Direct Excitation of Mitral Cells Via Activation of α1-Noradrenergic Receptors in Rat Olfactory Bulb Slices

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Hayar, Abdallah, Phillip M. Heyward, Thomas Heinbockel, Michael T. Shipley, and Matthew Ennis. Direct excitation of mitral cells via activation of α1-noradrenergic receptors in rat olfactory bulb slices. *J Neurophysiol* 86: 2173–2182, 2001. The main olfactory bulb receives a significant modulatory noradrenergic input from the locus coeruleus. Previous in vivo and in vitro studies showed that norepinephrine (NE) inputs increase the sensitivity of mitral cells to weak olfactory inputs. The cellular basis for this action of NE is not understood. The goal of this study was to investigate the effect of NE and noradrenergic agonists on the excitability of mitral cells, the main output cells of the olfactory bulb, using whole cell patch-clamp recording in vitro. The noradrenergic agonists, phenylephrine (PE, 10 μM), isoproterenol (Isop, 10 μM), and clonidine (3 μM), were used to test for the functional presence of α1-, β-, and α2-receptors, respectively, on mitral cells. None of these agonists affected olfactory nerve (ON)–evoked field potentials recorded in the glomerular layer, or ON-evoked postsynaptic currents recorded in mitral cells. In whole cell voltage-clamp recordings, NE (30 μM) induced an inward current (54 ± 7 pA, $n = 16$) with an EC_{50} of 4.7 μM. Both PE and Isop also produced inward currents (22 ± 4 pA, $n = 19$, and 29 ± 9 pA, $n = 8$, respectively), while clonidine produced no effect ($n = 6$). In the presence of TTX (1 μM), and blockers of excitatory and inhibitory fast synaptic transmission [gabazine 5 μM, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) 10 μM, and ($\pm$)-2-amino-5-phosphonopentanoic acid (APV) 50 μM], the inward current induced by PE persisted ($EC_{50} = 9$ μM), whereas that of Isop was absent. The effect of PE was also observed in the presence of the Ca^{2+} channel blockers, cadmium (100 μM) and nickel (100 μM). The inward current caused by PE was blocked when the interior of the cell was perfused with the nonhydrolyzable GDP analogue, GDPβS, indicating that the α1 effect is mediated by G-protein coupling. The current-voltage relationship in the absence and presence of PE indicated that the current induced by PE decreased near the equilibrium potential for potassium ions. In current-clamp recordings from bistable mitral cells, PE shifted the membrane potential from the downstate (-52 mV) toward the upstate (-40 mV), and significantly increased spike generation in response to perithreshold ON input. These findings indicate that NE excites mitral cells directly via α1 receptors, an effect that may underlie, at least in part, increased mitral cell responses to weak ON input during locus coeruleus activation in vivo.

INTRODUCTION

The mammalian main olfactory bulb (MOB) receives a significant noradrenergic input from the locus coeruleus (LC) (Fallon and Moore 1978; McLean and Shipley 1991; McLean et al. 1989; Shipley et al. 1985). Noradrenergic inputs to the MOB play important roles in olfactory function. Olfactory cues increase the discharge of LC neurons in behaving animals (Aston-Jones and Bloom 1981) and trigger rapid increases in norepinephrine (NE) levels in the olfactory bulb (Brennan et al. 1990; Rangel and Leon 1995; Rosser and Keverne 1985). LC-NE projections to the main and accessory olfactory bulb are critical for the formation and/or recall of specific olfactory memories, pheromonal regulation of pregnancy, and postpartum maternal behavior (Brennan et al. 1990; Dluzen and Ramirez 1989; Kaba et al. 1989; Rosser and Keverne 1985; Sullivan et al. 1989, 1992; Wilson and Leon 1988).

Despite several decades of research, the postsynaptic targets and neurophysiological actions of NE inputs to the MOB have remained elusive. Based on anatomical considerations, both the mitral cells and the granule cells are potential targets of NE inputs to MOB. Noradrenergic fibers are localized exclusively in the subglomerular layers where they terminate densely in the internal plexiform and the granule cell layers, and moderately in the external plexiform and mitral cell layers (McLean et al. 1989; Shipley et al. 1985). The glomerular layer is nearly devoid of noradrenergic fibers (McLean et al. 1989). In agreement with the distribution of NE fibers, both mitral cells and granule cells express several noradrenergic receptor subtypes, namely α1 and α2 receptors (Day et al. 1997; McCune et al. 1993; Pieribone et al. 1994; Winzer-Serhan et al. 1997).

Exogenous application of NE in the mammalian MOB has been reported to produce a number of effects. Iontophoretic application of NE was found to inhibit mitral cell spontaneous activity, presumably by excitation of granule cells (McLennan 1971). Field potential studies in the rat suggested that NE, acting at α1 receptors, depolarized granule cells (Mouly et al. 1995), an effect that would also inhibit mitral cells. Alternatively, in the turtle and dissociated rat MOB cultures, NE disinhibited mitral cells (Jahr and Nicoll 1982; Trombley 1992, 1994; Trombley and Shepherd 1992). This effect was attributed to α2 receptor–mediated presynaptic inhibition of granule and/or mitral cell dendrites. More recent electrophysiological studies in vivo and in vitro have demonstrated a consistent action of NE in the MOB. Endogenously released or exog-

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enously applied NE increased the responses of mitral cells to weak or perithreshold olfactory nerve (ON) input (Ciombor et al. 1999; Jiang et al. 1996). The specific site of action of NE in these later studies was not determined.

Taken together, the findings above indicate that the net influence of endogenously released NE in the MOB circuit is likely to result from direct postsynaptic actions on mitral cell output neurons as well as on granule cell interneurons. At present, however, there is no information about the cellular effects of NE on mammalian MOB neurons in vivo or in slice preparations. The goal of the present study therefore was to investigate the cellular actions of NE on mitral cells using whole cell patch-clamp recordings in rat olfactory bulb slices.

METHODS

Sprague-Dawley rats (18–22 days old), of either sex, were anesthetized with chloral hydrate and decapitated in accordance with Institutional Animal Care and Use Committee and National Institutes of Health guidelines. The olfactory bulbs were removed and immersed in sucrose–artificial cerebrospinal fluid (sucrose-ACSF) equilibrated with 95% O_2-5% CO_2 (pH 7.38) at 4–10°C. The sucrose-ACSF had the following composition (in mM): 26 $NaHCO_3$, 1 NaH_2PO_4, 3 KCl, 5 $MgSO_4$, 0.5 $CaCl_2$, 10 glucose, and 248 sucrose. Horizontal slices (400 μm thick) were cut with a microslicer (Ted Pella, Redding, CA). After a period of recovery (15–20 min) at 30°C, the slices were incubated until used at room temperature (22°C) in ACSF equilibrated with 95% O_2-5% CO_2 and composed of (in mM) 124 NaCl, 26 $NaHCO_3$, 3 KCl, 1.25 NaH_2PO_4, 2 $MgSO_4$, 2 $CaCl_2$, and 10 glucose. For recording, a single slice was placed in a recording chamber on an upright epifluorescent microscope (Olympus BX50WI, Tokyo) and submerged in normal ACSF equilibrated with 95% O_2-5% CO_2, perfused at the rate of 1.5–2.5 ml/min at 30°C.

Patch pipettes were pulled from borosilicate glass capillaries with an inner filament (1.5 mm OD, Clark, Kent, UK) on a pipette puller (Sutter P97) and were filled with a solution of the following composition (in mM): 114 K-gluconate, 17.5 KCl, 4 NaCl, 4 $MgCl_2$, 10 HEPES, 0.2 EGTA, 3 Mg_2ATP, and 0.3 Na_2GTP; in some experiments, 0.02% Lucifer yellow (Molecular Probes, Eugene, OR) was included in the pipette solution. Osmolarity was adjusted to 270 mOsm and pH to 7.3. The pipette resistance was 5–8 MΩ. Whole cell voltage- and current-clamp recordings were made using an Axopatch-200B amplifier (Axon Instruments, Foster City, CA). Liquid junction potential was 9–10 mV, and all reported voltage measurements were not corrected for this potential. Only recordings made with an access resistance of <30 MΩ were included in this study.

Electrical stimulation (Grass S8800 stimulator, Astro-Med, West Warwick, RI) was performed using two stainless steel wires (50 μm diam, A-M Systems, Everett, WA), insulated except at their tips positioned in the olfactory nerve (ON) layer. Stimulus pulses of 10–300 μA, were 100 μs duration and were applied at 0.05 Hz. Evoked field potentials were recorded in the glomerular layer using glass pipettes (0.5–2 MΩ) filled with 2 N NaCl.

Drugs and solutions of different ionic content were applied to the slice by switching the perfusion with a three-way electronic valve system (General Valve, Fairfield, NJ). Norepinephrine bitartrate, phenylephrine, clonidine, isoproterenol, prazosin, and propranolol were obtained from Sigma (St. Louis, MO). Tetrodotoxin (TTX), gabazine (SR95531), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and (±)-2-amino-5-phosphopentanoic acid (APV) were obtained from Research Biochemicals International (Natick, MA).

During the experiments, analog signals were low-pass Bessel filtered at 2 kHz (Axopatch 200B, Axon Instruments), digitized at 10 kHz (Instrutech, Long Island, NY), and stored on videotape for later analysis. They were also collected through a Digidata-1200A Interface (Axon Instruments), and digitized at 10–20 kHz. Group data, expressed as means ± SE, were statistically analyzed with paired *t*-tests unless otherwise stated.

RESULTS

Effects of noradrenergic receptor agonists on ON-evoked synaptic responses

The previously reported NE-induced increase in sensitivity of mitral cells to weak ON input (Ciombor et al. 1999; Jiang et al. 1996) could be due to enhanced postsynaptic responses of mitral cell to glutamatergic input from ON terminals. To test this hypothesis, we investigated the effect of noradrenergic agonists on ON-evoked field potentials (fEPSPs) recorded in the glomerular layer (Fig. 1, *A* and *B*). The peak amplitude of

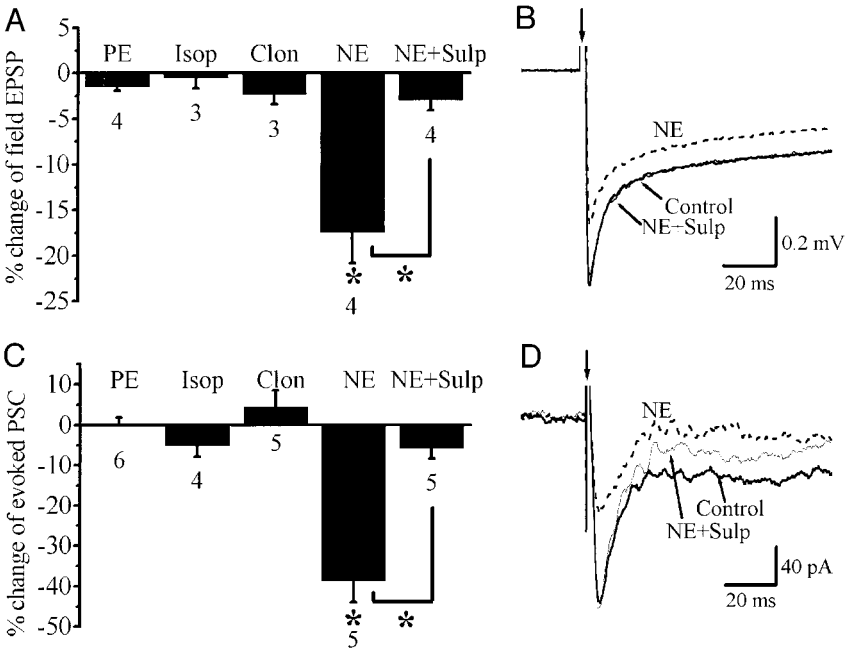

FIG. 1. Effects of norepinephrine (NE) and specific noradrenergic receptor agonists on olfactory nerve (ON)–evoked responses. *A* and *C*: group data showing that the selective noradrenergic receptor agonists tested [phenylephrine (PE), 10 μM; isoproterenol (Isop), 10 μM; clonidine (Clon), 3 μM] did not alter significantly the amplitude of the ON-evoked field excitatory postsynaptic potential (fEPSP) recorded in the glomerular layer (*A*), or the amplitude of the ON-evoked postsynaptic currents (PSC) in mitral cells (*C*). NE (30 μM), however, reduced the amplitude of the ON-evoked fEPSP and PSC. The effects of NE were reversed by the dopamine D2 receptor antagonist sulpiride (Sulp, 100 μM) as also shown in the fEPSP traces in *B* and PSC traces in *D*. Numbers beneath the columns in *A* and *C* indicate the number of cells tested. Asterisks indicate a significant difference from the control response unless otherwise indicated. * $P < 0.05$, paired *t*-test.

the fEPSPs was not changed by PE (10 μM, 0.72 ± 0.1 mV vs. 0.72 ± 0.1 mV, mean ± SE, $P = 0.09$, $n = 4$), isoproterenol (Isop; 10 μM, 0.78 ± 0.11 mV vs. 0.78 ± 0.11 mV, $P = 0.52$, $n = 3$), or clonidine (3 μM, 0.69 ± 0.1 mV vs. 0.67 ± 0.1 mV, $P = 0.19$, $n = 3$). However, NE (30 μM) slightly reduced the fEPSPs by 17 ± 7% (1.1 ± 0.08 mV vs. 0.91 ± 0.09 mV, $P = 0.007$, $n = 4$). Since this action was not mimicked by any of the selective noradrenergic receptor agonists, we wondered whether NE could activate other inhibitory receptors in the glomeruli. Dopamine, a transmitter present in periglomerular interneurons, was recently reported to presynaptically inhibit ON terminals via the D2 receptor subtype (Hsia et al. 1999). Therefore we tested the ability of the D2 dopamine receptor antagonist, sulpiride, to block the inhibitory effect of NE. Sulpiride (100 μM) reversed the inhibitory effect of NE on fEPSPs in four slices tested (NE: 17 ± 7% reduction, NE + sulpiride: 3 ± 1% reduction, $P = 0.02$). Moreover, sulpiride, applied alone, produced no change by itself on the fEPSP, but it prevented the effect of NE (sulpiride: 0.87 ± 0.16 mV; sulpiride + NE: 0.85 ± 0.15 mV, $n = 3$, not shown).

We also tested the effects of noradrenergic agonists on ON-evoked excitatory postsynaptic currents (EPSCs) recorded in mitral cells using whole cell recordings (Fig. 1, *C* and *D*). NE (30 μM) reduced the amplitude of the evoked EPSCs in all cells tested by an average of 46 ± 5% (188 ± 35 pA vs. 103 ± 73 pA, $n = 11$, $P = 0.004$). In five of five cells tested, the depressive effect of NE on the amplitude of the evoked EPSCs was reversed by application of sulpiride (NE: 39 ± 5% reduction, NE + sulpiride: 5 ± 3% reduction, $P = 0.003$). In contrast, there was no effect of PE (10 μM, 144 ± 35 pA vs. 146 ± 37 pA, $P = 0.62$, $n = 6$), Isop (10 μM, 222 ± 32 pA vs. 208 ± 24 pA, $P = 0.22$, $n = 4$), or clonidine (3 μM, 172 ± 35 pA vs. 177 ± 33 pA, $P = 0.35$, $n = 5$) on the amplitude of evoked EPSCs in all cells tested. Taken together, these results indicate that EPSCs induced by ON stimulation are not discernibly modulated by noradrenergic receptors. The apparent depressive effects of NE on ON-evoked responses are probably due to NE activation of inhibitory D2 dopaminergic receptors located on ON terminals, consistent with similar pharmacological findings in the substantia nigra (Grenhoff et al. 1995).

Effect of noradrenergic agonists on mitral cell membrane currents

NE produced an inward current in all mitral cells tested (range 23–110 pA, 54 ± 7 pA, $n = 16$, Fig. 2) in voltage-clamp mode at the holding potential of −60 mV. The magnitude of the response to NE was concentration dependent (1–30 μM; Fig. 2, *A* and *B*). The concentration of NE was increased consecutively at 4-min intervals in the same cells, and the EC_{50} of the NE response was 4.7 μM. Using this protocol, NE induced an inward current that was not significantly different from when it was applied at a single concentration of 30 μM (59 ± 13 pA, $n = 4$ vs. 54 ± 7 pA, $n = 16$, $P = 0.73$, unpaired *t*-test), indicating that there was no substantial desensitization of the response to NE with prolonged application.

The inward current caused by NE could be due to its interaction with different noradrenergic receptor subtypes, namely α1 and β receptors, which are known to produce excitatory effects in many neurons throughout the brain (for review, see Hein and Kobilka 1995). In three cells, a second application of NE in the presence of the α1 and β receptor antagonists (prazosin 1 μM, and propranolol 10 μM, respectively) produced no significant effect (<4 pA, Fig. 2*C*). The effect of NE recovered partially after wash out of the antagonists (>30 min). Next, we investigated which specific noradrenergic agonists could mimic the effect of NE (Fig. 3). Although prazosin has been reported to be an antagonist of α2B and α2C receptor subtypes (Hieble and Ruffolo 1996), α2 receptors do not appear to be involved in the inward current induced by NE in mitral cells because clonidine (3 μM) produced no detectable change in holding current (0.3 ± 1 pA, $n = 6$). Concentrations of clonidine higher than 3 μM were not tested because they can activate nonspecifically α1 receptors. In contrast, the α1 noradrenergic agonist, PE (10 μM), produced an inward current in 15 of 19 mitral cells tested (range

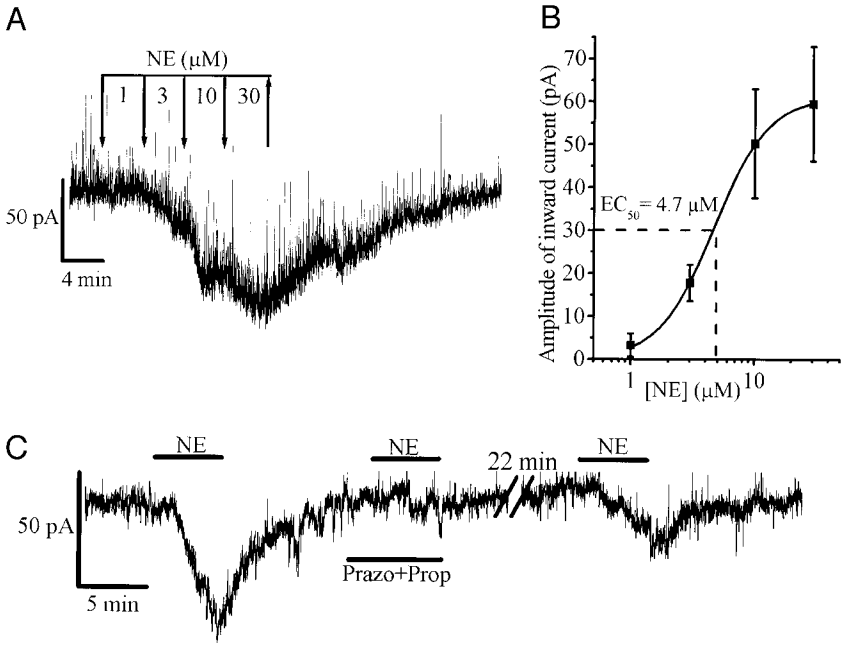

FIG. 2. Effect of norepinephrine (NE) in voltage-clamp recordings. *A*: inward currents evoked by NE at different concentrations (1, 3, 10, and 30 μM added cumulatively, 4 min at each concentration). *B*: a sigmoidal curve was fitted to the NE concentration-response data obtained from 4 cells. The holding potential was −60 mV in this and all subsequent figures showing voltage-clamp recordings. *C*: the response to a 2nd application of NE (30 μM) was blocked in the presence of the α1 receptor antagonist prazosin (Prazo; 1 μM) and the β receptor antagonist propranolol (Prop; 10 μM). The NE-induced inward current recovered partially after wash out of the antagonists (about 30 min).

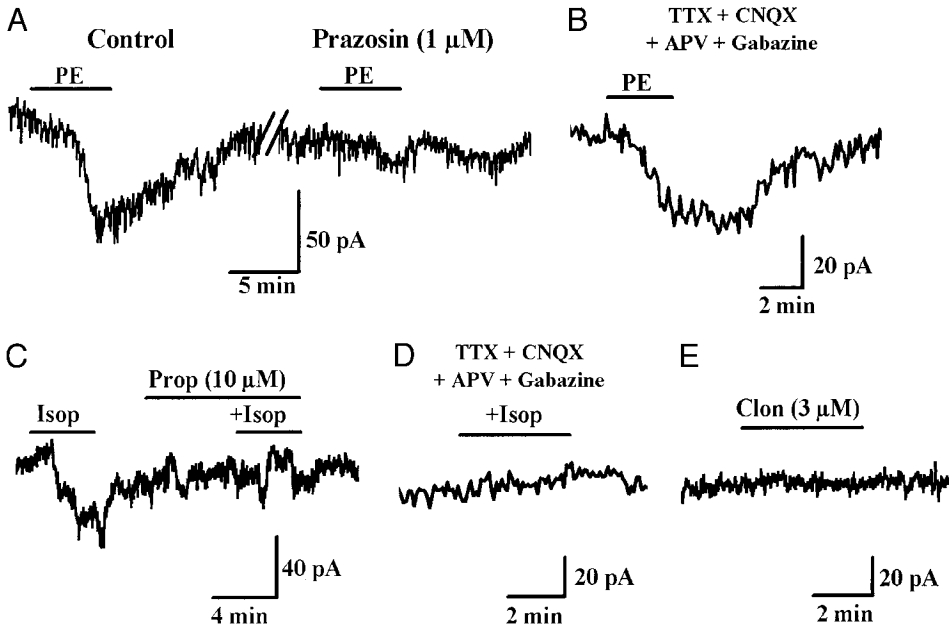

FIG. 3. Effect of specific noradrenergic receptor agonists on the holding current of mitral cells. *A*: phenylephrine (PE, 10 μM) produced an inward current. A 2nd application of PE in the presence of the α1 receptor antagonist prazosin produced no significant effect. *B*: PE-induced inward currents persisted in the presence of TTX, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), (±)-2-amino-5-phosphonopentanoic acid (APV), and gabazine. *C*: isoproterenol (Isop, 10 μM) produced an inward current. A 2nd application of Isop in the presence of the β receptor antagonist propranolol produced no significant effect. *D*: the effects of Isop on mitral cell currents were abolished in the presence of TTX, CNQX, APV, and gabazine. *E*: the α2 receptor agonist clonidine produced no effect on the holding current. Each panel represents a voltage-clamp recording from a different mitral cell.

5–55 pA, 22 ± 4 pA, $n = 19$). In the four remaining cells, PE produced no detectable effect (<4 pA). The effect of PE was reproducible on a second application with no evidence of desensitization ($n = 3$). In three mitral cells that responded significantly to PE (>25 pA), a second application of PE in the presence of the α1 receptor antagonist, prazosin (1 μM), produced no detectable current.

The β receptor agonist, Isop (10 μM) also induced an inward current in all cells tested (range 8–80 pA, 29 ± 9 pA, $n = 8$). A second application of Isop in the presence of the β receptor antagonist propranolol (10 μM) produced no significant inward current ($n = 3$, Fig. 3*C*). Moreover, Isop produced no significant effect when applied for the first time in the presence of propranolol ($n = 4$). Isop also increased the frequency of spontaneous EPSCs (sEPSCs). The sEPSCs were similar to "the long-lasting depolarizations" that have been described recently (Carlson et al. 2000). The inward current caused by Isop could therefore result, in part, from a network effect due to an increase in excitatory input to mitral cells. Alternatively, Isop might reduce tonic inhibition to mitral cells (disinhibition) by inhibiting inhibitory interneurons (namely, granule and periglomerular cells). To investigate these two possibilities, the effect of Isop on the holding current and on sEPSCs was examined in the presence of the $GABA_A$ receptor antagonist gabazine (5 μM); the *N*-methyl-D-aspartate (NMDA) receptor antagonist APV (50 μM) was included in the bath to prevent epileptic activity caused by application of gabazine alone (not shown). Under these conditions, the inward currents produced by Isop (10 μM) persisted (29 ± 6 pA, $n = 5$; Figs. 4*A* and 5), and the frequency of sEPSCs increased in all cells tested by an average of 81 ± 12% (from 0.31 ± 0.07 Hz to 0.54 ± 0.09 Hz, $n = 5$, $P = 0.004$, Fig. 5). All sEPSCs (recorded in APV and gabazine) were α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptor dependent and action potential dependent as they were blocked by CNQX ($n = 4$) or TTX (1 μM, $n = 3$, not shown) (see also Carlson et al. 2000). Although PE induced an inward current in gabazine and APV (32 ± 9 pA, $n = 5$; Fig. 4), it did not change the frequency of sEPSCs.

In the next experiment, we investigated whether the PE- and Isop-induced inward currents are mediated by direct activation of noradrenergic receptors on mitral cells. To eliminate possible indirect effects, we blocked action potential propagation by TTX (1 μM), and ionotropic glutamate and $GABA_A$ receptors were blocked by the antagonists APV (50 μM) and CNQX (10 μM), and gabazine (5 μM), respectively. Under these conditions, Isop (10 μM) had no detectable effect on the holding current of mitral cells (0.5 ± 1.3, $n = 6$; Figs. 3*D* and 4*A*),

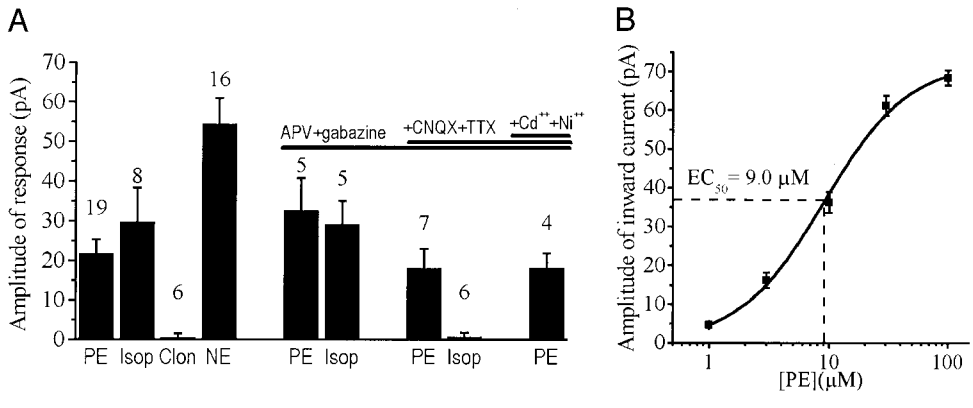

FIG. 4. Effects of adrenergic receptor agonists on the holding current of mitral cells. *A*: group data showing the mean amplitude of the inward currents elicited by the α1, β, and α2 receptor agonists (PE, 10 μM; Isop, 10 μM; and Clon, 3 μM, respectively). Note that in control conditions, Clon produced no effect and that NE (30 μM) produced an effect comparable to the sum of the effect of PE and Isop. The lines above some columns denote that the agonists were tested in the presence of the indicated blockers. The numbers on top of the columns indicate the number of cells tested. *B*: a sigmoidal curve was fitted to the PE concentration-response data obtained from 5 cells recorded in the presence of TTX, CNQX, APV, and gabazine.

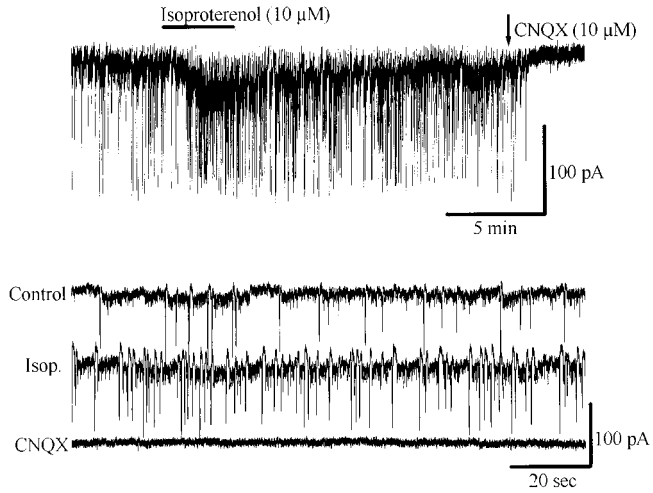

FIG. 5. Effect of isoproterenol under $GABA_A$ receptor blockade. Current trace showing the effect of isoproterenol on the holding current and the frequency of spontaneous excitatory postsynaptic currents (EPSCs) in the presence of the $GABA_A$ receptor antagonist gabazine (3 μM) and the *N*-methyl-D-aspartate (NMDA) receptor antagonist APV (50 μM). The *3 bottom traces* correspond to recordings from the *1st trace* displayed at faster time scale. Isoproterenol induced an inward current associated with an increase in the frequency of spontaneous EPSCs that were completely blocked by CNQX, indicating that they were mediated by non-NMDA receptors.

indicating that the effect of Isop is mediated by an indirect circuit action or requires TTX-sensitive sodium channels in mitral cells. However, in identical conditions, PE (10 μM) still induced an inward current (15–35 pA) in five of seven cells tested (18 $\pm$ 5 pA, $n = 7$; Figs. 3*B* and 4*A*). Because PE produced a direct effect on mitral cells, we examined the effect of PE at different concentrations (1–100 μM) independent of network interactions (in the presence of TTX, CNQX, APV, and gabazine). We measured the concentration-response relationship only in cells that responded with more than 15 pA of inward current to 10 μM of PE. In these cells, PE produced a small inward current (4.6 $\pm$ 0.8 pA) starting at 1 μM, and the estimated EC_{50} was 9.0 μM ($n = 5$, Fig. 4*B*).

Extracellular calcium influx could be one of several second-messenger pathways activated by α1 receptor (Han et al. 1987; Pan et al. 1994; Vaughan et al. 1996). To test this possibility, we applied PE (10 μM) in the presence of blockers of voltage-dependent Ca^{2+} channels, cadmium (100 μM) and nickel (100 μM), in addition to TTX, CNQX, APV, and gabazine. Under these conditions, mitral cells still responded to PE with an inward current (18 $\pm$ 4 pA, $n = 4$) comparable to that observed in the absence of Ca^{2+} channel blockers (Fig. 4). This result indicates that the inward current produced by PE does not result from calcium entry into mitral cells, and therefore voltage-dependent Ca^{2+} channels play little or no role in the α1 receptor–mediated response.

α1 receptor responses are mediated by G-protein–coupled signaling pathways (for review, see Hein and Kobilka 1995; Zhong and Minneman 1999). To determine whether the PE-induced inward current in mitral cells is G-protein mediated, we examined the effect of PE after inactivation of G-proteins by including GDPβS (1 mM) in the pipette solution (Chu and Hablitz 2000; Lin and Dun 1998; Schneider et al. 1998). In mitral cells perfused with an intracellular solution containing GDPβS (1 mM), PE (10 μM) produced no detectable effect on the holding current ($n = 3$, not shown). Therefore G-proteins are involved in the mechanism of action of PE in mitral cells.

The preceding results indicate that the PE-induced current is not carried primarily by influx of calcium through cadmium- and nickel-sensitive calcium channels or by influx of sodium through TTX-sensitive sodium channels. Therefore we investigated whether the response to PE could be explained by a modulation of a potassium conductance. The current-voltage relationship in the absence and presence of PE indicated that the current induced by PE tended to decrease, but did not reverse in polarity, at the equilibrium potential for potassium ions (Fig. 6*A*). We assume that under blockade of fast synaptic transmission, as well as sodium and calcium channel blockade, the major ion channels that contribute to the conductance of the membrane are potassium channels. In this case, the decrease in the slope of the current-voltage curve is indicative of an increase in membrane input resistance due to closure of potassium channels that were open at the range of holding potentials tested (-110 to -30 mV). The inability to obtain a reversal potential for the PE-induced current is probably due to an inadequate space clamp of the mitral cells that have long lateral and apical dendrites. Similar results were also obtained in dorsal raphe neurons (Pan et al. 1994) and ventrolateral rat periaqueductal gray neurons (Vaughan et al. 1996).

One possible mechanism for the action of PE is a reduction of the transient outward potassium conductance I_A as has been described for dorsal raphe serotonergic neurons (Aghajanian 1985). To investigate this possibility, we activated A-like currents by holding the membrane potential at -80 mV for 400 ms (to deinactivate I_A), followed by a depolarizing voltage step to -45 mV (Aghajanian 1985). This protocol generated a transient outward current of 300–1,500 pA that decayed to

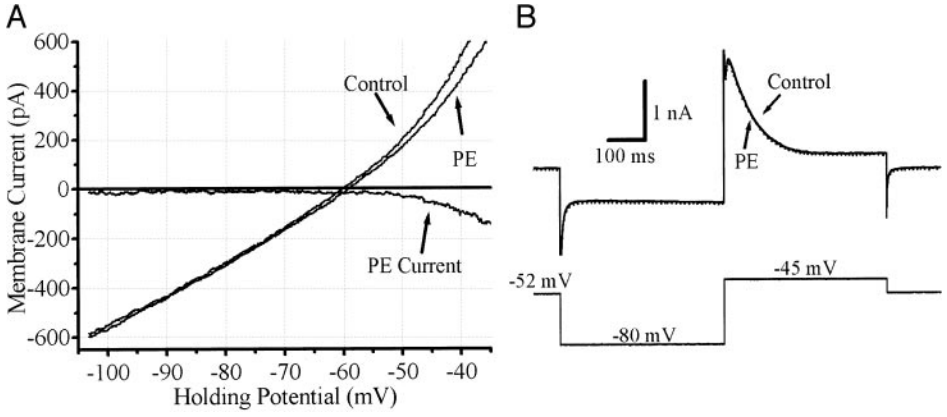

FIG. 6. In the presence of synaptic blockers, PE (10 μM) induced an inward current without affecting I_A. *A*: plot of current-voltage curves in control [in the presence of TTX (1 μM), CNQX (10 μM), APV (50 μM), gabazine (5 μM), cadmium (100 μM), and nickel (100 μM)], and after addition of PE. The PE-induced current was obtained by subtracting the 2 curves. *B*: PE (in the presence of TTX, CNQX, APV, and gabazine) did not alter the transient outward current I_A (*top traces*) induced by the voltage step protocol shown in the *bottom trace*. The current trace in PE (dotted line) was adjusted to offset the change in holding current induced by PE.

baseline level within 100–300 ms. PE had no effect on the amplitude (from 636 ± 343 pA to 634 ± 330 pA, $n = 4$, $P = 0.89$) or the decay time constant (from 91 ± 24 ms to 89 ± 20 ms, $n = 4$, $P = 0.82$) of the current evoked by this protocol (Fig. 6*B*). Taken together, these results suggest that the primary ionic mechanism for the PE-induced response is a decrease in a potassium conductance that is different from I_A.

Effects of α1 receptor activation on membrane potential, and spontaneous and ON-evoked discharge

A previous extracellular unit study showed that NE, acting through α1 receptors, increases responses of mitral cells to weak (i.e., perithreshold) ON shocks, by reducing the percentage of response failures to ON stimulation (Ciombor et al. 1999). The preceding results indicate that NE, via a direct α1 receptor–mediated effect, evokes an inward current in mitral cells. This suggests that α1 receptor activation may depolarize mitral cells, an action that could enhance mitral cell responsiveness to ON input. In the next experiments, therefore, we investigated the effects of PE on mitral cell membrane potential, and spontaneous and ON-evoked discharge.

Mitral cells recorded in vitro exhibit membrane potential bistability (Heyward et al. 2001). As shown in Fig. 7, mitral cells generate two levels of membrane potential separated by about 10 mV: a "down-state," subthreshold for spike generation, and a perithreshold "up-state," in which the cells are more responsive to ON input than the down-state (Fig. 8). Generation of the up-state is an active, voltage-dependent process, sensitive to membrane depolarization (Heyward et al. 2001). We hypothesized, therefore that α1-receptor activation might depolarize mitral cells and thereby increase the proportion of time spent by mitral cells in the up-state, an effect that would enhance responsiveness to weak ON input. This hypothesis was tested using current-clamp recording.

Figure 7 shows mitral cell spontaneous activity, and the membrane potential distributions of activity recorded before and during exposure to PE ($n = 9$, 10 μM). The membrane potential distributions show the proportion of time spent by the cell at each membrane potential. The distributions are bimodal; the two peaks corresponding to the down-state and up-state. PE application resulted in an overall depolarizing shift in the membrane potential distribution of about 1–2 mV (control: −53.4 ± 0.2 mV; PE: −51.5 ± 0.2 mV, $P < 10^{-7}$; control, PE: 1.9 mV, $n = 9$ cells), with a corresponding increase in time spent by the cell in the up-state potentials (from 16 ± 2.1% to 34 ± 5.4%, $P = 0.009$; see Fig. 7). This depolarization was not associated with a change in spontaneous firing rate (control: 3.7 ± 1.8 Hz; PE: 3.6 ± 1.4 Hz, $P = 0.47$).

The depolarization resulting from PE application was, however, associated with increased responsiveness of mitral cells to ON stimulation (Fig. 8). Single ON shocks were delivered at perithreshold intensity, sufficient to elicit short-latency action potentials in about 50% of trials. As shown in Fig. 8, short-latency spikes (latency <20 ms) were reliably elicited when ON shocks were delivered at up-state potentials, while ON shocks in the down-state resulted in either no response, or a spike at long latency (>20 ms). In the presence of PE, membrane potentials were depolarized, the probability of ON-evoked spikes increased, and the mean spike latency was reduced. The proportion of trials in which perithreshold stimulation elicited spikes was increased from 56 ± 9% to 82 ± 10% in the presence of PE ($P < 0.04$, $n = 217$ trials in 7 cells). In the presence of PE, there was a 1.8-fold increase in the proportion of trials in which short-latency spikes were generated in response to perithreshold ON stimulation (control: 38%; PE: 68%, $P < 0.02$, $n = 7$ cells), and a smaller reduction in the proportion of trials in which long-latency spikes were elicited (control: 18%, PE: 13%, $P < 0.03$, $n = 7$ cells). This change in the distribution of response latencies (Fig. 8) corresponded to a significant reduction in overall response latency ($P < 0.0005$, $n = 7$). These data are consistent with the results of earlier studies (Ciombor et al. 1999) and support the hypothesis that α1 receptor activation results in mitral cell depolarization, thereby increasing the probability of a short-latency response to ON stimulation.

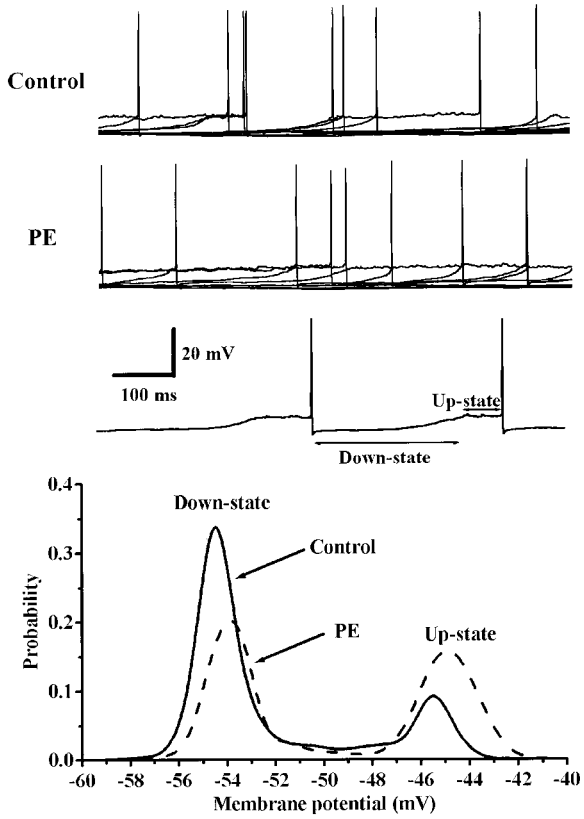

FIG. 7. Activation of α1 receptors results in mitral cell membrane depolarization. *Top 2 sets of traces* (9 superimposed sweeps recorded under each condition) show spontaneous activity recorded in the presence and absence of PE (10 μM). *Middle single trace* (recorded in the absence of PE) illustrates the up-state and down-state. The *histogram at bottom* shows the distribution of membrane potentials generated with and without PE (excluding action potential peaks, binned at 1-mV intervals). PE application resulted in a small membrane depolarization (of about 1 mV, seen as a rightward shift in the voltage distribution), a decrease in the proportion of time spent by the cell in the down-state, and an increase in the proportion of time spent in the up-state. The proportion of time spent in each state was estimated by bisecting the area under the curve between the 2 peaks (see RESULTS).

DISCUSSION

The present results demonstrate that NE, acting at the α1 receptor, induces an apparent inward current that depolarizes mitral cells. This apparent inward current and the accompanying depolarization appear to be due to a decreased potassium

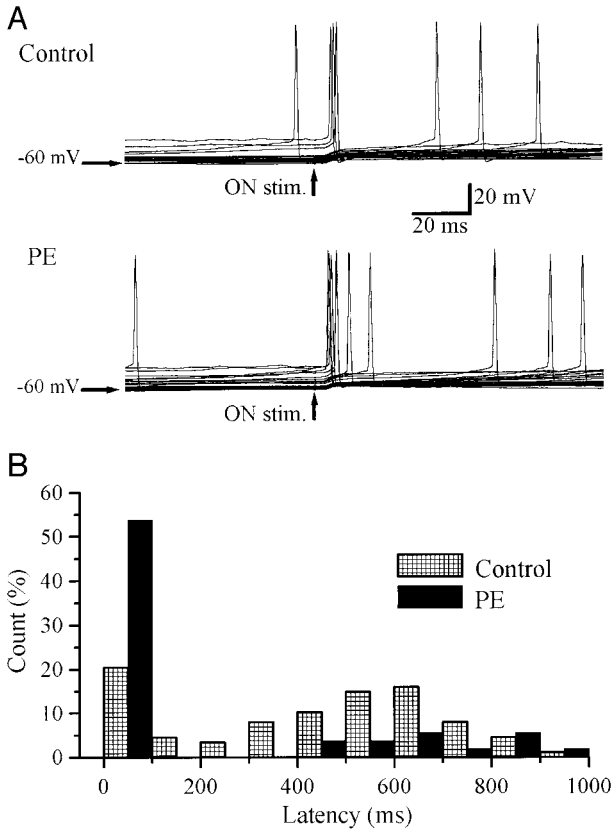

FIG. 8. Activation of α1 receptors increases ON-evoked spiking in mitral cells. *A*: mitral cell responses to perithreshold ON stimulation (see RESULTS). *Top record* (control): single ON shocks evoked short-latency spikes when the cell potential is close to or in the up-state. In the down-state, spikes were elicited at long latency, or did not occur. *Bottom record* (PE): the membrane depolarization produced by 10 μM PE increased the probability that ON stimulation occurred at potentials close to or in the up-state. PE application thus reduced the proportion of ON stimuli that failed to produce a spike (*bottom traces*) and reduced spike latency. Each panel contains 30 superimposed sweeps. *B*: histogram showing the percentages of total spikes over different onset latency intervals in control media and in the presence of PE. Note that the PE-evoked depolarization was associated with an increase in the proportion of short-latency spikes and a decrease in the proportion of long-latency spikes (see RESULTS).

conductance involving a G-protein signaling pathway. Together, these changes significantly modulate the excitability state of mitral cells by *1*) biasing the membrane potential to the perithreshold up-state and *2*) enhancing the generation of action potentials in response ON input.

Olfactory nerve terminals are not modulated by noradrenergic receptors

Electrical stimulation of the ON layer evoked in the glomerular layer fEPSPs that are produced by currents generated, for the most part, within the glomeruli (Aroniadou-Anderjaska et al. 1997). It also evoked long-lasting PSCs in mitral cells confirming previous results (Carlson et al. 2000; Chen and Shepherd 1997; Desmaisons et al. 1999; Ennis et al. 1996; Keller et al. 1998; Nickell et al. 1996). None of the specific noradrenergic agonists used in this study were effective in modulating these two types of ON-evoked excitatory responses. The lack of noradrenergic modulation of excitatory inputs from ON terminals is consistent with anatomical data showing that the glomerular layer, where axons of ON terminals synapse with mitral cell apical dendrites, is nearly devoid of NE fibers (McLean et al. 1989).

Unexpectedly, NE (30 μM) reduced ON-evoked fEPSPs recorded in the glomerular layer and ON-evoked EPSCs in mitral cells. This NE action appears to be mediated by nonnoradrenergic receptors because the depressive effects of NE on ON-evoked fEPSPs and EPSCs were prevented or reversed by the D2-dopamine receptor antagonist sulpiride. In the MOB, D2 receptors are localized exclusively in the ON and glomerular layers (Coronas et al. 1997; Koster et al. 1999; Nickell et al. 1991). Recent studies demonstrate that dopamine, a transmitter present in juxtaglomerular neurons, inhibits glutamate release by activation of presynaptic D2 receptors on ON terminals (Berkowicz and Trombley 2000; Hsia et al. 1999). Taken together, these findings suggest that exogenously applied NE may suppress glutamate release from ON terminals by activating inhibitory presynaptic dopamine D2 receptors present on ON terminals. These actions of NE at dopamine receptors are not without precedent. Interactions between NE and dopamine receptors have been reported in binding studies (Newman-Tancredi et al. 1997), and electrophysiological studies demonstrated that NE-induced hyperpolarizations in substantia nigra neurons were completely blocked by sulpiride (Grenhoff et al. 1995). However, because there are virtually no NE fibers in the glomerular layer, it is unlikely that NE released in the "infraglomerular layers" gains access to D2 receptors. These results suggest that care should be taken in interpreting the pharmacological effects of exogenously applied monoamines in the MOB.

β and α2-receptors

Low to moderate levels of β receptors are located in the glomerular and granule cell layers (Woo and Leon 1995). Application of the β receptor agonist Isop consistently caused an inward current in mitral cells. This current, however, was abolished when mitral cells were pharmacologically isolated from major circuit effects by application of TTX and blockers of fast synaptic transmission. This result suggests that the inward current induced in mitral cells by β receptor activation is most likely a circuit effect, perhaps resulting from increased glutamate release from excitatory inputs, or a decrease in inhibitory GABA inputs to mitral cells, or both. A decrease in GABA input is, however, an unlikely explanation since the effect of Isop persisted in the presence of the $GABA_A$ antagonist gabazine. This leaves increased glutamate release as the remaining possibility. The mechanism for such an effect is unclear as Isop did not alter responses evoked by stimulation of the ON, the only known glutamatergic synaptic input to mitral cells. Recent studies in vitro, however, have shown that in normal physiological media, mitral cells exhibit spontaneous depolarizing events that are mediated by glutamatergic recurrent intraglomerular dendrodendritic interactions among mitral/tufted cells (Carlson et al. 2000). It is therefore conceivable that Isop may enhance the release of glutamate from the apical or lateral dendrites of mitral or tufted cells. Since Isop did not directly evoke currents in mitral cells, this hypothesized effect of Isop may be caused by excitation of tufted cells or of, as of yet undiscovered, excitatory MOB interneurons. Direct excitation of tufted cells might enhance dendrodendritic excitatory

interactions among the tufts of both tufted cells and mitral cells within the glomeruli, leading to an increase in recurrent excitatory glutamate release. An additional possibility is that Isop may increase glutamate release by enhancing calcium currents in the dendrites of mitral or tufted cells. Activation of β receptors has been reported to facilitate glutamate release in the amygdala by increasing presynaptic calcium influx (Huang et al. 1996, 1998). The Isop-induced increase in the excitability of mitral cells observed here may play a role in NE-induced, β receptor–mediated facilitation of olfactory learning in neonatal animals (Sullivan et al. 1989, 1992, 2000).

Receptor localization studies indicate that mitral cell express α2 receptors (Winzer-Serhan et al. 1997). In the present study, the selective α2 receptor agonist, clonidine, did not produce any detectable currents in mitral cells at the holding potential of −60 mV. Our results, however, do not exclude the presence of functional α2 receptors in mitral cells. Although clonidine did not produce a detectable effect, α2 receptors could modulate high-threshold, voltage-gated channels that are closed at the holding potentials tested in the present study. In this regard, it is noteworthy that Trombley (1992, 1994) reported that clonidine decreased high-threshold calcium currents in mitral cells in culture, an effect that reduced glutamate release from these cells. It is also possible that potential actions of clonidine may have been prevented by dialysis of intracellular messengers by the patch pipette solution. Finally, it is possible that the effects of α2 receptors occur at dendritic sites too remote to be detected by somatic recordings.

Activation of α1 receptor induces an inward current in mitral cells

NE, the α1 receptor agonist PE and the β receptor agonist Isop induced relatively similar inward currents in mitral cells. In conditions that eliminate fast synaptic transmission (TTX, APV, CNQX, and gabazine), the inward currents elicited by PE persisted, whereas those elicited by Isop were abolished. This suggests that endogenously released NE may directly modify the membrane conductance of mitral cells via activation of the α1 receptor subtype. The calculated EC_{50} for the PE-evoked inward current in the present study was 9 μM. This value is higher than the EC_{50} for the PE-induced depolarization in dorsal raphe neurons (1.4 μM) (Pan et al. 1994). It is possible that the α1 receptor subtype in mitral cells has lower affinity to the agonist PE. Indeed, at least three subtypes of α1 receptors have been cloned so far (for review, see Docherty 1998).

Current-voltage curves generated in the presence and absence of PE demonstrated that the inward currents evoked by PE decreased at negative membrane potentials near the calculated equilibrium potential for K^+ ions (−96 mV). Space-clamp limitations in mitral cells, in addition to the relatively small magnitude of the PE response (about 20 pA in TTX), precluded accurate determination of the reversal potential of the conductance modulated by PE. Additionally, the current-voltage curves in the presence and absence of PE suggest that activation of α1 receptors is associated with increased input resistance. Taken together, these finding suggest that the α-induced currents in mitral cells are mediated by decreased K^+ conductance. In agreement with this, PE-evoked currents persisted in the presence of the Ca^{2+} channel blockers cadmium and nickel, indicating that Ca^{2+} or Ca^{2+}-dependent potassium channels were not involved. Chloride channels are probably not involved in the inward current produced by PE because their activation would produce an outward current at potentials more positive than −65 mV, which is the equilibrium potential for chloride ions in our conditions. However, we cannot rule out some other possibilities, such as a contribution from sodium TTX-insensitive channels.

Although activation of α1 receptors has been reported to reduce I_A in serotonergic neurons by 34% (Aghajanian 1985), A-like currents were not discernibly affected by PE in mitral cells. A reasonable candidate mechanism for the α1 receptor–mediated excitation is a decrease of a leak potassium conductance, which is decreased by activation of α1 receptors in several brain areas including the dorsal motor nucleus of the vagus (Fukuda et al. 1987), hypoglossal motoneurons (Parkis et al. 1995), dorsal raphe (Pan et al. 1994), thalamus, and cortex (Wang and McCormick 1993). The present study showed that the PE-induced inward current was prevented by intracellular dialysis with GDPβS, a manipulation that blocks G-protein activation. This indicates that the PE-induced inward current is mediated by an α1 receptor G-protein–coupled mechanism. This is similar to the signaling pathway mediating α1 receptor–dependent inhibition of the leak potassium current in other cell types (Grenhoff et al. 1995; Pan et al. 1994; Parkis et al. 1995).

Noradrenergic axons are very dense in the granule cell, mitral cell, and external plexiform (EPL) layers (Halasz et al. 1978; McLean et al. 1989). The EPL has the highest level of α1 receptor binding sites in the MOB (Jones et al. 1985b) and, indeed, the highest density of α1 receptors in the brain (Young and Kuhar 1980). The EPL contains the lateral dendrites of mitral cells and the apical dendrites of the granule cells. Thus both cell types are potential targets of NE fibers. In agreement with this, both mitral and granule cells express α1 receptor mRNA (Day et al. 1997; McCune et al. 1993; Pieribone et al. 1994). Previous studies reported α1 receptor–mediated changes in ON-evoked discharge of mitral cells or evoked field potential activity in the MOB (Ciombor et al. 1999; Mouly et al. 1995; Perez et al. 1987), although the specific site of the α1 receptor action was not determined. The present results indicate that the α1 receptor–mediated responses are due, at least in part, to direct α1 receptor–mediated modulation of mitral cells.

A previous study (Trombley and Shepherd 1992) of dissociated cultured mitral cells did not detect any NE-evoked changes in mitral cell holding currents over the range of voltages similar to those examined in the present study. That study was performed on immature MOB neurons harvested from 1- to 2-day-old rat pups. At birth, however, the MOB exhibits very little α1 receptor binding; the levels of α1 receptors subsequently increase during the second postnatal week and remain stable thereafter (Jones et al. 1985a). This postnatal developmental expression may explain, in part, why NE-evoked inward currents comparable to those observed in the present study were not detected in immature mitral cells.

Activation of α1 receptors depolarizes mitral cells and increases responses to ON input

In current-clamp recordings, rat mitral cells in vitro exhibit membrane potential bistability (Ennis et al. 1997; Heyward et al. 2001), generating two levels of membrane potential separated by about 10 mV: a "down-state," subthreshold for spike

generation, and an "up-state," perithreshold for spike generation. Mitral cell bistability is due to intrinsic membrane properties and persists in the presence of blockers of ionotropic glutamate receptors and $GABA_A$ receptors. Application of PE consistently elicited a relatively small membrane depolarization. This depolarization increased the amount of time spent by the mitral cells at the relatively depolarized, up-state potentials. This depolarization was not associated with a change in spontaneous firing rate of mitral cells, consistent with the results of bath-applied PE in previous studies (Ciombor et al. 1999).

The sensitivity of mitral cells to ON input differs dramatically in the up- and the down-states, such that mitral cells are more responsive to ON stimuli, and ON-evoked spikes occur at shorter onset latencies, in the up- versus the down-state (Heyward et al. 2001). Based on these findings, the depolarization evoked by PE, and the resulting increased time spent by mitral cells in the up- versus the down-state, should increase the excitability of mitral cells in response to ON input. In agreement with this, PE significantly increased the probability of ON-evoked spiking and decreased the overall latency of evoked spikes. Additionally, increased membrane resistance caused by PE may improve the ability of weak or subthreshold ON-evoked synaptic responses to initiate action potentials in mitral cells. As these experiments could not be performed in the presence of synaptic blockers, we cannot exclude that potential PE actions on other cells in the MOB network may have contributed to the mitral cell depolarization and increased responsivity to ON input. However, the present voltage-clamp experiments demonstrate that PE directly modulates mitral cells in a manner consistent with a depolarizing action. This suggests that the increased responsivity to ON input is at least partially due to direct effects of PE on mitral cells.

These results are in agreement with the effects of NE and PE on mitral cell responses to ON stimulation reported in previous extracellular recording studies. Activation of the pontine nucleus locus coeruleus (LC), the sole source of noradrenergic projections to MOB (Shipley et al. 1985), selectively and dramatically enhanced responses of mitral cells to weak or perithreshold ON stimulation in vivo (Jiang et al. 1996). Application of NE or PE similarly increased short-latency spikes in mitral cell produced by perithreshold ON shocks in vitro (Ciombor et al. 1999); these effects were prevented by α1 receptor antagonists. The later study demonstrated that the NE- and PE-evoked increase in ON-evoked responses was due to a reduction in response failures to perithreshold ON shocks, a finding confirmed in the present experiments.

Functional implications

NE inputs to the bulb play important roles in olfactory function. LC-NE projections to the main and accessory olfactory bulb are pivotal to the formation of and/or recall of specific olfactory memories, pheromonal regulation of pregnancy and postpartum maternal behavior (Brennan et al. 1990; Dluzen and Ramirez 1989; Kaba et al. 1989; Rosser and Keverne 1985; Sullivan et al. 1989, 1992; Wilson and Leon 1988). The present results suggest that direct α1 receptor–mediated actions of NE interact with intrinsic membrane properties (bistability) to increase the excitability of mitral cells in response to relatively weak levels of olfactory nerve input. While other actions of NE in the MOB network are possible, the present results taken together with previous studies (Ciombor et al. 1999; Jiang et al. 1996) suggest that endogenously released NE may increase the sensitivity of mitral cells to aid in the detection or discrimination of weak odors. Overall, the behavioral and electrophysiological findings indicate that NE plays a critical role in modulating olfactory function, including formation and/or recall of specific olfactory memories.

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