. *J Neurosci* 2002;22:396–403. [PubMed: 11784784]
- Schaefer ML, Bottger B, Silver WL, Finger TE. Trigeminal collaterals in the nasal epithelium and olfactory bulb: a potential route for direct modulation of olfactory information by trigeminal stimuli. *J Comp Neurol* 2002;444:221–226. [PubMed: 11840476]
- Sciancalepore M, Constanti A. Inward-rectifying membrane currents activated by hyperpolarization in immature rat olfactory cortex neurones in vitro. *Brain Res* 1998;814:133–142. [PubMed: 9838080]

- Senogles SE. The D2 dopamine receptor isoforms signal through distinct G α proteins to inhibit adenylyl cyclase. A study with site-directed mutant G α proteins. *J Biol Chem* 1994;269:23120–23127. [PubMed: 7916015]
- Trombley PQ, Westbrook GL. Voltage-gated currents in identified rat olfactory receptor neurons. *J Neurosci* 1991;11:435–444. [PubMed: 1992011]
- Vargas G, Lucero MT. Dopamine modulates inwardly rectifying hyperpolarization-activated current (I_h) in cultured rat olfactory receptor neurons. *J Neurophysiol* 1999;81:149–158. [PubMed: 9914276]
- Wachowiak M, Cohen LB. Presynaptic inhibition of primary olfactory afferents mediated by different mechanisms in lobster and turtle. *J Neurosci* 1999;19:8808–8817. [PubMed: 10516300]
- Wetzel CH, Spehr M, Hatt H. Phosphorylation of voltage-gated ion channels in rat olfactory receptor neurons. *Eur J Neurosci* 2001;14:1056–1064. [PubMed: 11683897]
- Wirsig-Wiechmann CR. Peripheral projections of nervus terminalis LHRH-containing neurons in the tiger salamander, *Ambystoma tigrinum*. *Cell Tissue Res* 1993;273:31–40. [PubMed: 8364959]
- Zufall F, Leinders-Zufall T, Greer CA. Amplification of odor-induced Ca²⁺ transients by store-operated Ca²⁺ release and its role in olfactory signal transduction. *J Neurophysiol* 2000;83:501–512. [PubMed: 10634891]

FIG. 1.

Confocal imaging of odor responses in olfactory epithelium slices. **A**: representative olfactory epithelium slice (300 μm) made from mouse (postnatal day 0–5). Scale bar = 500 μm . **B–D**: confocal images from a fluo-4 AM-loaded mouse olfactory epithelium (OE) slice before (**B**), during (**C**), and after (**D**) application of odors (10 μM *n*-amyl acetate + 10 μM *r*-carvone). Odor-responsive olfactory receptor neurons (ORNs) are outlined in white. Note that in this focal plane, some ORNs have only the soma outlined. The arrowhead indicates the cell used for odor transients shown in **E**. Scale bars = 10 μm . **E**: time course of odor- and high- K^+ -evoked Ca^{2+} transients elicited from one ORN in the slice shown in **B–D**. Black triangles correspond to the time of loop injection of the odors high K^+ or Ringer.

FIG. 2.

Dopamine reduces odor-evoked calcium transients. Fluo-4 AM loaded olfactory epithelium slices were superfused with multiple applications of 10 μ M *n*-amyl acetate + 10 μ M *r*-carvone (odor) in the absence (A and B) or presence (C and D) of 50 μ M dopamine (DA). A and C: representative odor-evoked Ca^{2+} transients in the absence (A) and presence (C) of 50 μ M dopamine. - - -, linear regressions of the peak amplitudes from the 1st and 3rd Ca^{2+} transients in this and all subsequent figures. B and D: average peak Ca^{2+} transient amplitudes are shown (mean $\pm$ SE), as are the predicted peak amplitudes for the 2nd application (■). B: $n = 48$ cells from 11 slices; D: $n = 21$ cells/3 slices. * $P < 0.05$, paired Student's *t*-test on predicted vs. observed values.

FIG. 3.

D2 dopamine receptors mediate dopamine-evoked reduction in odor response. *A*, *C*, and *E*: representative odor responses in the presence of 50–500 μ M D2 dopamine receptor antagonist sulpiride, or dopamine (DA; 50 μ M) + sulpiride. ORNs were exposed to dopamine and sulpiride for 5 min prior to odor superfusion. *B*, *D*, and *F*: average peak Ca^{2+} transient amplitudes are shown (means $\pm$ SE) as are the predicted peak amplitudes for the 2nd application (■). $n = 20$ cells/4 slices (*B*), 30 cells/7 slices (*D*), and 20 cells/4 slices (*F*). *B*: sulpiride (500 μ M) did not significantly affect odor responses: $P > 0.05$, paired Student's t -test performed on predicted vs. observed values. *D*: 50 μ M dopamine significantly reduced odor responses even in the presence of 50 μ M sulpiride: $*P < 0.05$, paired Student's t -test as in *B*. *F*: 500 μ M sulpiride blocked the suppressive effect of dopamine: $P > 0.05$, paired Student's t -test as in *B*.

FIG. 4. High-K⁺ Ringer-evoked calcium transients are reduced in the presence of dopamine. *A* and *C*: representative high-K⁺-evoked Ca²⁺ transients elicited by multiple applications of 100 mM K⁺ Ringer in the absence (*A*) or presence (*C*) of 50 μ M dopamine. *B* and *D*: average peak Ca²⁺ transient amplitudes are shown (means $\pm$ SE) as are the predicted peak amplitudes for the 2nd application (■). $n = 12$ cells/2 slices and 21 cells/4 slices, respectively. * $P < 0.05$, paired Student's *t*-test on predicted vs. observed values.

FIG. 5.

D2 dopamine receptor activation reduces high-K⁺-evoked Ca²⁺ transients. **A** and **C**: representative 100 mM K⁺-evoked Ca²⁺ transients in the presence of 500 μ M sulpiride or dopamine + sulpiride. ORNs were exposed to dopamine and sulpiride for 5 min prior to high-K⁺ superfusion. - - -, linear regressions on the peak amplitudes of the 1st and 3rd calcium transients. **B** and **D**: average peak Ca²⁺ transient amplitudes are shown (means $\pm$ SE) as are the predicted peak amplitudes for the 2nd application (■). $n = 19$ cells/3 slices and 10 cells/2 slices, respectively. * $P < 0.05$, paired Student's t -test on predicted vs. observed values. Sulpiride prevented both basal and exogenous dopamine reductions in high-K⁺-induced Ca²⁺ transients.

FIG. 6. Dopamine decreases the probability of obtaining a high-K⁺ response. *A*: representative Ca²⁺ responses to 50 mM K⁺ (transients not shown), 50 mM K⁺ + dopamine (0–500 μM), and a 3rd 50 mM K⁺ “recovery” were measured from fluo-4 AM loaded mouse olfactory epithelial slices using confocal microscopy. Note that the first high-K⁺-evoked Ca²⁺ transient is not shown. *B*: summary of the frequency of response to high K⁺ in the presence of dopamine. Note that the probability of obtaining a response decreases in the presence of increasing dopamine concentrations. The number of ORNs = 47, 65, and 48, respectively. *C*: the ratios of Ca²⁺ transient amplitudes from the 2nd high-K⁺ + dopamine (0 or 500 μM) response, over the 3rd high-K⁺ “recovery” response were binned at 0.5 intervals and plotted as a frequency histogram. Gaussian fits center over a ratio of 1 for the 0 μM dopamine control group, whereas ratios in the 500 μM dopamine group are shifted toward 0.

FIG. 7.

Nifedipine block of Ca^{2+} channels eliminates the suppressive effect of dopamine on odor-induced Ca^{2+} transients. **A:** representative odor responses in the presence and absence of Ringer or 100 μM nifedipine (100 nif). **B:** averaged Ca^{2+} transient amplitudes show that 100 μM nifedipine significantly inhibits observed odor responses compared with predicted (■; * $P = 0.01$, paired Student's t -test). **C** and **E:** representative odor responses during constant bath application of 50 or 100 μM nifedipine in the presence and absence of 50 μM dopamine (DA). **D** and **F:** averaged Ca^{2+} transient amplitudes show that in the presence of 50 μM nifedipine dopamine suppresses odor responses ($P = 0.01$, paired Student's t -test) but that in 100 μM nifedipine, dopamine does not suppress odor responses ($P = 0.63$, paired Student's t -test). **G:**

averaged percentage reduction of the observed odor responses compared with control (mean $\pm$ SE; $*P < 0.03$, paired Student's t -test).