Dopamine Modulates Inwardly Rectifying Hyperpolarization-Activated Current ($I_h$) in Cultured Rat Olfactory Receptor Neurons

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Vargas, Gricelly and Mary T. Lucero. Dopamine modulates inwardly rectifying hyperpolarization-activated current ($I_h$) in cultured rat olfactory receptor neurons. J. Neurophysiol. 81: 149–158, 1999. The presence of dopamine receptors in olfactory receptor neurons (ORNs) suggests that odor sensitivity may be modulated by neurotransmitters at the level of primary sensory neurons. Using standard patch-clamp techniques on rat ORNs, we found that 1 µM dopamine, 500 µM SQ 22536 (SQ, an adenylyl cyclase inhibitor), 20 and 50 µM quinpirole (a selective dopamine D$_2$ receptor agonist), and 1 mM adenosine 3',5'-cyclic monophosphate (cAMP) modulate the hyperpolarization-activated current $I_h$. On hyperpolarizing from a holding potential of −58 mV, a small Cs$^+$-sensitive inwardly rectifying current was observed. Increases in extracellular K$^+$ increased $I_h$ amplitude without shifting its voltage dependence of activation, whereas increases in temperature produced an increase in $I_h$ amplitude and a hyperpolarizing shift in the activation curve. Application of 1 µM dopamine reversibly shifted $I_h$ activation to more negative potentials and decreased $I_h$ current amplitudes. These effects were blocked by concomitant application of dopamine with sulpiride, a selective dopamine D$_2$ receptor antagonist. The effects of dopamine were mimicked by quinpirole. Quinpirole (20 µM) decreased $I_h$ current amplitude, but was without effect on $I_h$ voltage dependence of activation. However, 50 µM quinpirole produced both a reduction of $I_h$ peak currents and a hyperpolarizing shift in the activation curve for $I_h$. External application of the adenylyl cyclase inhibitor SQ 22536 produced a reversible decrease in peak currents but had no effect on $I_h$ voltage dependence of activation, whereas internal application of cAMP shifted $I_h$ activation to more depolarized potentials. Because $I_h$ modulates cell excitability and spike frequency adaptation, our findings support a role for dopamine in modulating the sensitivity and output of rat ORNs to odors.

INTRODUCTION

Extrinsic innervation by sympathetic fibers from the superior cervical ganglion is responsible for the high levels of norepinephrine (NE) and dopamine (DA) found in the mammalian olfactory mucosa (Kawano and Margolis 1985). The actions of these neurotransmitters on olfactory sensitivity were tested in rat behavioral studies. Although NE was without behavioral effect (Doty et al. 1988), activation of dopamine D$_2$ receptors with quinpirole (a D$_2$ receptor agonist) significantly depressed odor detection performance (Doty and Risser 1989). Both D$_1$ and D$_3$ dopamine receptors are present in most regions of the olfactory pathway, but only D$_2$ receptors have been identified in olfactory receptor neurons (ORNs). (Coronas et al. 1997b; Mania-Farnell et al. 1993b; Shipley et al. 1991). The presence of dopamine D$_2$ receptors in ORNs suggests that odor sensitivity may be modulated at both the soma in the periphery and the nerve terminal in the olfactory bulb. The mechanisms by which peripheral dopamine D$_2$ receptors may modulate olfactory function are not known. However, activation of dopamine D$_2$ receptors in ORNs is associated with several effects, such as inhibition of adenylyl cyclase activity (Mania-Farnell et al. 1993) and induction of apoptosis or differentiation (Coronas et al. 1997a). In other preparations, D$_2$ receptor activation is associated with reduction of voltage-dependent Ca$^{2+}$ currents (Brown and Seabrook 1995; Chronwall et al. 1995; Stack and Surprenant 1991; Yan et al. 1997), increases in K$^+$ conductance (Johnson and North 1992; Lacey et al. 1987; Stack and Surprenant 1991), and modulation of the hyperpolarization-activated current, $I_h$ (Akopian and Witkovsky 1996; Jiang et al. 1993).

Because $I_h$ is present in rat ORNs (Lynch and Barry 1991a), we explored the possible modulatory role of DA on this current. $I_h$ is a hyperpolarization-activated current that was first described as $I_h$ in the heart (Brown and DiFrancesco 1980; DiFrancesco et al. 1986; Yanagihara and Irisawa 1980) and as $I_h$ in peripheral and central neurons (Akasu et al. 1993; Bobker and Williams 1989; Crepel and Penit-Soria 1986; Halliwell and Adams 1982; Hestrin 1987; Maccaferri et al. 1993; Mayer and Westbrook 1983; Pape and McCormick 1989; Spain et al. 1987; Takahashi 1990) including ORNs (Corotto and Michel 1994; Lynch and Barry 1991a). Despite small variations in the voltage range of activation and kinetics, the current shares some general characteristics: it is a slowly activating, noninactivating current that activates at or close to the resting membrane potential. The current is resistant to block by tetrodotoxin, tetraethylammonium, BaCl$_2$ or 4-aminopyridine, but is readily blocked by CsCl (Lynch and Barry 1991a; Tokimasa and Akasu 1990). $I_h$ is a mixed K$^+$-Na$^+$ current with a permeability ratio of 5.2:1 and a reversal potential of −36 mV in physiological conditions (Lynch and Barry 1991a). Thus when active, $I_h$ will depolarize the cell. Functionally, $I_h$ is important in setting the resting membrane potential (Bal and McCormick 1997; Trotier and Dovington 1996; Wang et al. 1997; Womble and Moises 1993) and in controlling cell excitability and neuronal firing pattern (Akasu et al. 1993; Maccaferri et al. 1993; Maccaferri and McBain 1996; McCormick and Pape 1990b; Tabata and Ishida 1996; Wang et al. 1997). With its important role in shaping cell output, $I_h$ was a likely candidate for DA modulation in rat ORNs. We found that DA shifted $I_h$ voltage dependence of activation to more hy-
perpolarized potentials and reduced its peak currents through activation of a dopamine D₂ receptor. These findings suggest a mechanism by which activation of D₂ receptors in ORNs may set resting properties as well as modulate odor sensitivity.

**METHODS**

**Cell preparation and culture**

Rat ORNs were dissociated with a modification of previously described procedures (Lynch and Barry 1991a; Ronnett et al. 1991). Briefly, adult male Simonsen albino rats (~200 g) were handled according to the policy of The American Physiological Society regarding the use and care of animals. Rats were deeply anesthetized with 150 mg/kg ketamine + 15 mg/kg rompum (Mallinckrodt Veterinary; Mundelein, IL) and killed by decapitation. The olfactory epithelium from the nasal septum and turbinates was dissected under 100% oxygen vapor. The tissue was placed in divalent cation-free rat Ringer solution ([in mM] 145 NaCl, 5.6 KCl, 10 Na₂-hydroxethylpiperazine-2'-ethanesulfonic acid (HEPES), 10 glucose, 4 ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) (pH 7.4), 300 mM) containing 10 mg/ml bovine serum albumin, 1 mg/ml collagenase (GIBCO BRL; Grand Island, NY), 50 μg/ml deoxyribonuclease II, and 44 U/ml dispase (GIBCO BRL) and incubated with gentle shaking (80 rpm) at 37°C for 60 min. Following incubation, the tissue was transferred to fresh divalent cation-free rat Ringer and incubated with gentle shaking at 37°C for 5 min. The tissue was then triturated and filtered using a 53-μm monofilament cloth (Small Parts, Miami Lakes, FL). Cells were plated onto concanavalin A (10 mg/ml; Sigma type IV) coated glass coverslips placed in 35-mm petri dishes. The dishes were placed at 37°C in a CO₂ incubator until used (up to 4 days). There was no change in the properties of Iₜ over time in culture. The culture media was replaced daily and consisted of Dulbecco’s modified Eagle medium (GIBCO BRL) supplemented with 100 μM ascorbic acid, 1 x insulin-transferrin-selenium-X (GIBCO BRL) and 2 mM glutamine, 100 U/ml penicillin G, 100 mg/ml streptomycin (Irvine Scientific, Santa Ana, CA). All chemicals were obtained from Sigma Chemical (St. Louis, MO) unless stated otherwise.

**Electrophysiological recordings**

Standard whole cell voltage-clamp and current-clamp recording techniques (Hamill et al. 1981) were performed. Electrodes (10–12 MΩ resistance in 25 mM K⁺ rat Ringer) were pulled on a Flaming/Brown P87 puller from thick-walled (0.64 mm) borosilicate filament glass (Sutter Instrument, San Rafael, CA). Coverslips with adherent cells were placed into the recording chamber and perfused with external bath solution at a rate of 1–2 ml/min. Test solutions were delivered either by exchange of the bath solution perfusing the recording chamber or by a multibarrel, rapid solution changer. Both the external bath solution and the test solutions were maintained at 35°C unless stated otherwise. The bath solution was grounded with either a silver–silver chloride pellet bath electrode or a KC1 agar bridge to a silver chloride wire.

Voltage-clamp and current-clamp recordings were performed and digitized with a Digidata 1200 interface, a 200A patch-clamp amplifier, pClamp software (Axon Instruments, Foster City, CA), and a 486-33 IBM clone computer. Data was sampled at 1 kHz and filtered off-line at 500 Hz. Data analysis was performed using Channel software (Biodiversity, Park City, UT) and Microcal Origin (Microcal Software, Northampton, MA). Conductance-voltage (g-V) relationships were calculated according to $g = \frac{I}{V - V_r}$, where $I$ was the peak current activated at the end of each hyperpolarizing voltage step ($V$), and $V_r$ was the reversal potential (~27 mV) (Lynch and Barry 1991a). The normalized g-V relationships were fit with a Boltzmann distribution: $g = \frac{1}{1 + \exp \left( \frac{V - V_{1/2}}{k} \right)}$, where $V_{1/2}$ was the potential of half-maximal activation and $k$ was the slope factor.

Passive properties were determined by averaging the currents ($I$) from 32 repeated 4 ms voltage ($V$) pulses stepping from ~78 to ~88 mV. Membrane input resistance ($R_I$) was calculated by determining $\Delta I/\Delta V$ during the sustained phase of the current response and dividing $\Delta V$ by $\Delta I$. Cell capacitances were determined according to $Q/\Delta V$ where $Q$ was the amount of charge moved (determined by integrating the area under the capacity transient) and $\Delta V$ was the voltage change (10 mV). Average cell capacitance and $R_I$ were 4.5 ± 0.1 pF and 3.7 ± 1.1 MΩ ($n = 88$), respectively. The average uncompensated series resistance ($R_S$) was 29.4 ± 1.4 MΩ ($n = 88$). The residual voltage error associated with the $R_S$ remaining after compensation ranged from 1–3 mV and was not corrected for. The liquid junction potential between either the 25 mM K⁺ rat Ringer or normal rat Ringer and the internal solution was ~8 mV (calculated using Axoscope, Axon Instruments) and was subtracted from all records. All averages are reported as means ± SE, $n$ = number of cells tested for which a complete protocol was obtained. Statistical comparisons were made using Student’s t-test and P values < 0.05.

**Solutions**

The external and internal solutions used in these experiments were similar to solutions used previously to characterize $I_N$ in rat ORNs (Lynch and Barry 1991a). The external bath solution included 140 mM NaCl, 5 KCl, 1 MgCl₂, 2 CaCl₂, 10 HEPES, 10 glucose (pH 7.4), 300 mOsm; 25 mM K⁺ rat Ringer (in mM): 120 NaCl, 25 KCl, 1 MgCl₂, 2 CaCl₂, 10 HEPES, 10 glucose (pH 7.4), 300 mOsm. The internal patch solution was (in mM) 125 KF, 15 KCl, 11 EGTA, 10 HEPES, 3 MgCl₂, 2 NaATP, 1 glutathione, 5 tetraethylammonium (TEA) (pH 7.2), and 310 mOsm. For adenosine 3’5’-cyclic monophosphate (cAMP) experiments, a stock solution of cAMP was made fresh and was added to the internal solution. Stock solutions of DA, SQ 22536 (SQ, Calbiochem-Novabiochem, San Diego, CA), quinpirole, sulpiride, and CsCl were made fresh and added to the external solution. Ascorbic acid (2 μM) was added to the DA solution to retard oxidation and had no effect on $I_N$ when applied in control solutions. All chemicals were obtained from Sigma Chemical unless stated otherwise.

**RESULTS**

**Characterization of $I_N$ in rat ORNs**

Whole cell recordings were made from cultured adult rat ORNs. Hyperpolarization of rat ORNs to test potentials more negative than ~68 mV from a holding potential of ~58 mV activated a slowly activating, inwardly rectifying current (Fig. 1Aa). The magnitude of the current increased as the membrane was hyperpolarized to more negative potentials. No inactivation of the current was observed. Application of 5 mM CsCl to the cell during the hyperpolarizing voltage steps caused a reversible block of the current and revealed a small Cs⁺-insensitive current component (Fig. 1, Ab and B). Control voltage-clamp experiments were performed to test if CsCl could block voltage-gated Na⁺ or K⁺ currents; no effect of CsCl was observed (data not shown). These characteristics are indicative of the hyperpolarization-activated current $I_h$. Hyperpolarization of the membrane poten-
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Fig. 1. Isolation of $I_h$ in rat olfactory receptor neurons (ORNs). A: $I_h$ was activated by hyperpolarizing voltage steps up to $-163$ mV from a holding potential of $-58$ mV. $Aa$ and $Ac$: total current ($I_{total}$) was measured at the end of each 1.2-s voltage step. Control solution was 25 mM K+ rat Ringer. $Ab$: application of 5 mM CsCl reversibly blocked $I_h$ and revealed a small, Cs+-insensitive current component ($I_{Cs^+-insensitive}$). $Ad$: Voltage step from $-58$ to $-138$ mV activated a small inward current ($I_{inst}$). An example is illustrated for obtaining $I_{hist}$ from $I_{total}$. Calibration bars apply to traces in $Aa$–$Ad$. Voltage protocol applies to $Aa$–$Ac$. Bath temperature was 35°C. B: current-voltage ($I$-$V$) relationships for the current components illustrated in A. $I_{h}$ component was calculated by subtraction of either $I_{Cs^+-insensitive}$ or $I_{hist}$ from $I_{total}$.

$I_h$ current amplitude was reported to depend on extracellular K+ concentration ($[K^+]_o$) (DiFrancesco and Pape 1990a) and bath temperature (DiFrancesco and Barry 1991a; Mayer and Westbrook 1983; McCormick and Pape 1990a) and bath temperature (DiFrancesco and Barry 1991a; Mayer and Westbrook 1983; McCormick and Pape 1990a) and bath temperature (DiFrancesco and Barry 1991a; Mayer and Westbrook 1983; McCormick and Pape 1990a) and bath temperature (DiFrancesco and Barry 1991a; Mayer and Westbrook 1983; McCormick and Pape 1990a). Therefore in all subsequent experiments the $I_h$ component of the total current elicited by hyperpolarization was isolated by subtraction of either the Cs+-insensitive current or the $I_{inst}$. In addition, cells that showed changes in $I_{inst}$ over the course of the experiments were not included in the data analyses.

$I_h$ current amplitude was reported to depend on extracellular K+ concentration ($[K^+]_o$) (DiFrancesco and Barry 1991a; Mayer and Westbrook 1983; McCormick and Pape 1990a) and bath temperature (DiFrancesco and Ojeda 1980; Hart 1983; Watts et al. 1996). We investigated the effects of raising $[K^+]_o$ and bath temperature on the amplitude and voltage dependence of $I_h$. In agreement with previous observations, we found that raising the $[K^+]_o$ concentration from 5 to 25 mM significantly increased $I_h$ peak current amplitude. Figure 2A shows the $I$-$V$ relationships for $I_h$ recorded from a cell perfused with 5 and 25 mM K+ rat Ringer. In this cell, increasing $[K^+]_o$ caused a $40 \pm 1\%$ ($n = 6$ trials) increase in $I_h$ peak current amplitude at $-118$ mV. On average, at $-118$ mV, increasing $[K^+]_o$ from 5 to 25 mM produced a $145 \pm 42\%$ ($n = 4$) increase in $I_h$ current amplitude. A similar effect was observed by increasing bath temperature: when the bath temperature was increased from 25 to 35°C, $I_h$ current density was significantly increased by $180 \pm 1\%$ ($n = 5$) at a voltage of $-108$ mV (Fig. 2B). We also investigated the effects of increasing $[K^+]_o$ and bath temperature on the voltage dependence of $I_h$ activation. The potential for half-maximal activation ($V_{1/2}$) was calculated as described in the METHODS and in Figs. 3 and 4. In cells perfused with 25 mM K+ rat Ringer, the $V_{1/2}$ ranged from $-89$ to $-124$ mV; the average $V_{1/2}$ and slope factor were $-118 \pm 5$ mV and $-9 \pm 2$ mV, respectively ($n = 10$). Increasing $[K^+]_o$ produced no change in the $V_{1/2}$, whereas increasing the bath temperature from 25 to 35°C produced a $13 \pm 2$ mV ($n = 5$) hyperpolarizing shift in the averaged $V_{1/2}$ (data not shown). Thus to maximize the amplitude of $I_h$, all experiments shown were conducted at 35°C with 25 mM K+ rat Ringer as the external solution (control conditions).

Dopamine decreases $I_h$ peak currents and shifts voltage dependence of activation

$I_h$ can be modulated by a variety of mechanisms including changes in intracellular levels of cAMP (Tokimasa and Akasu 1990). Neurotransmitters that alter basal adenylyl cyclase activity have been shown to modulate $I_h$ (Banks et al. 1993; Bobker and Williams 1989; DiFrancesco and Tromba 1988; Kiehn and Harris-Warrick 1992; Larkman and Kelly 1997; McCormick and Pape 1990a). Because DA decreases adenylyl cyclase activity in rat ORNs (Mania-Farnell et al. 1993), we tested whether DA modulates $I_h$ in these neurons. Figure 3A shows an example of the effect of DA on $I_h$ recorded from a cell perfused with control solution and 1 $\mu$m DA. Application of 1 $\mu$m DA reversibly decreased the amplitude of $I_h$ (Fig. 3A). The inhibitory effect of DA was observed at all potentials at which $I_h$ was activated. In this...
cell, DA produced a 50 ± 1% (n = 3 trials) reduction of $I_h$ peak currents at −128 mV. Figure 3B shows the $I-V$ relationships for $I_h$ recorded when the cell was perfused with 5 and 25 mM K+ rat Ringer. $I_h$ was activated by hyperpolarizing voltage steps up to −163 mV from a holding potential of −88 mV. Data shown are mean values of 6 successive trials recorded in both external solutions. Error bars represent SE; * significance at $P < 0.05$ in this and all subsequent figures. B: $I_h$ was recorded from cells bathed in 25 mM K+ rat ringer at room temperature (25°C, n = 4) and at 35°C (n = 5). Currents were activated by hyperpolarizing voltage steps up to −178 mV from a holding potential of −58 mV. Peak currents measured at the end of each 1.2-s voltage step were divided by cell capacitance to obtain current density.

The inhibition of $I_h$ peak currents could be due to a reduced conductance, a shift in the voltage dependence of activation, or both. To determine which parameters DA modulated, we examined $I_h$ conductance–voltage relationships ($g-V$, activation curve) in the absence and presence of 1 μM DA. The conductance at each potential was calculated as described in the experimental procedures, and $g-V$ relationships in DA were normalized to the control. The maximum relative conductance ($g_{max}$) in DA for each cell was averaged and plotted in Fig. 3C. Application of 1 μM DA reduced the $g_{max}$ of $I_h$ by 37 ± 6% (n = 5).

To determine if DA also shifted the voltage dependence of activation of $I_h$, all of the $g-V$ relationships were normalized to their maximum, and the potential for half-maximal activation ($V_{1/2}$) was obtained from the Boltzmann fit of the $g-V$ relationships. In voltage-clamp experiments, four of five cells that responded to DA with a decrease in current amplitude showed a hyperpolarizing shift in the $V_{1/2}$ of activation. Figure 3D shows the average $V_{1/2}$ for $I_h$ recorded from cells under control conditions and from the same group of cells bathed in 1 μM DA. Under control conditions, the average $V_{1/2}$ and slope factor ($k$) for $I_h$ were −128 ± 5 and −8 ± 2 mV, respectively (n = 5). When DA was applied, the average $V_{1/2}$ and $k$ for $I_h$ were −137 ± 4 and −8 ± 1 mV (n = 5), respectively. Dopamine produced a significant 9 mV hyperpolarizing shift in the voltage-dependence of activation of $I_h$ without a significant change in the slope factor, indicating that the sensitivity of $I_h$ to changes in voltage was unaffected. To verify the physiological relevance of these findings, we performed similar experiments in normal rat Ringer (5 mM external K+). We found that 1 μM DA reduced $I_h$ current amplitude at −100 mV by as much as 47% (average reduction at −100 mV was 23 ± 12%, n = 3) and $I_h g_{max}$ by 21 ± 6% (n = 3). In addition, DA produced a 5 mV hyperpolarizing shift in the $V_{1/2}$ in one of three cells that responded (data not shown).

SQ 22536 and cAMP modulate $I_h$

In other preparations, changes in intracellular levels of cAMP ([cAMP]$_i$) modulate the activation of $I_h$. An increase in [cAMP]$_i$ produces a depolarizing shift (Larkman and Kelly 1997; McCormick and Pape 1990a; Tokimasa and Akasu 1990), whereas a decrease produces a hyperpolarizing shift in the $V_{1/2}$ of $I_h$ (Chang and Cohen 1992; DiFrancesco and Tromba 1988a,b). However, the recent cloning of several members of the $I_h$ channel superfamily (Gauss et al. 1998; Ludwig et al. 1998; Santoro et al. 1998) indicates that cAMP can have variable effects on $I_h$ properties. To evaluate if changes in [cAMP]$_i$ are involved in the dopaminergic modulation of $I_h$ observed in rat ORNs, we studied the effects of the adenylyl cyclase inhibitor SQ and of adding a high concentration of cAMP to the internal solution. Figure 4A shows an example of the effect of SQ on $I_h$ recorded at −110 mV from a cell perfused with control solution and 500 μM SQ. In this cell, application of SQ produced a 20% decrease in the peak current amplitude at −110 mV. This reduction was reversed after a 2-min wash in control solution (data not shown). Figure 4B shows the $I-V$ relationships for the cell illustrated in Fig. 4A. SQ produced a significant decrease in $I_h$ peak current amplitude. Similar effects were observed in four of seven cells tested. On average, at a potential of −100 mV, 500 μM SQ produced a 26 ± 12.6% (n = 4) reduction of $I_h$ current amplitude.

To test if, like dopamine, SQ also shifted the voltage dependence of activation of $I_h$, the Boltzmann fits of normalized $g-V$ relationships were analyzed. The inset in Fig. 4B shows the $g-V$ relationships for the cell shown in Fig. 4A. The $V_{1/2}$ and slope factor under control conditions and in SQ for this cell were identical: −108 and −6 mV, respectively. Figure 4C shows the average $V_{1/2}$ under control conditions and in 500 μM SQ for the four cells that showed a reduced current amplitude when perfused with SQ. The average $V_{1/2}$ and slope factor for $I_h$ under control conditions were −102 ± 3 and −6 ± 0.4 mV (n = 4), respectively. When the cells were perfused with 500 μM SQ, the average $V_{1/2}$ and slope factor (−101 ± 4 and −5 ± 1 mV, respectively;
FIG. 3. Dopamine decreases $I_h$ peak currents and shifts voltage dependence of activation. 

$A$: effect of dopamine (DA) on $I_h$. $Aa$: $I_h$ was activated by hyperpolarizing voltage steps up to $-168$ mV from a holding potential of $-58$ mV. Peak currents were measured at the end of each 1.2-s voltage step. Control solution was 25 mM K⁺ rat Ringer. $Ab$: application of 1 µM DA to the cell reduced $I_h$ peak current amplitude. $Ac$: effect of DA on $I_h$ was reversed by a 3 min wash in control solution. Calibration bars and voltage protocol apply to traces in $Aa$–$Ac$. $B$: $I-V$ relationships for the cell shown in $A$. Data shown are mean values of 3 successive trials recorded from the same cell under control conditions and in DA. $C$: maximal conductance in 1 µM dopamine was normalized to control and averaged across 5 cells. $D$: average $V_{1/2}$ was obtained from the same group of cells under control conditions and in 1 µM DA ($n = 5$).

FIG. 4. SQ 22536 (SQ) reduces $I_h$ current amplitude without a shift in voltage dependence of activation. $A$: $I_h$ was activated by a hyperpolarizing voltage step to $-110$ mV from a holding potential of $-50$ mV. Peak currents were measured at the end of each 1.2-s voltage step after subtraction of the Cs⁺-insensitive current. Control solution was 25 mM K⁺ rat Ringer. $B$: $I-V$ relationships for the cell shown in $A$. Data shown are mean values of 4 and 6 successive trials recorded from the same cell under control conditions and in SQ respectively. Inset: conductance-voltage ($g-V$) relationships for the same cell under control conditions (■) and in SQ (○). $C$: average $V_{1/2}$ under control conditions and in 500 µM SQ for the 4 cells that responded to application of SQ. No significant difference was observed. $D$: maximal conductance in 500 µM SQ was normalized to control and averaged across 4 cells.
**Adenosine 3',5'-cyclic monophosphate (cAMP) modulates the g-V relationships of Iₜ.** A: current traces from 2 different cells (control and internally perfused with 1 mM cAMP) were normalized to their peak currents at maximal conductance (−158 mV) and superimposed. Cells were held at −58 mV and stepped to either −88 or −128 mV. Scale bars, normalized amplitude (IHmax). B: g-V relationships are shown for 3 different cells under control (1 μM DA) and internal 1 mM cAMP. V1/2 of activation and slope factor for the cell under control conditions in DA and with cAMP were −105, −7; −121, −6; −91, and −4 mV, respectively.

n = 4) were not significantly different from control. We also tested whether SQ decreased Iₜ maximal conductance. For each cell that responded to SQ, the gmax in SQ was normalized to the control value. The average value is shown in Fig. 4D. Application of 500 μM SQ produced a 26 ± 4% (n = 4) reduction on Iₜ maximum conductance. Therefore the reduction of Iₜ peak current amplitude produced by 500 μM SQ resulted from a reduction of Iₜ maximal conductance rather than from a shift in the voltage dependence of activation.

To further evaluate if changes in [cAMP], can modulate Iₜ activation in rat ORNs, we studied the effects of adding 1 mM cAMP to the internal solution: Iₜ was recorded in voltage-clamp mode. Current traces (at 2 voltages) from a control cell with a V1/2 of −112 mV are superimposed on traces from a cAMP-dialyzed cell with a V1/2 of −79 mV (Fig. 5A). Activation curves from three different cells under three different conditions (control, 1 μM DA and 1 mM cAMP) are shown in Fig. 5B. The V1/2 of activation for Iₜ when cAMP was internally applied ranged from −70 to −90 mV; the average V1/2 and slope factor were −90 ± 2 and −7 ± 1 mV (n = 10), respectively. Compared to control (V1/2 = −118 ± 5 mV, slope factor = −9 ± 2, n = 10), internal application of cAMP produced a 28 mV depolarizing shift in the activation curve of Iₜ without a significant change in the slope factor. Because cAMP perfused rapidly into the cell, we were unable to assess whether cAMP also modulated the gmax as shown for DA in Fig. 3C. These data demonstrate that intracellular levels of cAMP can modulate Iₜ activation in rat ORNs.

**Dₐ agonist mimics effect of DA on Iₜ while antagonist blocks the DA effects**

Dopamine can activate several types of DA receptors. In particular, activation of D₂ dopamine receptors decreases [cAMP], by inhibition of adenylyl cyclase activity (Baldes-sarini and Tarazi 1996). Because our results with SQ showed that inhibition of adenylyl cyclase mimics some of the effects observed with DA, we used quinpirole (a selective dopamine D₂ receptor agonist) to test if D₂ receptor activation and subsequent inhibition of adenylyl cyclase mediate the effect of DA on Iₜ. In voltage-clamp experiments, Iₜ was activated by hyperpolarizing voltage steps from a holding potential of −58 mV under control conditions and when the cells were perfused with either 20 μM or 50 μM quinpirole. In three of seven cells, application of 20 μM quinpirole produced a 35 ± 11% (n = 3) reduction on Iₜ current amplitude at a potential of −100 mV, and a 38 ± 5% (n = 3) decrease of gmax without producing a significant shift in the voltage dependence of Iₜ activation (data not shown). When the higher concentration (50 μM) of quinpirole was used, both effects were observed; 50 μM quinpirole produced a reversible decrease in Iₜ current amplitude in 5 out of 11 cells and a hyperpolarizing shift in the voltage-dependence of activation of Iₜ in three out of the five cells that responded to 50 μM quinpirole (Fig. 6, A and B). In the cell shown in Fig. 6A, 50 μM quinpirole produced a 100% inhibition of Iₜ peak currents at −88 mV. On average, at −100 mV, quinpirole produced a 38 ± 16% (n = 5) reduction in Iₜ current amplitude. Conductance-voltage relationships for quinpirole were analyzed as described for Figs. 3–5. 50 μM quinpirole produced a 22 ± 4% (n = 5) decrease in gmax and an 11 ± 4 mV (n = 5) hyperpolarizing shift in the V1/2 with no change in the slope factor (−7 ± 1 and −7 ± 1 in control and 50 μM quinpirole, respectively; n = 5). Figure 6B shows the g-V relationships for the cell shown in Fig. 6A. In this cell, quinpirole reversibly produced a 13-mV hyperpolarizing shift in the V1/2 of Iₜ activation with no change in the slope factor.

We also tested the effect of 50 μM quinpirole on Iₜ in current-clamp experiments. Cells were perfused with control solution and 50 μM quinpirole; the effect of Cs⁺ on the steady-state voltage response was recorded. At −88 mV, application of Cs⁺ hyperpolarized the cell by 40 mV in control conditions, whereas in quinpirole, Cs⁺ only produced a 26-mV hyperpolarization (Fig. 6B, inset). This 14-mV reduction was reversed after a 2-min wash with the control solution. The quinpirole-induced reduction in Cs⁺-sensitive voltage responses at −100 mV was observed in four of five cells and averaged 14 ± 3 mV (n = 4).

The involvement of dopamine D₂ receptors in the modulatory effect of DA on Iₜ was also tested by using bromocrip-
DOPAMINE MODULATES $I_h$ IN ORNs

![Graphs showing DA effects on $I_h$.](Image)

**FIG. 6.** DA effects on $I_h$ are mimicked by quinpirole and blocked by sulpiride. **A:** $I-V$ relationships for $I_h$ recorded under control conditions ( ■ and ▲ represent pre- and postquinpirole application of control solution) and in 50 μM quinpirole (○). $I_h$ was activated by hyperpolarizing voltage steps up to −168 mV from a holding potential of −58 mV. Peak currents were measured at the end of each 1.2 s voltage step. Inset: current traces obtained at −108 mV when the cell was perfused with control solution (C) and 50 μM quinpirole (Q). Raw current traces were smoothed by a 10 point adjacent averaging (Microcal Origin). **B:** $g-V$ relationships for cell shown in A. ■, control conditions ($V_{1/2} = −120$ mV, slope factor = −8); ○, 50 μM quinpirole ($V_{1/2} = −133$ mV, slope factor = −8). Recovery $g-V$ relationship not shown. Inset: effect of quinpirole on steady-state voltage response of a different cell. **C:** $I-V$ relationships for cell shown in Fig. 6C. ■, control solution (C) and 50 μM quinpirole (Q). 5 mM CsCl was applied during the time indicated by the top bar. Because activation of $I_h$ produces a depolarization, its blockage with CsCl results in a membrane hyperpolarization. When quinpirole perfused the cell, the amplitude of the CsCl-induced hyperpolarization was reversibly reduced. **D:** $g-V$ relationships for cell shown in C. ■, 5 μM DA + 5 μM sulpiride ($V_{1/2} = −97$ mV, slope factor = −8); ○, 5 μM DA ($V_{1/2} = −106$ mV, slope factor = −8). Inset: maximal conductance in 5 μM DA (D) was normalized to the maximal conductance in 5 μM DA + 5 μM sulpiride (D + S) and averaged across 3 cells.

Dopamine, another selective D$_2$ dopamine receptor agonist, on two cells that responded, 20 μM bromocriptine produced a 20 and a 23% reduction in $I_h$ current amplitude (at −120 mV) without a shift in the voltage dependence of $I_h$ activation (data not shown). Because of problems with precipitation of the solutions, bromocriptine was not tested further.

To further examine the involvement of D$_2$ dopamine receptors in the dopaminergic modulation of $I_h$, we tested the effects of DA when it was concomitantly applied with sulpiride, a selective D$_2$ receptor antagonist. In voltage-clamp experiments, $I_h$ was activated by hyperpolarizing voltage steps from a holding potential of −50 mV when the cells were first perfused with 5 μM DA + 5 μM sulpiride and also when the cells were perfused with 5 μM DA alone. Following the perfusion of DA + sulpiride, application of DA alone produced a significant decrease in $I_h$ current amplitude (Fig. 6C). In this cell, at −90 mV, perfusion of DA activated only 27 ± 1.2% ($n = 6$ trials) of the current activated when the cell was perfused with DA + sulpiride. Similar effects were observed in three of eight cells tested.

On average, at −90 mV, DA activated only 63 ± 18% ($n = 3$) of the current activated when the cells were perfused with DA + sulpiride. Conductance-voltage relationships were also analyzed. Figure 6D shows the $g-V$ relationships for the cell shown in Fig. 6C. In this cell, 5 μM DA produced a 9-mV hyperpolarizing shift in the $V_{1/2}$ of activation with no change in the slope factor compared with DA + sulpiride. No difference in the $V_{1/2}$ was observed in the other two cells that responded to sulpiride (data not shown). To test for changes in maximal conductance, we normalized the maximal conductance activated when cells were perfused with 5 μM DA to the maximal conductance activated when the cells were perfused with 5 μM DA + 5 μM sulpiride. DA alone produced a 21 ± 3.5% ($n = 3$) reduction in $g_{max}$ (Fig. 6D, inset).

Collectively, the data presented in Fig. 6 show that activation of D$_2$ dopamine receptors in rat ORNs mimicked the effects of DA on $I_h$, whereas blockade of the receptors blocked the dopaminergic effects. Lower doses of D$_2$ agonists decreased $I_h$ current amplitude, whereas higher doses...
DISCUSSION

We investigated the dopaminergic modulation of the hyperpolarization-activated current ($I_h$) in rat ORNs. We found that $I_h$ activated slowly on hyperpolarization to test potentials more negative than $-68$ mV, showed no inactivation during test pulses lasting $>1$ s, and was reversibly blocked by CsCl. Increasing $[K^+]_o$ enhanced $I_h$ current amplitude as predicted by the increase in driving force, without shifting its voltage range of activation. These characteristics are in agreement with those previously described for $I_h$ in rat ORNs (Lynch and Barry 1991a). The effect of temperature on $I_h$ was not previously explored in rat ORNs, but its effects are similar to the increase in current amplitude described in rabbit sinoatrial node cells (DiFrancesco and Ojeda 1980) and the hyperpolarizing shift in the activation curve described in sheep Purkinje fibers (Hart 1983).

We have demonstrated that DA modulates $I_h$ in rat ORNs. Dopamine produced a $37 \pm 6\%$ ($n = 5$) decrease in $I_h$ relative conductance and shifted $I_h$ $V_{1/2}$ from $-128 \pm 5$ mV ($n = 5$) to $-137 \pm 4$ mV ($n = 5$). Although the current amplitudes were much smaller, similar DA effects were observed when using a physiological $[K^+]_o$. Because rat ORNs have high-input resistances, even small current changes profoundly affect membrane potential. For example, in 5 mM $[K^+]_o$ and with an $R_i$ of $3.7 \pm 1.1$ GΩ ($n = 88$), a 3 pA reduction of $I_h$ by DA will result in an 11-mV hyperpolarization of the cell membrane potential. In addition, rat ORNs have a resting potential negative to $-90$ mV (Rajendra et al. 1992) indicating that the potentials at which we observed the DA effects are within a physiological range. Dopamine’s effects were mimicked by quinpirole (a selective dopamine D$_2$ receptor agonist) and blocked by sulpiride (a selective D$_2$ receptor antagonist). Inhibition of adenylyl cyclase activity with 500 μM SQ produced a significant decrease in $I_h$ current amplitude and maximal relative conductance without a shift in the $V_{1/2}$ of activation; while internal perfusion of 1 mM CAMP produced a depolarizing shift in the activation range.

Mechanisms of dopamine-mediated modulation of $I_h$

Modulation of $I_h$ by neurotransmitters and intracellular second messengers through multiple mechanisms has been demonstrated in many other preparations including cardiac cells (DiFrancesco and Mangoni 1994; DiFrancesco and Tortora 1991; DiFrancesco and Tromba 1988a,b) and neurons (Bobker and Williams 1989; Ingram and Williams 1996; Larkman and Kelly 1997; McCormick and Pape 1990a; Tokimasa and Akasu 1990). We found that DA modulates $I_h$ through activation of a dopamine D$_2$ receptor. In rat ORNs, DA activates a dopamine D$_2$ receptor resulting in inhibition of adenylyl cyclase activity via an inhibitory G protein and subsequent reduction in cAMP production (Mania-Farnell et al. 1993). We propose that the dopamine-induced decrease in cAMP levels is responsible for the modulation of $I_h$. This represents a novel mechanism by which DA modulates $I_h$. An inhibitory action of DA on $I_h$ has been described in rod photoreceptors and ventral tegmental neurons. However, in both cases the dopaminergic inhibition did not involve a cAMP cascade, but was secondary to a Ca$^{2+}$-dependent activation of a G-protein (Akopian and Witkovsky 1996) or activation of a potassium conductance (Jiang et al. 1993; Watts et al. 1996). Dopamine also modulates $I_h$ in the lateral pyloric neuron of the spiny lobster Pandalus interruptus; however, DA produced an enhancement of $I_h$ currents and a depolarizing shift in its voltage dependence for activation (Harris-Warrick et al. 1995).

Thus in rat ORNs, DA inhibits $I_h$ through a different, novel mechanism.

Further studies are needed to elucidate the third messenger pathway involved in the dopaminergic modulation of $I_h$. We propose that a dopaminergic-induced inhibition of adenylyl cyclase activity and subsequent decrease in cAMP, is involved, but the events downstream from this step are still obscure. In Purkinje cells and in sympathetic neurons, protein kinase A inhibitors shifted $I_h$ activation to hyperpolarized potentials, reduced the magnitude of $I_h$, and prevented the effects of adenylyl cyclase activation (Chang et al. 1991; Tokimasa and Akasu 1990). These observations implicate protein kinase A in the tonic and receptor-mediated regulation of $I_h$ current amplitude and activation curve. However, in substantia nigra pars compacta neurons, neurotensin induced only a decrease in $I_h$ amplitude, which was blocked by specific protein kinase C inhibition; protein kinase A inhibition was without effect (Cathala and Paupardin-Tritsch 1997). Other pathways were also proposed. In sinoatrial node cells, both activated G-protein α-subunits (Yatani et al. 1990) and a phosphorylation-dependent mechanism (Accili et al. 1997) increased $I_h$ current amplitude. This observation suggests a direct modulation of the $I_h$ channel by G proteins. In addition, a direct action of cAMP on the $I_h$ channel was demonstrated in inside-out patches excised from sino-atrial node myocytes: cAMP activated $I_h$ by shifting its activation curve to more positive voltages (DiFrancesco and Tortora 1991) and induced facilitation of channel opening (DiFrancesco and Mangoni 1994). The findings observed in sino-atrial node cells demonstrate a differential regulation of $I_h$. Thus the dopaminergic modulation of $I_h$ that we have described might involve any or several of these pathways. Our data obtained from the experiments with DA, SQ, and quinpirole consistently showed that a decrease in current amplitude is not always accompanied by a shift in the voltage dependence of activation, suggesting that the two are differentially regulated in rat ORNs. Further studies will test the involvement of each pathway described above.

Physiological significance

Neurotransmitters have been shown to modulate firing frequency of sino-atrial node cells and cell excitability in neurons through modulation of $I_h$. In the heart, $I_h$ participates in the spontaneous diastolic depolarization responsible for pacemaking function and is modulated by NE and acetylcholine (DiFrancesco et al. 1989; Guth and Dietze 1995). In thalamic relay neurons, enhancement of $I_h$ by activation of β-adrenergic or serotonergic receptors results in a decreased response to hyperpolarizing stimuli, a subsequent dampening of rhythmic burst discharges, and a facilitation of single-
spike activity (McCormick and Pape 1990a). Similar observations have been made in neurons of the medial nucleus of the trapezoid body in which modulation of \( I_h \) by NE plays a role in increasing the temporal acuity of the auditory pathways during a heightened state of arousal (Banks et al. 1993). In rat ORNs, \( I_h \) has been postulated to contribute to the modulation of cell excitability and to spike frequency adaptation during the excitatory response to odorants (Lynch and Barry 1991a). Thus the modulation of \( I_h \) by DA that we have described can play an important role in setting the sensitivity of ORNs in response to odorous stimuli.

Our experiments clearly show that \( I_h \) is modulated by DA through \( D_2 \) receptors in rat ORNs; however, we cannot pinpoint the cellular region of the interaction without knowing \( I_h \) channel distribution. The presence of DA in olfactory mucosa (Kawano and Margolis 1985) and mucus (Lucero and Squires 1998) and functional \( D_2 \) dopamine receptors in peripheral ORNs (Mania-Farnell et al. 1993), along with the dependence of \( I_h \) on cAMP, point to the ciliary-dendritosominal region because these components are close to the odor transducing machinery. If \( I_h \) channels are localized to the dendritic knobs and cilia, the high \([K^+]_i\) in olfactory mucus (69 mM) (Reuter et al. 1998) would enhance \( I_h \) amplitude, and the subsequent reduction by DA may be even greater than that observed using 25 mM \([K^+]_i\). However, the finding that dopamine \( D_2 \) receptors are also expressed at ORN terminals (Coronas et al. 1997b) indicates regulation of cyclic nucleotide levels at this region as well. If \( I_h \) is also present at the nerve terminal, then dopamine’s modulation of \( I_h \) at this site may be through the same dopamine \( D_2 \) receptor activation determined by this study.

In conclusion, we have demonstrated the modulation of \( I_h \) by DA in rat ORNs. Our data represent the first evidence for a peripheral role of dopamine \( D_2 \) receptors present in ORNs and indirectly affect functional importance to the DA present in olfactory mucosa and mucus.

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