Presynaptic α-adrenoceptors in median preoptic nucleus modulate inhibitory neurotransmission from subfornical organ and organum vasculosum lamina terminalis

Miloslav Kolaj and Leo P. Renaud
Neurosciences, Ottawa Health Research Institute and University of Ottawa, Ottawa, Ontario, Canada

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Kolaj M, Renaud LP. Presynaptic α-adrenoceptors in median preoptic nucleus modulate inhibitory neurotransmission from subfornical organ and organum vasculosum lamina terminalis. Am J Physiol Regul Integr Comp Physiol 292: R1907–R1915, 2007. First published January 11, 2007; doi:10.1152/ajpregu.00763.2006.—The median preoptic nucleus (MnPO) in the lamina terminalis receives a prominent catecholaminergic innervation from the dorsomedial and ventrolateral medulla. The present investigation used whole cell patch-clamp recordings in rat brain slice preparations to evaluate the hypothesis that presynaptic adrenoceptors could modulate GABAergic inputs to MnPO neurons. Bath applications of norepinephrine (NE; 20–50 μM) induced a prolonged and reversible suppression of inhibitory post synaptic currents (IPSCs) and reduced paired-pulse depression evoked by stimulation in the subfornical organ and organum vasculosum lamina terminalis. These events were not correlated with any observed changes in membrane conductance arising from NE activity at postsynaptic α1- or α2-adrenoceptors. Consistent with a role for presynaptic α2-adrenoceptors, responses were selectively mimicked by an α2-adrenoceptor agonist (UK-14304) and blockable with an α2-adrenoceptor antagonist (idazoxan). Although the α1-adrenoceptor agonist cirazoline and the α1-adrenoceptor antagonist prazosin were without effect on these evoked IPSCs, NE was noted to increase (via α1-adrenoceptors) or decrease (via α2-adrenoceptors) the frequency of spontaneous and tetrodotoxin-resistant miniature IPSCs. Collectively, these observations imply that both presynaptic and postsynaptic α1- and α2-adrenoceptors in MnPO are capable of selective modulation of rapid GABAA receptor-mediated inhibitory synaptic transmission along the lamina terminalis and therefore likely to exert a prominent influence in regulating cell excitability within the MnPO.

α1-adrenoceptors; α2-adrenoceptors; inhibitory postsynaptic currents

THE LAMINA TERMINALIS, the narrow band of tissue forming the anterior wall of the third cerebral ventricle, contains three groups of neurons that serve important homeostatic functions. Two of these, the organum vasculosum lamina terminalis (OVLT) situated ventrally and the subfornical organ (SFO) located dorsally, are circumventricular organs, sites where fenestrated capillaries permit blood-born molecules access to central neurons (23). Information received by neurons residing in these two circumventricular organs is deemed to be transmitted along axonal pathways for integration within a third cell group forming the median preoptic nucleus (MnPO; also called MnPN, POMe, or nucleus medianus) located around the anterior commissure at the midpoint along the lamina terminalis. The connectivity of MnPO neurons is suitable for reception and integration of messages derived from both hemal and neural origins. MnPO is reciprocally connected with SFO and OVLT (24, 28) and has afferent and efferent connectivity with regions known for their involvement in neuroendocrine, hydromineral, thermal, and cardiovascular regulation and sleep-waking cycles (e.g., Refs. 2, 5, 15, 20, 21, 28, 30, 31, 33, 41). Lesions focused on MnPO and neighboring ventral periventricular tissues result in profound disruptions in cardiovascular regulation, fluid balance, angiotensin-induced drinking, and salt appetite (8, 10, 11, 22), attesting to the importance of neurons in this nucleus in maintaining homeostasis in autonomic, neuroendocrine, and cardiovascular systems. Recent Fos mapping studies have led to implications that neurons in this nucleus are also involved in the homeostatic regulation of sleep (13, 14).

The lamina terminalis displays a rich catecholaminergic innervation originating mainly from brain stem A1 and A2 cell groups (30, 38). At the level of the MnPO, the ultrastructure reveals catecholaminergic terminals forming axosomatic and axodendritic contacts with MnPO neurons (16, 17). Whole animal studies designed to evaluate function associated with this innervation have concluded that catecholamine fibers and receptors along the lamina terminalis are required for the elicitation of drinking and pressor responses to exogenous angiotensin (4, 6, 7). Injections of norepinephrine (NE) into MnPO induce an increase in urine output, sodium excretion, arterial pressure, and bradycardia (9). Microdialysis studies indicate a significant increase in NE release in the MnPO area in response to electrical stimulation in OVLT (39) or SFO (35), hemorrhage (34), and hypovolemia (25). GABA-synthesizing neurons, abundant within neurons and axon terminals in MnPO (e.g., Refs. 12, 26), have been proposed to modulate NE release in MnPO (29, 39). At the cellular level, stimulation in the A1 area in vivo is reported to evoke an α-adrenoceptor-mediated increase in the excitability of MnPO neurons (36), whereas in vitro studies in slice preparations reveal both inhibitory and excitatory actions attributed to postsynaptic α2- and α1-adrenoceptors on MnPO neurons, respectively (3).

Incorporating these observations into an operative schema of NE’s role in the functions attributed to MnPO neurons is hampered in part because of the lack of detailed cellular information about catecholamine receptors and their interactions with the various inputs to MnPO, including those that originate from SFO and OVLT. This prompted our initial evaluation of the nature of postsynaptic adrenoceptors in MnPO neurons, leading to the conclusion that NE acts at
α-adrenoceptors to attenuate cell excitability through modulation of potassium and calcium channels (3, 18). GABAergic neurotransmission is a prominent response to electrical stimulation of SFO afferents to MnPO neurons (19). Reports of an interaction between GABA and NE release in MnPO (29, 39) prompted us to evaluate the hypothesis that presynaptic catecholamine receptors might modulate GABAergic neurotransmission in MnPO. We now report that adrenoceptors can attenuate evoked, spontaneous, and activity-independent GABA_A receptor-mediated inhibitory postsynaptic currents (IPSCs) in MnPO neurons.

METHODS

Experiments used Wistar rats weighing 50–100 g. All experiments conformed to Canadian Council for Animal Care and approved by the Ottawa Health Research Institute Committee on the ethical use of animals in research.

Recordings were made from MnPO neurons in acutely prepared sagittal or coronal brain slice preparations using methods described in detail previously (19). Briefly, animals were decapitated, and the brain was quickly removed and immersed in oxygenated (95% O₂-5% CO₂) artificial cerebrospinal fluid (ACSF) of the following composition (in mM): 127 NaN₃, 3.1 KCl, 1.3 MgCl₂, 2.4 CaCl₂, 26 NaHCO₃, and 10 glucose, pH 7.3, osmolality of 300–310 mosmol/kgH₂O. The brain was sliced in the sagittal or coronal plane at a constant level in a given neuron. The analysis was performed by using cumulative probability plots, and statistical comparisons were evaluated with the Kolmogorov-Smirnov (KS) test.

Drugs were bath applied at the concentrations indicated. These included (−)-bicuculline methochloride, (−)-2-amino-5-phosphonovaleric acid, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzof[f]quinoline-7-sulfoamide disodium (NBQX), UK-14303, prazosin, and cirazoline from Tocris Cookson (Ballwin, MO); NE, isoprenaline, idazoxan, MgATP, and NaGTP from Sigma (St. Louis, MO); and TTX from Alomone Labs (Jerusalem, Israel). To minimize oxidation, NE was prepared as a 1 mM solution immediately before use.

RESULTS

Data were obtained from MnPO neurons located dorsal (n = 5) or ventral (n = 58) to the anterior commissure. We detected no difference based on cell location, so the data were pooled. All recordings were performed in ACSF containing glutamate receptor antagonists NBQX (10 μM) and D-(−)-2-amino-5-phosphonovaleric acid (20 μM) to pharmacologically isolate GABA_A receptor-mediated IPSCs. In response to single stimuli applied in SFO or OVLT, evoked IPSCs were recorded as outward currents (holding voltage of −45 mV; in some experiments −40 mV) with the following properties: for SFO (n = 30), amplitude = 39.2 ± 5.3 pA, latency = 6.7 ± 0.3 ms, rise time = 5.3 ± 0.5 ms, decay time = 36.3 ± 1.9 ms; for OVLT (n = 19), amplitude = 34.2 ± 6.9 pA, latency = 8.0 ± 0.6 ms, rise time = 6.3 ± 0.6 ms, decay time = 34.2 ± 2.4 ms (P > 0.05 for all comparisons). In any given neuron, IPSCs displayed a constant latency over a range of stimulation intensities and ability to follow three pulses at 20 Hz, features we deemed consistent with a monosynaptic connection (see Ref. 19).

**NE acts at α₂-adrenoceptors to suppress SFO- and OVLT-evoked IPSCs.** Bath-applied NE (20–50 μM, 1–3 min) induced a slowly developing, prolonged, and reversible suppression in both SFO-evoked IPSCs (14 of 15 cells; Fig. 1, A and C) and OVLT-evoked IPSCs (4 of 5 cells; Fig. 1B). On average, IPSC amplitudes were reduced to 62.5 ± 4.4% (P < 0.001) and 65.2 ± 8.5% (P < 0.05) for SFO- and OVLT-evoked events, respectively. Fifty percent of cells were tested with both NE and one or more specific agonists. As illustrated in Fig. 1, the NE effect was mimicked by a specific α₂-adrenoceptor agonist, UK-14303, but not by cirazoline, a specific α₁-adrenoceptor agonist, or by isoprenaline, a specific β-adrenoceptor agonist.

The NE-induced reduction in evoked IPSC amplitudes was reduced or abolished by pretreatment (5 min) with idazoxan (5–10 μM), a specific α₂-adrenoceptor antagonist (Fig. 2, A and C; P < 0.05). By contrast, pretreatment with prazosin (10 μM), an α₁-adrenoceptor antagonist (Fig. 2, B and C; P > 0.05) that effectively blocks NE-evoked postsynaptic α₁-adrenoceptor-mediated depolarization in MnPO neurons (3), was without effect, a further indication that these results were mediated via α₂-adrenoceptors. Interestingly, under idazoxan, addition of NE was followed by a small but nonsignificant increase in SFO-evoked (2 cells; 107 and 115% of control) and OVLT-evoked IPSCs (1 cell, 108% of control; not illustrated).

**Lack of correlation between postsynaptic and presynaptic effects of NE.** Consistent with earlier observations from our laboratory (3), applications of NE were also associated with a postsynaptic action. In recordings in gluconate-based internal solutions, outward currents were recorded in 25 cells, inward currents in 8 cells, both inward and outward currents in 3 cells, and no response in 5 cells. The outward currents were mimicked by UK-14303 (5 of 9 cells tested) and inward currents by cirazoline (2 of 10 cells tested). Although it is possible that...
events at these postsynaptic adrenoceptors could influence the NE-induced reductions in evoked IPSC amplitudes, we observed no statistical correlation between postsynaptic conductances induced by NE and changes in IPSC amplitudes ($n = 15$; $P = 0.238$, $r^2 = 0.105$, Pearson correlation), and IPSC amplitude reductions were present in neurons demonstrating both outward or inward currents. In addition, superimposition of normalized traces before and during NE applications (Fig. 1, A and B) did not reveal any change in rise or decay time kinetics. These features indicate roles for NE receptors at both pre- and postsynaptic sites.

**α2-Adrenoceptor inhibition of SFO- and OVLT-evoked IPSCs involves presynaptic mechanisms.** To obtain further insight into possible presynaptic sites of action, we next applied paired-pulse protocols. In these preparations, when tested at an interstimulus interval of 200 ms (Fig. 3, A and B), SFO- and OVLT-evoked IPSCs displayed varying degrees of paired-pulse depression (PPD): for SFO, the ratio of the second evoked IPSC (P2) to the first evoked IPSC (P1) was 0.77 ± 0.03 (23 of 30 cells); for OVLT, the P2-to-P1 ratio was 0.81 ± 0.03 (15 of 19 cells). Less common was evidence of paired-pulse facilitation: for SFO, the P2/P1 was 1.34 ± 0.19 (4 of 30 cells); for OVLT, the P2/P1 was 1.09 ± 0.01 (3 of 19 cells). As illustrated in Fig. 3, NE significantly reduced PPD, largely due to suppression of the P1 amplitude relative to that of P2. These effects were mimicked by the α2-adrenoceptor agonist UK-14303 (Fig. 3C), consistent with a modulating action of presynaptic α2-adrenoceptors.

To assess the possibility that ambient levels of NE in the extracellular space might be sufficient to activate presynaptic adrenoceptors, we next examined whether blocking these receptors by application of idazoxan for 6–10 min would induce any changes. Although the majority of cells showed no significant change in amplitude of IPSCs or pair-pulse ratio (Fig. 4B), in three cells (two SFO- and one OVLT-evoked IPSCs), application of idazoxan was followed by a small (123, 113, and 107% of control) and reversible increase (Fig. 4A) in IPSC amplitude, suggesting that endogenously released NE could influence inhibitory inputs to a subpopulation of MnPO neurons. Applications of prazosin were without effect in all cells tested (Fig. 4B) at concentrations that effectively blocked NE activation of postsynaptic α1-adrenoceptors in MnPO (3).

**α1- and α2-Adrenoceptors influence spontaneous IPSCs.** In a subpopulation of MnPO neurons, applications of NE were observed to influence the frequency of spontaneous IPSCs (sIPSCs). In 18 MnPO neurons, control sIPSC frequency measured 1.6 ± 0.3 Hz and mean amplitude was 8.3 ± 0.7 pA. In 8 of these 18 neurons, addition of NE was followed by a significant increase ($P$ value from KS test <0.05) in sIPSC frequency (Fig. 5A); 5 other cells revealed a decrease in sIPSC frequency, with no change in the remaining 5 cells. NE was also active at postsynaptic receptors, inducing inward currents in 5 cells and outward currents in 11 cells, with no effects in the other 2 cells. However, there was no significant correlation between NE-induced current amplitudes and sIPSC frequency changes ($n = 18$; $P = 0.528$, $r^2 = 0.0254$, Pearson correlation), implying that the postsynaptic adrenoceptor-induced changes were independent of the NE-induced change in sIPSC frequencies. In four of the eight neurons that displayed an increase in sIPSC frequency, we also observed a significant increase in sIPSC amplitude (133 ± 8%, from 9.51 ± 2.50 to

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**Fig. 1.** Norepinephrine (NE) suppresses subfornical organ (SFO)- and organum vasculosum lamina terminalis (OVLT)-evoked inhibitory postsynaptic currents (IPSCs). A: sample traces (average of 4) illustrate NE (30 μM, 90 s)-induced reduction in SFO-evoked (single stimulus every 20 s) IPSC amplitudes [holding voltage ($V_h$) of −45 mV; gray trace]. Superimposed traces on right (control in black; normalized trace obtained in the presence of NE in gray) reveal no change in IPSC rising and decay kinetics. B: sample traces from another median preoptic nucleus (MnPO) neuron (average of 4) illustrate NE (40 μM, 90 s)-induced reduction in OVLT-evoked (single stimulus every 20 s) IPSC amplitudes ($V_h$ of −45 mV; gray trace). Superimposed traces on right (control in black; normalized trace obtained in the presence of NE in gray) reveal no change in IPSC rising and decay kinetics. C: averaged data from 14 MnPO neurons illustrate a slowly developing, prolonged, and reversible NE effect on SFO-evoked IPSC amplitudes. IPSCs are normalized with control = average of first 6 IPSCs. D: summary histograms. NE effect is mimicked by the α2-adrenergic receptor agonist UK-14303 (40–60 μM); IPSC amplitudes are unaffected by the α1-adrenergic receptor agonist cirazoline (30–60 μM) or the β-adrenergic agonist isoprenaline (50 μM). Numbers of cells in each category are indicated in bar columns. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. 

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12.27 ± 2.66 pA; \( P \) from KS test < 0.05). We postulate that this arose because of an increase in the synaptic (inhibitory) activity as a consequence of NE’s excitatory actions (via \( \alpha_1 \)-adrenoceptors) that depolarized GABAergic neurons whose axons projected to the recorded MnPO neurons. None of the remaining 14 cells demonstrated a significant change in sIPSC amplitude (from 7.72 ± 0.41 to 7.80 ± 0.51 pA). Increase in sIPSC frequency was also present in cells perfused with the \( \alpha_2 \)-adrenoceptor antagonist idazoxan (in 2 of 3 cells), and decrease was present during perfusion with \( \alpha_1 \)-adrenoceptors antagonist prazosin (in 2 of 3 cells). In one cell, a second application of NE without prazosin induced an increase in sIPSC frequency, suggesting that in a subpopulation of MnPO neurons there are both \( \alpha_1 \)- and \( \alpha_2 \)-adrenoceptors contributing to presynaptic modulation of GABAergic fast inhibitory transmission.

Both \( \alpha_1 \)- and \( \alpha_2 \)-adrenoreceptors modulate TTX-independent GABA release. To investigate with more precision a role for presynaptic adrenoceptors, we tested the effects of NE on calcium- and TTX-independent miniature IPSC (mIPSCs), which represent quantal release of transmitter. Using a CsCl-based internal solution to amplify mIPSCs, we found that the mean frequency of mIPSCs (\( n = 20; 2.02 ± 0.73 \text{ Hz} \)) was not increased when compared with the mean frequency of sIPSCs (\( n = 18; 1.57 ± 0.27 \text{ Hz}; P > 0.05 \)). As noted above for sIPSCs, a mixed response followed addition of NE: increased mIPSC frequency to 207.7 ± 24.9% of control (from 1.33 ± 0.39 Hz to 2.67 ± 0.64 Hz; \( P \) from KS test < 0.05) in seven cells, decreased mIPSC frequency to 42.8 ± 11.4% of control (from 2.36 ± 1.48 Hz to 1.14 ± 0.73 Hz; \( P \) from KS test < 0.05) in four cells, and no effect in the remaining nine cells (Fig. 6). The amplitude of mIPSCs was not changed in all three groups (from 42.62 ± 3.99 pA to 42.95 ± 4.59 pA, \( n = 20; P \) from KS test > 0.05; Fig. 6C). A lack of change in rise times or decay times further suggested that kinetics of these responses were independent of any postsynaptic conductance changes. Similar to results with sIPSCs, the frequency increase could also be observed by NE perfusion in the presence of the \( \alpha_2 \)-adrenoceptor antagonist idazoxan (2 of 4 cells tested; Fig. 6, A and C), and the decrease could be noted during NE perfusion with the \( \alpha_1 \)-adrenoceptor antagonist prazosin (2 of 4 cells tested; Fig. 6, B and C), with other cells showing no significant change. In one cell, the NE-induced frequency decrease was completely reversed under idazoxan, and a second NE application yielded a large mIPSC frequency increase. On average, neither idazoxan (\( n = 5 \)) nor prazosin (\( n = 5 \)) alone induced changes in mIPSC frequency (idazoxan: 1.74 ± 0.59 to 1.66 ± 0.72 Hz, prazosin: 2.11 ± 0.52 to 2.14 ± 0.62 Hz; exception was one cell showing a reduction by 10.220.33.2 on October 14, 2017 http://ajpregu.physiology.org/ Downloaded from
DISCUSSION

The catecholaminergic innervation of the ventral lamina terminalis is deemed important for various functions, including angiotensin-induced drinking, cardiovascular regulation, extracellular fluid balance, and sleep-wake behavior [e.g., Refs. 4, 7, 26]. In an earlier electrophysiological evaluation of adrenoceptors focused on in MnPO neurons, our group [3] sought to characterize the properties of postsynaptic adrenoceptors and observed that a majority of MnPO neurons responded to bath-applied NE with \(\alpha_2\)-adrenoceptor-mediated membrane hyperpolarization or, less frequently, with \(\alpha_1\)-adrenoceptor-mediated membrane depolarization. The observations presented in the present analysis indicate a role for presynaptic adrenoceptors. In slice preparations where glutamate ionotropic receptors are blocked pharmacologically, we noted that presynaptic adrenoceptors mediate a significant NE-induced reduction in the amplitude of IPSCs evoked by stimulation of two identifiable afferent pathways to MnPO that arise from neurons in the SFO and OVLT. These effects of NE develop and recover slowly. Although often accompanied by currents induced in the recorded neuron (see Ref. 3), these events are not correlated with NE-induced actions at postsynaptic \(\alpha_1\) or \(\alpha_2\) adrenoceptors. Selective blockade of the NE-induced suppression by idazoxan but not by prazosin suggests that only \(\alpha_2\)-adrenoceptors are located presynaptically on the SFO and OVLT GABAergic afferents to MnPO. Whereas adrenoceptor antagonists alone had no significant collective effect on these evoked IPSCs (Fig. 4B), individual instances where evoked IPSC amplitudes were enhanced in the presence of idazoxan (see Fig. 4A) suggest that ambient levels of NE are sufficient to tonically activate presynaptic \(\alpha_2\)-adrenoceptors.

Although GABAergic pathways to MnPO that arise from SFO and OVLT show no evidence of presynaptic \(\alpha_1\)-adrenoceptor modulation in our analysis, an evaluation of sIPSC and mIPSC data confirm that other unspecified GABAergic inputs (possibly local interneurons and/or inputs from the medial preoptic area) may be subject to differential modulation by presynaptic \(\alpha_1\) and \(\alpha_2\)-adrenoceptors. Thus changes in the amplitude of sIPSCs induced by NE (as mentioned under RESULTS) are likely the result of NE acting at \(\alpha_1\)-adrenoceptors to increase firing of GABAergic neurons that are “presynaptic” to the cell under observation. At present, our interpretation of these observations must remain speculative, although the data do raise an appreciation of the possible complexity of sites where a combination of pre- and postsynaptic adrenoceptors...
could potentially modulate inputs and outputs of this lamina terminalis cell group.

Anatomic tracer studies indicate that noradrenergic neurons located within the nucleus tractus solitarii and ventrolateral medulla are the major sources for the catecholaminergic innervation to MnPO (30, 38). These ascending catecholaminergic pathways are but one constituent of a more complex afferent neural circuitry that provides subpopulations of MnPO neurons with information about cardiovascular and/or hydromineral perturbations as well as sleep-related states, as evidenced by changes in their membrane excitability (1, 32, 37) and c-Fos expression (13, 14). It is notable that the medullary catecholaminergic innervation is closely linked not only with the ability to elicit angiotensin-induced drinking behavior (6, 7) but also with the status of extracellular fluid volume and arterial blood pressure; hemorrhage induces a sharp rise in NE release in the MnPO area, a feature that is significantly attenuated after local anesthetic injections into the A1 area (34, 40).

In MnPO, terminal profiles immunoreactive for tyrosine hydroxylase, NE, or epinephrine have been reported to form axosomatic and axodendritic synapses with neurons whose axons project to several identified targets, i.e., the hypothalamic paraventricular nucleus, the SFO, and the ventrolateral medulla (16, 17). The fact that these terminals may display symmetric or asymmetric contacts and contain both small clear and large-cored vesicles (16) suggests the possibility of multiple molecules participating in afferent neurotransmission from these brain stem sites. Indeed, in addition to NE, ATP and neuropeptide Y have already been deemed as possibilities (16, 42). At the ultrastructural level, the profile of catecholaminergic terminal boutons abutting on neuronal somata and dendrites is consistent with the notion that synaptically released NE can directly activate postsynaptic adrenoceptors (3). By contrast, the morphological profile in support of the presynaptic action of NE remains to be identified, since catecholaminergic terminals have not been observed to form axoaxonic contacts in MnPO. This may indicate that synaptically released catecholamines reach their presynaptic receptors (as described herein) by a process of diffusion.

In a physiological context, various microdialysis experiments have revealed that a rise in NE release in the MnPO area occurs in response to hemodynamic changes (e.g., hemorrhage, hypovolemia) as well as to activation of SFO afferents (25, 34, 35). Interestingly, the levels of NE measured in the dialysate in these studies are two- to threefold lower than the micromolar concentrations that are required to elicit electrophysiological evidence of activation of presynaptic or postsynaptic adrenoceptors reported in brain slice or dissociated cell preparations of MnPO (see Refs. 3, 18). Although consistent with effective concentrations of NE reported in the electrophysiological literature, reasons for such discrepancies remain unclear, perhaps reflecting the uniqueness of the microdialysis technique and its ability to reflect ambient levels of NE in the extracellular space. It is worth noting however that behavioral responses to

Fig. 5. NE produces both increase and decrease of spontaneous IPSCs (sIPSCs) in MnPO. A: sample trace from an MnPO neuron illustrates a large increase in the frequency of sIPSCs induced by NE (30 μM for 1 min). Bottom row illustrates the NE-induced effect in expanded traces. Right: cumulative fraction graphs showing that the interevent interval is significantly changed by NE [Kolmogorov-Smirnov (KS) test: interspike interval P < 0.001]. In this neuron, amplitude was also significantly changed (KS test: amplitude P < 0.01; not illustrated). B: summary histogram demonstrates NE action on frequency (right) and amplitude (left) of sIPSCs. Increase in sIPSC frequency is present during application of the α2-adrenoceptor antagonist idazoxan (IDA; 3–6 μM), and decrease in sIPSC frequency is present during application of the α1-adrenoceptor antagonist prazosin (PRA; 5–10 μM). Note the small increase (P < 0.05) in the amplitude of sIPSCs for MnPO neurons displaying NE-induced increase in frequency, possibly due to postsynaptic α1-adrenoceptor-induced depolarization and increased firing of GABAergic neurons that are “presynaptic” to the recorded cell.
infusions of NE into MnPO (e.g., Ref. 9) do appear to require levels of NE that more closely approximate the micromolar concentrations that elicit electrophysiological evidence for adrenoceptor activation.

**Perspectives**

A catecholaminergic innervation to the MnPO and ventral lamina terminalis is vital to functions attributed to this region. Catecholamine depletion induced by 6-hydroxydopamine selectively blocks ANG II-induced drinking behavior and pressor responses (e.g., Ref. 4), effects that can be functionally restored after central NE infusions (7). Whereas these and numerous other lesion, infusion, and microdialysis studies provide information attesting to physiological and behavioral outcomes associated with NE in this region, details now unfolding at the cellular level begin to shed light on a complexity of events that may underlie catecholamine-mediated functions of this region. Unraveling the details of these events is no simple task, recognizing that MnPO is heterogeneous, with subpopulations that respond differentially to osmotic stimuli, systemic ANG II, baroreceptor activation, and during sleep (e.g., Refs. 13, 32), and project their axons to diverse targets (2, 5, 15–17, 20, 21, 28, 31, 33, 41). Interestingly, the fact that MnPO and neighboring regions demonstrate an abundance of neurons with a GABAergic phenotype, some of which display adrenoceptors (26), suggests that GABAergic transmission features prominently in the neural mechanisms underlying MnPO-mediated functions. Whereas it has already been proposed that GABAergic transmission modulates NE release in vivo (29), our in vitro observations by contrast support a modulatory role for NE in regulating cell excitability in MnPO through actions that may be directly mediated via postsynaptic adrenoceptors on the neurons themselves and/or through presynaptic adrenoceptor-mediated actions on GABAergic afferents. At this site, NE appears to act through α-adrenoceptors, with the α₂-subtype exerting an inhibitory role and the α₁-subtype the converse action. One might envision various scenarios as to how these receptors may affect MnPO functions. Acting at postsynaptic adrenoceptors, locally released NE might either suppress (via α₂-adrenoceptors) cell excitability and suppress impulse traffic through MnPO or, alternatively, enhance (via α₁-adrenoceptors) cell firing and facilitate cell firing within MnPO. The present analysis implies the possibility that locally released NE might also act via presynaptic α₂-adrenoceptors to suppress rapid GABAergic afferent neurotransmission from circumventricular structures (SFO and OVLT), thereby attenuating information that might be derived from the circulation. The α₂-adrenoceptor-mediated preferential reduction in the P1 response in the paired pulse trials that converts PPD to paired-pulse facilitation (Fig. 3, A and B), although unlikely to represent a normal modus operandi in the central nervous system, could conceptually achieve a prolongation of GABAergic synaptic inhibition in an actively firing MnPO neuron, thereby augmenting any existing postsynaptic suppressive actions of NE. Arguably, at the moment, these are items for speculation.

In the broader context, one might also consider how locally released NE could potentially influence MnPO outputs. In an earlier in vivo analysis, we reported that stimulation in MnPO evoked GABA_A-mediated inhibition in target neurons in the supraoptic nucleus (27), implying that at least this output of MnPO was inhibitory in nature. Consistent with this notion are
subsequent ultrastructural observations that the great majority of anterogradely labeled terminals from MnPO to supraoptic neurons form symmetric (i.e., inhibitory) synapses (2). One might postulate that local release of NE from ascending catecholaminergic afferent fibers has the potential to modulate neuronal excitability and the efferent activity of MnPO by a variety of mechanisms, with differing consequences. For example, hemorrhage-induced NE release could act via postsynaptic α2-adrenoceptors to suppress firing of MnPO neurons, thereby reducing (by disinhibition) the GABAergic MnPO output to supraoptic neurons. Concordant local release of NE in the supraoptic nucleus would also facilitate the ability of vasopressinergic neurons to respond to hemorrhage with increased firing and vasopressin release. This is but one of the possibilities that need to be considered at the cellular level when assessing function of this region. More detailed analyses are likely to provide a more accurate picture of the operative circuitry within the lamina terminalis.

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