In vivo voltammetry monitoring of electrically evoked extracellular norepinephrine in subregions of the bed nucleus of the stria terminalis

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Herr NR, Park J, McElligott ZA, Belle AM, Carelli RM, Wightman RM. In vivo voltammetry monitoring of electrically evoked extracellular norepinephrine in subregions of the bed nucleus of the stria terminalis. J Neurophysiol 107: 1731–1737, 2012. First published December 21, 2011; doi:10.1152/jn.00620.2011.—Norepinephrine (NE) is an easily oxidized neurotransmitter that is found throughout the brain. Considerable evidence suggests that it plays an important role in neurocircuitry related to fear and anxiety responses. In certain subregions of the bed nucleus of the stria terminalis (BNST), NE is found in large amounts. In this work we probed differences in electrically evoked release of NE and its regulation by the norepinephrine transporter (NET) and the α2-adrenergic autoreceptor (α2-AR) in two regions of the BNST of anesthetized rats. NE was monitored in the dorsomedial BNST (dmBNST) and ventral BNST (vBNST) by fast-scan cyclic voltammetry at carbon fiber microelectrodes. Pharmacological agents were introduced either by systemic application (intraperitoneal injection) or by local application (iontophoresis). The iontophoresis barrels were attached to a carbon fiber microelectrode to allow simultaneous detection of evoked NE release and quantitation of iontophoretic delivery. Desipramine (DMI), an inhibitor of NET, increased evoked release and slowed clearance of released NE in both regions independent of the mode of delivery. However, the effects of DMI were more robust in the vBNST than in the dmBNST. Similarly, the α2-AR autoreceptor inhibitor idazoxan (IDA) enhanced NE release in both regions but to a greater extent in the vBNST by both modes of delivery. Since both local application by iontophoresis and systemic application of IDA had similar effects on NE release, our results indicate that terminal autoreceptors play a predominant role in the inhibition of subsequent release.

fast-scan cyclic voltammetry; iontophoresis; norepinephrine transporter; adrenergic receptors; catecholamines

THE BED NUCLEUS of the stria terminalis (BNST) is a heterogeneous brain region that relays information from cortical, hippocampal, and amygdalar nuclei to subcortical, hypothalamic, and brain stem regions (Cullinan et al. 1993; Dong et al. 2000, 2001a, 2001b). Various studies have explored the role of the BNST in mediating a host of behavioral responses ranging from fear and anxiety to addiction and reward (Cecchi et al. 2002; Delfs et al. 2000; Epping-Jordan et al. 1998; Erb et al. 2000; Fendt et al. 2005; Sullivan et al. 2004; Walker et al. 2009). Neurons releasing dopamine (DA) and norepinephrine (NE) have projections to the BNST and have demonstrated modulatory effects on signaling in this region (Forray and Gylysing 2004; McElligott and Winder 2009; Meloni et al. 2006a). In the rat, the BNST spans ~1 mm on the medial-lateral axis and ~1.5 mm on the dorsal-ventral axis, including the anterior commissure (AC), which transects the BNST, dividing it into dorsal and ventral portions. BNST subnuclei reside in close proximity to the caudate putamen (CPU), ventral pallidum (VP), and the preoptic areas (PA), all of which contain catecholamines (Fig. 1A). Because BNST subregions containing appreciable amounts of NE or DA are often only a few hundred micrometers across, techniques with high spatial resolution and sensitivity are required to investigate the individual subregions.

Immunohistochemical experiments have demonstrated that noradrenergic and dopaminergic fibers are anatomically segregated in the BNST. The ventral BNST (vBNST), located ventral to the AC, has the highest noradrenergic innervation from the A6, A2, and A1 cell groups in the brain but little DA content (Delfs et al. 2000; Kozicz 2002; Park 2009). Emerging immunohistochemical and neurochemical evidence, however, suggests that both NE and DA are present in the dorsal BNST (Egli et al. 2005; Freedman and Cassell 1994; Kilts and Anderson 1986). Specifically, the dorsolateral region of the BNST receives dopaminergic projections from the ventral tegmental area (VTA) and the periaqueductal gray (Hasue and Shammah-Lagnado 2002; Meloni et al. 2006b). In contrast, the dorsomedial BNST (dmBNST) receives mainly noradrenergic inputs (Fendt et al. 2005; Freedman and Cassell 1994). These immunohistochemical studies, however, do not establish whether the projections stained contain functional release sites. Microdialysis studies provide supporting evidence that DA and NE are found within the BNST (Carboni et al. 2000; Cecchi et al. 2002), but the technique lacks the spatial resolution necessary to determine functional innervations of subnuclei.

Previously, we demonstrated that stimulus-evoked NE release in the vBNST can be measured by utilizing in vivo fast-scan cyclic voltammetry (FSCV) at carbon fiber microelectrodes (Park et al. 2009). A key advantage of this experimental approach is that carbon fiber microelectrodes (~75 μm in length × 5 μm in diameter) provide the spatial resolution necessary to selectively monitor catecholamines in small BNST subregions. Empirical evidence demonstrates that <100 μm can separate functional release sites where catecholamine is observed and nonfunctional release sites where no catecholamine is observed. In addition, rapid changes (subsecond) in the extracellular catecholamine concentration can be monitored in real time, allowing critical differences in the dynamics of release and uptake among the BNST subregions to be probed.
As predicted by the anatomy, we functionally demonstrate here that the dmBNST and vBNST contain release and uptake sites for NE. We also use iontophoresis, a localized drug delivery tool, to electrically deliver small amounts of drug directly onto the terminals being studied. Iontophoretic drug application results in an almost immediate drug response because absorption or distribution is not necessary for the drug to take effect at the site of measurement. Additionally, because of the localized nature of iontophoretic drug delivery, negligible amounts of drug are present at distances > 100 μm from the site of delivery. Thus surrounding areas will be drug naive. For quantitative delivery, iontophoresis barrels are coupled to a carbon fiber microelectrode and the amount of an electroactive marker compound is monitored (Herr et al. 2008, 2010). With this approach, drug effects can be compared and averaged across subregions and animals because similar amounts of drug are quantitatively delivered. In this work, we examine differences in evoked NE release in the vBNST and dmBNST with FSCV. In addition, NE regulation by the norepinephrine transporter (NET) and the NE autoreceptor, the α2-adrenergic receptor (α2-AR), is compared in both regions with desipramine (DMI), an inhibitor of NET, and idazoxan (IDA), an α2-AR autoreceptor antagonist. Both drugs were administered either by intraperitoneal (ip) injection or by iontophoresis to differentiate regulation occurring at local terminals from that occurring systemically. Analysis of systemic and localized drug effects, as well as comparison of the vBNST and dmBNST, provides new insight into the differential neurotransmission of NE in the BNST.

MATERIALS AND METHODS

Chemicals and drugs. Unless noted, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. Solutions were prepared with deionized water. A physiological buffer solution (pH 7.4; in mM: 15 Tris, 126 NaCl, 2.5 KCl, 25 NaHCO3, 2.4 CaCl2, 1.2 NaH2PO4, 1.2 MgCl2, 2.0 Na2SO4) was used in all calibration experiments. Acetaminophen (AP), desipramine-HCl, raclopride-HCl, and idazoxan-HCl were dissolved in saline. GBR 12909-HCl was dissolved in deionized water and then diluted with saline.

Animals and surgery. Male Sprague-Dawley rats (225–350 g; Charles River, Wilmington, MA) were anesthetized with urethane (1.5 g/kg ip) and placed in a stereotaxic frame (Kopf, Tujunga, CA). Holes were drilled in the right hemisphere of the skull for the working and stimulating electrodes at coordinates selected from the atlas of Paxinos and Watson (Paxinos and Watson 2005). A Ag/AgCl reference electrode was inserted in the left hemisphere. Working electrodes were placed either in the dmBNST [+1.2 mm medial-lateral (M-L), 0 mm anterior-posterior (A-P), and 6.0–6.6 mm dorsal-ventral (D-V) from bregma] or the vBNST (+1.2 mm M-L, 0 mm A-P, 7.0–7.4 mm D-V). These coordinates were chosen because NE release can be evoked at both sites and both lie on the same anterior-posterior axis.
simplifying the experimental design. The stimulating electrode was placed in the ventral noradrenergic bundle (VNAB) as it passes through the VTA and substantia nigra (+1.0 mm M-L, −5.2 mm A-P, 8.0–9.0 mm D-V from bregma). The carbon fiber and stimulating electrodes were individually adjusted in the dorsal-ventral coordinate to locate the optimal locations to measure stimulated NE release. All procedures were approved by the Institutional Animal Care and Use Committee of the University of North Carolina.

Electrical stimulation. An untwisted bipolar stimulating electrode (Plastics One, Roanoke, VA) was used to stimulate neurons with a pair of linear constant-current stimulus isolators (model NL80A, NeuroLog System, Digitimer). The stimulation consisted of 40 biphasic pulses (±300 μA, 2 ms/phase) applied at 60 Hz. The pulses were generated by a computer and applied between the cyclic voltamograms to avoid electrical interference.

Iontophoresis probes. The probes were fabricated as described previously (Herr et al. 2008, 2010). A glass capillary (part no. 624503, 0.60-mm OD, 0.4-mm ID, 4 in. long; A-M Systems, Sequim, WA) was loaded with a carbon fiber (T-650; Thornel, Amoco, Greenville, SC) that served as the working electrode. This capillary containing the carbon fiber was then inserted into one barrel of a four-barrel capillary (part no. 50644, 1-mm OD, 0.75-mm ID, 4 barrel GF pipettes, 4 in. long; Stoelting, Wood Dale, IL). The four-barrel assembly contained glass filaments (GF) in each barrel that aid in filling the barrel by capillary action. The capillaries were bundled together with heat shrink and tapered to a sharp tip with a micropipette puller (Narashige, Tokyo, Japan) in a two-step pull process. The protruding carbon fiber was cut with a scalpel under a ×10 microscope objective to a shorter length than normal (30–50 μm) to ensure close proximity of the iontophoresis barrels to releasing terminals. The resulting probe consists of a glass-encased carbon fiber that is 5–7 μm in diameter and three iontophoretic barrels, each with a tip diameter of ~1 μm. Before use, the barrel containing the carbon fiber was backfilled with electrolyte (4 M potassium acetate, 150 mM potassium chloride) and fitted with wires for electrical contact. The remaining barrels for iontophoresis were filled with solutions containing drugs to be ejected.

Electrochemical data acquisition and presentation. For all experiments, a triangular waveform was applied at a scan rate of 400 V/s with a rest potential of ~0.4 V (vs. Ag/AgCl) between scans, a linear scan to 1.3 V, followed by a scan back to the rest potential. The scans were repeated every 100 ms, and collection was typically for 15–60 s. Cyclic voltammograms were acquired with data-acquisition hardware and locally written software in LabVIEW (National Instruments, Austin, TX). The cyclic voltammetry waveform was generated and the voltammetric signal was acquired with a computer interface board, the PCI-6052E (National Instruments). A PCI-6711E D/A board (National Instruments) was used to synchronize waveform application and data acquisition and to trigger the iontophoretic current applied and the loop injector in the flow injection apparatus. The voltammometric waveform was applied to a custom-built instrument for current transduction (Univ. of North Carolina at Chapel Hill). Separations were carried out in a 50-μm-diameter fused silica capillary, 96.0 cm in total length, with the UV detector placed 87.5 cm from the inlet. Experiments were done in cationic mode (anode at the inlet and cathode at the outlet). Samples were run at a concentration of 2 mM in 17 mM phosphate buffer (0.25% monosodium phosphate and 0.04% disodium phosphate) with a pH of 5.8 as in previous iontophoresis experiments. UV detection was measured at 195 and 240 nm, and electrophoretic mobilities were calculated as previously described (Herr et al. 2008).

Histology. At the end of experiments electrode placements were verified by electrolytic lesions made with the carbon fiber microelectrodes as previously described (Park et al. 2009). Animals were euthanized with urethane (2.0 g/kg ip), and a lesion was made at the recording site by applying a constant current (20 μA for 10 s) to the carbon fiber microelectrode. Brains were removed from the skull and stored in 10% formaldehyde for at least 3 days. Coronal slices (40 μm) were made with a cryostat. Sections were mounted on slides stained with 0.2% thionin and coverslipped before viewing under a light microscope.

Data analysis. Clampfit 8.1 as part of the pCLAMP 8.1 software package (Axon Instruments, Foster City, CA) was used to determine [NE]max, fmax, and t1/2 according to procedures previously described (Park et al. 2009). [NE]max is the maximal evoked norepinephrine concentration, and fmax is the time to reach [NE]max from the end of the electrical stimulation. The t1/2 or clearance half-life is measured as the time required for [NE] to decay to half the [NE]max value. Data are represented as means ± SE, and n values indicate the number of rats. Mean values were compared by using the two-tailed Student’s t-test (GraphPad Software, San Diego, CA), and P < 0.05 was regarded as statistically significant.

RESULTS

Catecholamine detection within the BNST. Figure 1A shows a coronal cross section of the BNST, highlighting the placement of the carbon fiber microelectrode in the vBNST and dmBNST. The schematic in Fig. 1A, left, also shows the
proximity of neighboring dopaminergic sites, the CPU and the VP, stressing the need for a probe with high spatial resolution. In Fig. 1A, right, a representative brain slice is shown, indicating the position of the carbon fiber microelectrode by an electrolytic lesion (white dotted circle). In Fig. 1B, evoked catecholamine release at different depths is shown. Electrodes placed in the dmBNST revealed an increase in oxidation current at ~6.0 mm from the skull surface due to NE release upon electrical stimulation. No signal was evoked around 7.5 mm because of the electrode passing through the AC. The signal was most robust in the vBNST at ~7.5 mm near the parasagittal and fusiform subnuclei. Figure 1C shows a representative trace of electrically evoked NE release in the dmBNST and vBNST. Across animals, [NE]max in the dmBNST was approximately half of that in the vBNST (147 ± 45 nM vs. 291 ± 26 nM; P < 0.05, n = 6 animals). Similarly, t1/2 was significantly longer for the dmBNST than in the vBNST (t1/2 = 2.37 ± 0.07 s and 1.36 ± 0.07 s, respectively, P < 0.001, n = 6 animals) as was tmax (dmBNST tmax = 1.3 s; vBNST tmax = 0.7 s).

Effects on NE release in BNST due to systemic application of NE drugs. DA and NE cannot be distinguished on the basis of their voltammograms alone (Heien et al. 2003), but pharmacology and histology can aid in this distinction (Park et al. 2009). Once the carbon fiber microelectrode was placed at a site of reproducible evoked release, the baseline [NE]max and t1/2 were determined. Figure 2 shows the effects of systemically administered (ip injection) DA and NE drugs on the dmBNST and vBNST. The D2 antagonist raclopride and dopamine transporter (DAT) inhibitor GBR 12909 did not alter [NE]max or t1/2 in the dmBNST or vBNST, indicating that the measured signal was not due to release from DA neurons. In contrast, in both the dmBNST and vBNST, [NE]max and t1/2 increased and maximized within 20 min after administration of IDA (5 mg/kg ip), an α2-AR autoreceptor antagonist. Systemic IDA significantly increased [NE]max in the dmBNST and vBNST to 143 ± 5% and 171 ± 7% of the predrug value, respectively (n = 6 and 5 animals, respectively, P < 0.05 for each location). Systemic IDA also significantly increased t1/2 in the dmBNST and vBNST to 168 ± 10% and 166 ± 6% of the predrug value, respectively (n = 6 and 5 animals, respectively, P < 0.05 for each location). [NE]max and t1/2 after IDA remained significantly lower and slower in the dmBNST compared with the vBNST (P < 0.01 and P < 0.05, respectively). After the evaluation of IDA effects, DMI (15 mg/kg ip) was given to determine the role of NET in each region. In the dmBNST, DMI did not significantly change [NE]max (156 ± 10%; n = 6 animals, P > 0.05) or t1/2 (239 ± 23%; n = 6 animals, P > 0.05), both values relative to predrug values following IDA. Conversely, in the vBNST, DMI further increased both [NE]max and t1/2 (253 ± 14% (P < 0.001) and 323 ± 29% (n = 5 animals, P < 0.001), respectively, both values relative to predrug values). Increases in [NE]max following ip administration of DMI (post-IDA) were significantly greater in the vBNST than the dmBNST (P < 0.001).

Local regulation of NE release in BNST with quantitative iontophoresis. To examine local effects of DMI and IDA on [NE]max and t1/2, we used quantitative iontophoresis at BNST terminals. Quantitative iontophoresis of electroactive drugs such as IDA and DMI is accomplished by voltammetrically monitoring ejection of a neutral and electroactive substance that serves as a marker for EOF (Herr et al. 2008). AP was chosen as the EOF marker for these experiments because it showed no significant effect on NE neurotransmission in the BNST (data not shown) and was previously shown to have no effect on DA neurotransmission in the striatum (Herr et al. 2010).

CE was used to determine the relative mobility of IDA and DMI in an electric field. Electrophoretic mobilities were determined for IDA, DMI, uric acid, AP, and DA and used to calculate the relative iontophoretic rate of delivery. The results from this experiment are shown in Fig. 3. The data indicate that DMI and IDA are transported at rates that are 2.21 and 2.42 times as fast as the EOF marker AP, respectively. These rates were used to calculate the amount of IDA and DMI delivered when coejected with AP.
The effects of iontophoretically delivered DMI and IDA were evaluated in the dmBNST and vBNST. Since iontophoresis allows for multiple drugs and sites to be evaluated in one animal, the effects of both drugs were first investigated in the dmBNST and then the probe was lowered to the vBNST. An example experiment recorded in the vBNST is shown in Fig. 4. First, release was evoked repeatedly until constant amplitude was achieved on repeated stimulations. Then, IDA was iontophoretically coapplied with AP for 30 s. At the end of the ejection period, the average concentration of IDA across the electrode and is equivalent to 12 μM IDA. C: current trace for stimulated release 120 s after ejection seen in B. At the time of stimulation (open box), the concentration of AP has decreased to 2% of its original value, corresponding to a decrease in IDA concentration to 240 nM. The extracellular concentration of NE seen in C is significantly increased from that observed predrug.

The average concentration of IDA across the electrode was 12 μM as calculated from the measured AP concentration and relative iontophoretic ejection values (Fig. 3). Release was evoked 2 min after iontophoretic application, where previous work has shown that the ejection signal has diminished to ~2% of the ejected value (Herr et al. 2010). Thus at the time of stimulation the average concentration of IDA present at the electrode surface is ~240 nM.

Figure 5 shows the effects of iontophoretically administered DA and NE drugs on the dmBNST and vBNST. Across animals in the dmBNST, IDA significantly increased [NE]max (134 ± 10%; n = 4 animals, P < 0.05) and t1/2 (123 ± 3%; n = 3 animals, P < 0.05), DMI was iontophoretically applied following IDA injections after evoked NE release was allowed to return to its original value over a time course of 10–20 min. In the dmBNST, there was a trend for DMI to increase [NE]max (140 ± 26%; n = 3 animals) but only t1/2 increased significantly (126 ± 8%; n = 3 animals, P < 0.05). In the vBNST, IDA had significant effects on [NE]max (191 ± 17%; n = 5 animals; P < 0.01) and t1/2 (304 ± 84%; n = 4 animals, P < 0.05) compared with predrug values. Similarly, DMI (administered after IDA and following a return to baseline NE release) also increased [NE]max to 168 ± 20% (n = 5 animals, P < 0.05) of the predrug value and increased t1/2 by 405 ± 145% of baseline (n = 4 animals, P < 0.05). IDA iontophoretic administration, like systemic administration, significantly increased [NE]max to a lesser extent in the dmBNST versus the vBNST (P < 0.05). The DA D2 antagonist raclopride had no effect on [NE]max or t1/2 in either region.

Systemic and iontophoretic drug delivery revealed similar drug effects in both the dmBNST and vBNST. In both regions, the average increase in [NE]max observed by iontophoretically and systemically delivered IDA were similar. Additionally, in the vBNST, iontophoretic administration of DMI following IDA iontophoresis showed a drug effect similar to systemic administration of DMI alone for [NE]max and t1/2. Conversely, the time for drug effect onset was different for the two modes of drug delivery. Figure 6 shows a comparison between systemic (ip) and iontophoretic drug delivery time courses of IDA and DMI effects in the vBNST with drugs administered at t = 0. In Fig. 6A, IDA and DMI were evaluated in individual animals to determine the time course of each drug without influence from the other. The results indicate that the effects of both drugs reach a steady state after ~15 min. In contrast, when IDA and DMI are administered iontophoretically (Fig. 6B), steady state is reached within the first 2 min after delivery.

**DISCUSSION**

The BNST is a heterogeneous brain region made up of various subregions that may differentially modulate behaviors...
related to stress, addiction, anxiety, pain, and other affective disorders. The subregions span merely a few hundred micrometers, requiring measuring techniques with high spatial resolution. Using carbon fiber microelectrodes with and without iontophoretic barrels, we are able to selectively monitor and modulate NE release in subregions of the BNST (Fig. 1). Systemic (raclopride and GBR 12909) and iontophoretic (raclopride) delivery of DA drugs do not affect $[\text{NE}]_{\text{max}}$ or $t_{1/2}$ in either region, while systemic and iontophoretic delivery of NE drugs (IDA and DMI) increase $[\text{NE}]_{\text{max}}$ and/or $t_{1/2}$ in both the dmBNST and the vBNST. This indicates that the measured signal is predominantly due to NE release with little contribution from DA and confirms previous anatomical findings regarding the distribution of catecholaminergic input into the BNST (Egli et al. 2005; Smith and Aston-Jones 2008).

As predicted by the density of dopamine-$\beta$-hydroxylase (Smith and Aston-Jones 2008) and NET (Egli et al. 2005) expression in the dmBNST and vBNST, we found differential catecholamine content and uptake between the two regions. The $t_{\text{max}}$ and $t_{1/2}$ following the termination of stimulation were almost twice as long in the dmBNST as in the vBNST. Reduced release and uptake may result in an altered profile of noradrenergic signaling in these distinct subregions. Lower concentrations of released NE in the dmBNST may preferentially act on different adrenergic receptor populations on NE terminals, other afferent terminals, and postsynaptic cells in the BNST. Similarly, reduced uptake may allow for greater diffusion of NE into neighboring subregions of the BNST under instances of prolonged NE neuron activation, as is observed in restraint stress (Cecchi et al. 2002; Pacak et al. 1995).

In addition to the differences in basal properties of release observed between the dmBNST and the vBNST, we observed differences in the response to the $\alpha_2$-AR antagonist IDA between the two regions. While IDA increased the $[\text{NE}]_{\text{max}}$ in both subregions, both systemic administration and iontophoretic administration of IDA led to a greater increase in the vBNST versus the dmBNST. Conversely, systemic IDA increased $t_{1/2}$ to a greater extent in the dmBNST than in the vBNST, although no statistical difference between regions was observed for iontophoretic delivery of IDA. It is possible that the systemic administration of IDA may have altered signaling on several circuits that impinge on the BNST and that this altered signaling could account for the different pattern observed with systemic versus local iontophoretic administration. Regardless, the increases observed in both regions to iontophoretically applied IDA strongly indicate that local $\alpha_2$-ARs regulate NE signaling in the BNST.

Our data indicate that NET plays a small role governing the neurotransmission of NE in the dmBNST. Systemic administration of DMI following IDA failed to result in a further significant increase in either $[\text{NE}]_{\text{max}}$ or $t_{1/2}$. Iontophoretic administration of DMI in the dmBNST, however, resulted in an increase in $t_{1/2}$ but not $[\text{NE}]_{\text{max}}$. In contrast, DMI administration (both systemically and iontophoretically) increased both measures in the vBNST. The differential neurotransmission of NE in these subregions may have a consequence on different aspects of animal behavior. Neurons within these two areas have distinct targets within and outside of the BNST. Adrenergic receptor activation in the BNST has been demonstrated to modulate synapses in various ways including increasing and decreasing glutamatergic and GABAergic transmission depending on the receptors that are activated (Dumont and Williams 2004; McEllegant et al. 2010). Additionally, NE can alter synaptic plasticity in the BNST via $\alpha_1$-ARs in a manner that is dependent on the duration of exposure to NE (McElligott and Winder 2008). Therefore, if clearance rates differ in subregions, the concentration of NE present in the extracellular space that is available to act on receptors after behaviorally evoked release and the propensity for this type of synaptic plasticity may also be variable in the subregions.

Finally, the use of iontophoresis in combination with FSCV at carbon fiber microelectrodes allows direct pharmacological characterization of BNST subregions without influence from systemic feedback mechanisms. This approach gives definitive evidence that NE release in the dmBNST and the vBNST is primarily controlled at releasing terminals. Using an internal EOF marker during iontophoresis we are able to control and confirm the amount of drug delivered. The results from Fig. 3 are used to calculate the amount of IDA or DMI delivered in relationship to the amount of AP measured at the electrode’s surface. Although this does not give us a direct measure of drug concentration at the receptors, it allows us to deliver similar quantities of drug at each location we study. In addition, because the region affected by drug is only a few hundred micrometers in diameter, multiple sites can be studied within the same animal. Figure 6 shows the differences in time course obtained for systemic and iontophoretic drug delivery. Systemic effects of IDA and DMI take tens of minutes to reach steady state, while iontophoretic drug effects are observed within the first stimulation following drug administration. The rapid onset allows for multiple sites and drugs to be characterized in these often lengthy studies.

Conclusions. The results presented demonstrate that catecholamine release can be monitored in two subregions of the
BNST (vBNST and dmBNST). Pharmacological characterization indicates that NE is the catecholamine released in the vBNST and dmBNST. The combination of systemic drug delivery and quantitative iontophoresis reveals that there are significant differences in release and clearance of NE in the vBNST and dmBNST. Specifically, evoked NE release is greater in the vBNST, as are the effects due to the $\alpha_2$-AR antagonist IDA and the NET inhibitor DMI. Quantitative iontophoresis was used to demonstrate that regulation of NE is primarily controlled at terminals by $\alpha_2$-ARs and NET. Thus the observed differences in evoked release and clearance are likely due to the greater density of noradrenergic nerve terminals found in the vBNST versus the dmBNST and perhaps altered expression of NET and autoreceptors on the terminals in each region.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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