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ELECTROPHYSIOLOGICAL PROPERTIES OF CATECHOLAMINERGIC NEURONS IN THE NOREPINEPHRINE-DEFICIENT MOUSE

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Abstract—To determine how norepinephrine affects the basic physiological properties of catecholaminergic neurons, brain slices containing the substantia nigra pars compacta and locus coeruleus were studied with cell-attached and whole-cell recordings in control and dopamine β-hydroxylase knockout (*Dbh* −/−) mice that lack norepinephrine. In the cell-attached configuration, the spontaneous firing rate and pattern of locus coeruleus neurons recorded from *Dbh* −/− mice were the same as the firing rate and pattern recorded from heterozygous littermates (*Dbh* +/−). During whole-cell recordings, synaptic stimulation produced an α-2 receptor-mediated outward current in the locus coeruleus of control mice that was absent in *Dbh* −/− mice. Normal α-2 mediated outward currents were restored in *Dbh* −/− slices after pre-incubation with norepinephrine. Locus coeruleus neurons also displayed similar changes in holding current in response to bath application of norepinephrine, UK 14304, and methionine-enkephalin. Dopamine neurons recorded in the substantia nigra pars compacta similarly showed no differences between slices harvested from *Dbh* −/− and control mice. These results indicate that endogenous norepinephrine is not necessary for the expression of catecholaminergic neuron firing properties or responses to direct agonists, but is necessary for auto-inhibition mediated by indirect α-2 receptor stimulation.

Key words: dopamine β-hydroxylase knockout, locus coeruleus, substantia nigra pars compacta, cocaine.

The activity of norepinephrine (NE) neurons in the locus coeruleus (LC) has an important role in selective attention, general arousal, and stress reactions upon challenging environmental situations (Foote et al., 1983; Levine et al., 1990; Berridge and Waterhouse, 2003; Aston-Jones and Cohen, 2005), and NE depletion may underlie some affective disorders and disease states (Ressler and Nemeroff, 1999). In addition, the LC projects directly to, and modulates the activity of, midbrain dopamine (DA) neurons (Swanson and Hartman, 1975; Jones and Moore, 1977; Grenhoff et al., 1993; Grenhoff and Svensson, 1993; Liprando et al., 2004), which are critical for reward- and motor-related behaviors (Girault and Greengard, 2004; Montague et al., 2004). In an intact animal, NE neurons recorded *in vitro* fire spontaneously in a single-spike pattern at a rate of up to five spikes per second (Williams et al., 1984). However, it is not known whether NE depletion affects the basic physiological properties of NE or DA neurons.

Dopamine β-hydroxylase knockout (*Dbh* −/−) mice completely lack NE and have many brain-mediated behavioral abnormalities, including impaired maternal and social behavior, defects in memory retrieval, increased seizure susceptibility, dysregulation of DA signaling, and hypersensitivity to psychostimulants (Thomas et al., 1995, 1998; Thomas and Palmiter, 1997a,b; Szot et al., 1999; Weinshenker and Szot, 2002; Murchison et al., 2004; Marino et al., 2005; Schank et al., 2005). NE neurons are intact and make proper connections in *Dbh* −/− mice, and receptor levels and co-transmitter expression are remarkably normal (Weinshenker et al., 2002b; Jin et al., 2004; Sanders et al., 2005; D. Weinshenker, unpublished observations). Many *Dbh* −/− phenotypes are reversible by acute pharmacological restoration of NE, indicating that the phenotypes are specifically due to NE deficiency (Thomas and Palmiter, 1997b; Thomas et al., 1998; Szot et al., 1999; Weinshenker et al., 2000). However, it is unclear whether NE replacement truly restores the NE system to a "wild-type" state. In addition, other *Dbh* −/− phenotypes cannot be pharmacologically rescued (Thomas and Palmiter, 1997b; Weinshenker et al., 2002a), provoking the question of whether the chronic absence of NE affects the firing properties of LC neurons and contributes to these deficits. Here we assessed the properties of NE neurons from *Dbh* −/− mice to gain insights into how this neurotransmitter influences LC activity. We also examined the firing properties of DA neurons to determine whether the dysregulation of DA release in *Dbh* −/− mice is an inherent abnormality in DA neurons.

EXPERIMENTAL PROCEDURES

Animals

Mice were used in accordance with guidelines for animal care and use established by the National Institutes of Health, and the Animal Care Committees at the University of Texas at San Antonio, Emory University, and Oregon Health and Science University. All experimental procedures were designed to minimize the num-

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Abbreviations: DA, dopamine; *Dbh*, dopamine β-hydroxylase; DOPS, L-3,4-dihydroxyphenylserine; GIRK, G protein-coupled inward rectifier potassium channel; LC, locus coeruleus; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f]-quinoxaline; NE, norepinephrine; SNC, substantia nigra pars compacta.

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ber of animals used and their suffering. *Dbh* −/− mice were bred as described and maintained on a mixed C57BL6/J and 129SvEv genetic background (Thomas et al., 1998). *Dbh* +/− mice have normal brain NE content and are behaviorally indistinguishable from wild-type mice, and were used as controls (Thomas and Palmiter, 1997a, 1998; Szot et al., 1999; Marino et al., 2005). Because complete NE deficiency is lethal embryonically in mice (Thomas et al., 1995), NE was restored to knockout embryos by supplementing the drinking water of pregnant dams with adrenergic agonists from E 9.5 to E 14.5, and L-3,4-dihydroxyphenylserine (DOPS) from E 14.5 until birth (Thomas et al., 1995, 1998). DOPS can be converted to NE by the enzyme aromatic acid decarboxylase, thus bypassing the requirement for DBH. NE is not required for postnatal survival, so the mice used in the study lacked NE since birth. *Dbh* −/− and littermate *Dbh* +/− mice used for recordings *in vitro* were 6–12 weeks old.

Slice recordings. Horizontal brain slices (200–300 μm) were prepared from 26 mice (11 *Dbh* −/− mice and 15 *Dbh* +/− mice) as described previously (Torrecilla et al., 2002). The experimenter was blind to the genotype of the animal until after the experiments were completed. Horizontal slices were placed in a chamber (0.5 ml) superfused with physiological saline (35 °C) at a rate of 1.5 ml/min. The solution was equilibrated with 95% O_2 25% CO_2 (pH 7.4) and contained 126 mM NaCl, 2.5 mM KCl, 1.2 mM $MgCl_2$, 2.4 mM $CaCl_2$, 1.4 mM NaH_2PO_4, 25 mM $NaHCO_3$, and 11 mM D-glucose. The internal solution used for NE neuron whole-cell recordings contained 115 mM K-methyl sulfate, 20 mM KCl, 1 mM $MgCl_2$, 10 mM Hepes, 0.1 mM EGTA, 2 mM ATP, 0.3 mM GTP, and 10 mM creatine phosphate. DA neuron recordings contained the same internal solution except 10 mM BAPTA was used instead of 0.1 mM EGTA. Patch recordings were made by using an Axopatch 1D amplifier (Axon Instruments, Foster City, CA, USA). Midbrain DA neurons were identified by their electrical properties, which included slow spontaneous activity and a hyperpolarization-induced inward current (H-current; Johnson and North, 1992; Mercuri et al., 1995; Neuhoff et al., 2002). NE neurons were identified visually within the LC and fired action potentials at a rate up to 5 Hz (Williams et al., 1984; Alvarez-Maubecin et al., 2000).

Evoked responses. Iontophoretic pipettes (20–50 MΩ) were filled with NE or DA (0.5 M, pH 7.5) and placed within 10 μm of the soma or proximal dendrite. Iontophoretic pulses (50 nA, 50 ms; −1 nA backing current) were applied once per minute. Synaptic currents were evoked with bipolar tungsten stimulating electrodes with a tip separation of 300–600 μm and placed rostral to the recording site (within 1 mm). A train of five to 10 stimuli (400 μs at 0.3–0.5 mA) was delivered at 66 Hz once every 60 s. Evoked responses were measured as the peak amplitude and width of the response at 50% of amplitude, relative to baseline holding current directly before stimulation. Picrotoxin (100 μM) and strychnine (1 μM) were used to block GABA type A ($GABA_A$) and glycine receptors, respectively. The α-2-adrenergic receptor inhibitory postsynaptic potential was isolated by using 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f]-quinoxaline (NBQX) (5 μM), MK-801 (50 μM), and CGP 56999a (100 nM) to block AMPA, *N*-methyl-D-aspartate (NMDA), and GABA type B ($GABA_B$) receptors, respectively.

Drugs. Drugs were applied to the slice by superfusion, except when NE or DA was iontophoresed (see above). ATP, baclofen, DA–HCl, guanosine trisphosphate, NE, methionine-enkephalin, picrotoxin, yohimbine, and strychnine were from Sigma-Aldrich (St. Louis, MO, USA). S(−)-eticlopride and MK-801 were from Research Biochemicals (Natick, MA, USA). NBQX, (S)-methyl-4-carboxyphenylglycine (MCPG), and (S)-3,5-dihydroxyphenylglycine were from Tocris Cookson (St. Louis, MO, USA). CGP 56999a was a gift from Novartis Pharmaceuticals (Basel, Switzerland). Cocaine–HCl was from the National Institute on Drug Abuse.

Data analysis

Values are given as means±S.E.M. For all experiments, $P<0.05$ was considered as a significant difference. The firing pattern coefficient of variation, defined as the standard deviation divided by the mean firing rate, was also measured. For *in vitro* recordings, the change produced by a drug was calculated as the mean holding evoked current amplitude 30 s after equilibrium had been reached relative to the holding current before drug superfusion. Unpaired comparisons between two groups were made with a Mann-Whitney *U* test, whereas paired comparisons were made by using a Wilcoxon signed-rank test. Concentration-response curves were compared with a two way repeated-measures ANOVA. Maximum D2 receptor-mediated currents and I_H were analyzed with unpaired Student's *t*-test.

RESULTS

Basic electrophysiological properties of LC neurons

NE neurons in the LC fire spontaneously *in vitro* in a single-spike pattern. A typical firing pattern of a LC neuron recorded in the cell-attached mode for both a *Dbh* −/− and control mouse is shown in Fig. 1A. The average firing rates from *Dbh* −/− and control mice were the same (Fig. 1B; *Dbh* −/−: 0.8±0.2 spikes per s, $n=15$; *Dbh* +/−: 0.9±0.1 spikes per s, $n=17$; $P=0.10$). There was also no difference in firing pattern between *Dbh* −/− and control mice. The coefficient of variation in control mice and *Dbh* −/− was 49.0±12.2% and 47.6±18.8%, respectively ($n=12$, 9; $P=0.95$). NE neurons also display a characteristically linear current response to hyperpolarizing voltage commands (10 mV steps from −50 to −80 mV) (Williams et al., 1984). The currents were measured and compared between *Dbh* −/− and control mice, and no difference in amplitude could be detected (Fig. 1C, D; $n=24$; $P=0.62$). Thus, the postnatal formation and maintenance of these basic electrophysiological properties of LC neurons are similar in *Dbh* −/− and control mice.

α-2 Receptor-mediated currents in LC neurons and cocaine

Iontophoretic application of NE on the recorded LC neuron induced an α-2-mediated outward current in control mice (36.3±7.9 pA, $n=4$). Synaptic stimulation of the slice in the presence of antagonists for GABAergic and glutamatergic inputs also induced an average outward current of 28.4±3.0 pA ($n=12$). The effects of cocaine on the iontophoretically- and synaptically-induced currents were also measured. By interacting with biogenic amine transporters, like the NE transporter, cocaine blocks neurotransmitter reuptake, resulting in increased extracellular concentrations of neurotransmitter (Amara and Sonders, 1998). Cocaine-induced increase of NE concentration in the midbrain is known to inhibit the activity of LC neurons through activation of α-2 autoreceptors (Pitts and Marwah, 1987). When cocaine was perfused in the slice, the peak amplitude of the outward currents induced by electrical stimulation (control mice only) was increased to 137±9% of base-