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ACTIVATION OF α1 AND α2 NORADRENERGIC RECEPTORS EXERT OPPOSING EFFECTS ON EXCITABILITY OF MAIN OLFACTORY BULB GRANULE CELLS

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Abstract—The mammalian main olfactory bulb (MOB) receives a dense noradrenergic innervation from the pontine nucleus locus coeruleus that is important for neonatal odor preference learning and odor processing in mature animals. Modulation of GABAergic granule cells (GCs) is thought to play a key role in the net functional impact of norepinephrine (NE) release in the MOB, yet there are few direct studies of the influence of NE on these cells. In the present study we investigated noradrenergic modulation of GC excitability using electrophysiological approaches in rat MOB slices. A moderate concentration of NE (10 μM) and the α1 receptor agonist phenylephrine (10 μM) depolarized and increased spontaneous or current injection-evoked spiking in GCs. By contrast, low NE concentrations (0.1–1.0 μM) or the α2 receptor agonist clonidine (Clon, 10 μM) hyperpolarized and decreased the discharge of GCs. The effects of NE (10 μM) were blocked by antagonism of α1 and α2 receptors. Inhibitory effects of low NE concentrations were blocked or converted to excitatory responses by α2 receptor blockade, whereas excitatory effects of the moderate NE concentration were converted to inhibitory responses after α1 receptor blockade. NE (10 μM) and phenylephrine elicited inward currents that reversed near the potassium equilibrium potential. The effects of NE and phenylephrine were associated with increased membrane input resistance. Clonidine elicited an outward current associated with decreased membrane input resistance that reversed near the potassium equilibrium potential. These results indicate that α1 and α2 receptor activation exert opposing effects on GC excitability. Low concentrations of NE acting via α2 receptors suppress GC excitability, while higher concentrations of NE acting at α1 receptors increase GC excitability. These findings are consistent with recent findings that α1 and α2 receptor activation increase and decrease, respectively, GABAergic inhibition of mitral cells. The differential affinities of α1 and α2 noradrenergic receptor subtypes may allow for differential modulation of GABA release and olfactory processing as a function of the level of NE release, which in turn, is regulated by behavioral state.

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Abbreviations: ACSF, artificial cerebrospinal fluid; APV, 2-amino-5-phosphonopentanoic acid; Clon, clonidine; CNQX, 6-cyano-2,3-dihydroxy-7-nitro-quinoxaline; GC, granule cell; GCL, GC layer; Idaz, idazoxan; LC, locus coeruleus; mGluR1, metabotropic glutamate receptor; MOB, main olfactory bulb; NE, norepinephrine; PBS, phosphate buffered saline; PE, phenylephrine; Praz, prazosin.

Key words: norepinephrine, main olfactory bulb, adrenergic receptor, granule cells, GABA.

The noradrenergic nucleus locus coeruleus (LC) provides the sole source of noradrenergic innervation of the main olfactory bulb (MOB) (Shipley et al., 1985). Noradrenergic fibers terminate in all but the most superficial MOB layers, densely targeting the internal plexiform and granule cell (GC) layers with a more moderate density in the mitral cell and external plexiform layers (McLean et al., 1989). The LC system plays well documented roles in modulation of behavioral state and sensory processing including olfactory function. Olfactory cues activate LC neurons and trigger norepinephrine (NE) release in the MOB (Ennis and Hayar, 2008). The widespread distribution of noradrenergic fibers in the MOB network provides a structural basis for potentially diverse physiological effects of NE release on olfactory processing. Consistent with this, noradrenergic input to the MOB is critical for the formation of conditioned odor preferences in neonates, as well as odor habituation and discrimination in mature animals (Sullivan et al., 2000; Harley et al., 2006; Doucette et al., 2007; Veyrac et al., 2007; Guérin et al., 2008; Mandairon et al., 2008).

The dense LC-NE innervation of the GC layer (GCL) suggests that modulation of GABAergic inhibition is a major component involved in noradrenergic modulation of olfactory processing in the MOB network. However, there are relatively few direct physiological studies on the impact of NE on the excitability of GABAergic GCs in the mammalian MOB. NE was reported to suppress GABAa receptor-mediated inhibition of mitral cells in the turtle MOB, an effect attributed to direct inhibition of GCs (Jahr and Nicoll, 1982). NE, acting at α2 receptors, was similarly found to reduce GABAergic inhibition of cultured rat mitral cells due to presynaptic inhibition of transmitter release from mitral or GCs (Trombley, 1992; Trombley and Shepherd, 1992). However, α2 receptor activation did not directly influence GC cell excitability in the *Xenopus laevis* MOB (Czesnik et al., 2001). Smith et al. (2009) observed that NE or α1 receptor activation depolarized accessory olfactory bulb GCs. Recent studies in MOB slices demonstrated that GABAa receptor-mediated inhibition of mitral cells is bidirectionally regulated in a concentration-dependent manner by NE. Low NE concentrations in the sub-micromolar range, acting at α2 receptors, suppressed GABAergic inhibition while low micromolar concentrations increased GABAergic inhibition via α1 receptors (Nai et al., 2009). NE-induced, α1 receptor-mediated increase in GABAergic

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inhibition of mitral cells has also been observed in the accessory olfactory bulb (Araneda and Firestein, 2006). In both studies, the effects of NE were mediated on neuronal elements presynaptic to mitral cells, presumably by direct modulation of GC excitability.

Taken together, the preceding studies suggest that NE may modulate GABAergic inhibition of mitral cells via presynaptic actions on GCs involving both α1 and α2 receptors. The goal of the present study was to assess the direct postsynaptic effects of α1 and α2 receptor activation on the excitability of GCs using patch clamp electrophysiology in rat MOB slices. Our results indicate that α1 receptor activation increases, and α2 receptor activation decreases, spontaneous and evoked discharge in GCs in a manner consistent with recently observed effects of activation of these receptor subtypes on GABAa receptor-mediated inhibition of mitral cells (Nai et al., 2009).

EXPERIMENTAL PROCEDURES

Slice preparation

Male and female 14–28-day-old Sprague–Dawley rats were decapitated in accordance with Institutional Animal Care and Use Committee and National Institute of Health guidelines. Horizontal 400 μm-thick olfactory bulb slices were prepared as previously described (Dong et al., 2007). Briefly, the olfactory bulbs and a portion of the forebrain were dissected free from the surrounding skull, removed and immersed in oxygenated chilled sucrose-artificial cerebrospinal fluid (ACSF) composed of (in mM): 26 $NaHCO_3$, 1 NaH_2PO_4, 3 KCl, 4 $MgSO_4$, 0.1 $CaCl_2$, 20 glucose and 234 sucrose; pH 7.3, 310 mOsm. Slices were cut using a Vibratome 3000 (Vibratome, St. Louis, MO, USA) and transferred to an incubation chamber filled with normal ACSF saturated with 95% O_2 and 5% CO_2 and composed of (in mM): 124 NaCl, 26 $NaHCO_3$, 1 NaH_2PO_4, 3 KCl, 2 $MgSO_4$, 2 $CaCl_2$, 10 glucose, 0.4 ascorbic acid, 2 sodium pyruvate (pH 7.3, 310 mOsm); osmolarity was 310 mOsm. NaH_2PO_4 was omitted in experiments where nickel and cadmium were added to the ACSF. Slices were held at 33 °C for 15 min, and then at room temperature (22 °C) until used. For recording, a single slice was placed in a recording chamber and continuously perfused with ACSF equilibrated with 95% O_2 and 5% CO_2.

Electrophysiology

Whole-cell recordings were performed at 30 °C. Drugs were applied by bath perfusion (1.5–2 ml/min) for 4 min. The recording pipette contained (in mM): 120 potassium gluconate, 6 KCl, 2 NaCl, 2 $MgCl_2$, 10 phosphocreatine ditris salt, 3 MgATP, 0.3 Na_2GTP, 0.2 EGTA, 10 HEPES, and (pH 7.3, 290 mOsm). The intracellular solution also contained 0.4% biocytin and 0.02% Lucifer Yellow. Neurons were visualized using an upright microscope (BX51WI; Olympus Optical, Tokyo, Japan) equipped with epifluorescence and near-infrared differential interference contrast optics.

Whole-cell current and voltage clamp techniques were used to record membrane potential and currents. Analog signals were low-pass filtered at 2 kHz (Axopatch 200B) and digitized at 5 kHz using a Digidata-1322A interface and Clampex 9.0 software (Molecular Devices, Sunnyvale, CA, USA). In current clamp membrane input resistance was determined by voltage changes elicited by negative current pulses (−2 to −100 pA, 500 ms). I–V relations of NE and agonist evoked currents were studied with a voltage ramp protocol (−120 to −10 mV, 70 mV/s, 1.4 s duration) from a holding potential (HP) of −80 mV; the current attributable to NE or agonists was determined by subtracting the curves recorded in their presence and absence. The membrane potential and firing frequency were analyzed with ClampFit 9.0 (Molecular Devices) and Mini Analysis program (Synaptosoft, Decatur, GA, USA). OriginPro 8 (OriginLab Corporation, Northampton, MA, USA) was used for further data analysis.

GCs were identified by soma location, and distinct morphological (small soma size, single apical dendrite ramifying in the external plexiform layer) and electrophysiological (high input impedance, relatively hyperpolarized resting membrane potential, low rate of spontaneous spiking) properties. The majority of cells recorded in the present study were superficial GCs located in the mitral cell and internal plexiform layers. Recordings were also obtained from a smaller subset of deep GCs within the GCL proper.

Data analysis

Data, expressed as mean±SEM, were statistically analyzed using one-way repeated measurement ANOVA followed by post hoc comparisons (Newman–Keuls tests), or with Student's *t*-tests (SigmaStat, Aspire Software International, Ashburn, VA, USA). Percentage data from different groups were analyzed with the Mann–Whitney U test.

Immunohistochemistry and microscopy

Slices containing the recorded cells were treated using methods described previously (Dong and Buonomano, 2005). Briefly, the slices were fixed with 4% paraformaldehyde overnight and then rinsed thoroughly with 0.1 M phosphate buffered saline (PBS, pH 7.4) at room temperature. Slices were incubated with 0.6% Triton X-100 (0.1 M PBS) at room temperature for 1 h, then in 0.1 M PBS with 1:200 Avidin-Oregon Green 488 conjugate at 4 °C overnight. After rinsing in 0.1 M PBS for three times, 10 min each, the slices were mounted with the Vectashield mounting medium with DAPI (H-1200, Burlingame, CA, USA). Images of labeled cells were captured using an upright Olympus BX50 microscope equipped with a BioRad MRC 1024 Confocal system with krypton-argon laser (Hemel Hempstead, UK).

Drugs and solutions

Drugs were applied by switching the bath perfusion solution with a three-way valve system. Biocytin and Avidin-Oregon Green 488 were purchased from Molecular Probes (Carlsbad, CA, USA). Clonidine (Clon), prazosin (Praz), idazoxan (Idaz), LY367385, tetrodotoxin (TTX), 2-amino-5-phosphonopentanoic acid (APV), and 6-cyano-2,3-dihydroxy-7-nitro-quinoxaline (CNQX) were obtained from Tocris Bioscience (Ellisville, MO, USA). NE, phenylephrine (PE), gabazine and other chemicals were purchased from Sigma (St. Louis, MO, USA).

RESULTS

Recordings were obtained from 109 superficial and 17 deep GCs. The mean resting membrane potential (−64.5± 1.1 mV vs. −64.9±2.0 mV) and input impedance (1.2±0.1 GΩ vs. 1.7±0.3 GΩ) did not differ between the two GCs subtypes ($P>0.05$, *t*-tests). Unless specified otherwise, the data reported were obtained from superficial GCs. An example of a biocytin-filled superficial GC is shown in Fig. 1A.

Effects of NE and receptor agonists on GC membrane potential and discharge

A previous study from our laboratory reported that α1 and α2 receptor activation modulates GABAergic input to mitral