NO inhibits supraoptic oxytocin and vasopressin neurons via activation of GABAergic synaptic inputs

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Stern, Javier E., and Mike Ludwig. NO inhibits supraoptic oxytocin and vasopressin neurons via activation of GABAergic synaptic inputs. Am J Physiol Regulatory Integrative Comp Physiol 280: R1815–R1822, 2001.—To study modulatory actions of nitric oxide (NO) on GABAergic synaptic activity in hypothalamic magnocellular neurons in the supraoptic nucleus (SON), in vitro and in vivo electrophysiological recordings were obtained from identified oxytocin and vasopressin neurons. Whole cell patch-clamp recordings were obtained in vitro from immunochemically identified oxytocin and vasopressin neurons. GABAergic synaptic activity was assessed in vitro by measuring \( \text{GABA}_A \) miniature inhibitory postsynaptic currents (mIPSCs). The NO donor and precursor sodium nitroprusside (SNP) and L-arginine, respectively, increased the frequency and amplitude of \( \text{GABA}_A \) mIPSCs in both cell types \((P \leq 0.001)\). Retrodialysis of SNP (50 mM) onto the SON in vivo inhibited the activity of both neuronal types \((P \leq 0.002)\), an effect that was reduced by retrodialysis of the \( \text{GABA}_A \)-receptor antagonist bicuculline \((2 \text{ mM}, P \leq 0.001)\). Neurons activated by intravenous infusion of 2 M NaCl were still strongly inhibited by SNP. These results suggest that NO inhibition of neuronal excitability in oxytocin and vasopressin neurons involves pre- and postsynaptic potentiation of GABAergic synaptic activity in the SON.

sodium nitroprusside; bicuculline; hypothalamus; retrodialysis

THE ACTIVITY OF MAGNOCELLULAR neuroendocrine neurons is regulated by a number of intrinsic factors, synaptic inputs, and dendrodendritic interactions within the supraoptic (SON) and paraventricular nuclei (PVN). There is growing evidence that nitric oxide (NO) functions as a local modulator of magnocellular neuronal activity. Neuronal NO synthase (NOS) is expressed densely in the SON and PVN (1), where it is colocalized with oxytocin (OT) and vasopressin (VP) synthesizing neurons and its expression is functionally regulated \((3, 32, 36, 47)\). In the SON and PVN, the expression of neuronal NOS mRNA is increased in response to osmotic stimuli \((16, 44, 46)\) and after hypovolemia \((45)\), and staining for NADPH-diaphorase changes during late pregnancy and parturition \((29)\). In rats and in humans, NO inhibits OT secretion \((7, 15)\). Electrophysiological studies in vitro \((23)\) and in vivo \((40)\) have shown that the NO donor sodium nitroprusside (SNP) and the NO precursor L-arginine inhibit SON neurons, whereas the NOS inhibitor nitro-L-arginine methyl ester (L-NAME) and the NO scavenger hemoglobin excite them. These data suggest that NO is a major inhibitory regulator of SON neurons. However, it was recently reported that NO may be functionally excitatory, by increasing dye coupling among supraoptic neurons \((49)\). One mechanism by which NO influences signaling in the central nervous system is by modulating neurotransmitter release, including in particular the inhibitory neurotransmitter GABA \((29, 38)\). GABAergic synapses comprise ~40% of all synaptic contacts in the SON \((43)\), and GABA plays a key role in controlling the firing activity both of OT and VP neurons \((27, 34, 48)\). The NO and GABA systems seem to strongly interact in the PVN \((19)\). For instance, perfusion of the PVN with NO-containing medium by microdialysis or micro-injection of SNP increases local GABA release \((13)\). NO has also been shown to inhibit renal sympathetic outflow by modulating local GABAergic activity within the PVN \((50)\). Similarly, NMDA-receptor activation in the PVN increased GABAergic activity in magnocellular neuroendocrine neurons, an effect mediated by local NO production \((2)\). Recently, Ozaki et al. \((31)\) showed that NO potentiated GABAergic but not glutamatergic synaptic activity in SON magnocellular neurons, providing evidence for a similar synaptic interaction between NO and GABA in this nucleus.

To further study NO-GABA interactions and their physiological relevance in modulating the activity of SON magnocellular neurons, we combined in vitro and in vivo electrophysiological studies on identified magnocellular neurons. Our studies demonstrate that NO inhibitory actions on both OT and VP neurons in the SON are largely mediated by local activation of GABAergic synaptic activity.

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METHODS

In vitro recordings and data analysis. Coronal hypothalamic slices (350 μm thick) containing the SON were obtained from female rats (Holtzman, Harlan) aged 21–60 days with a vibroslicer (D.S.K. Microslicer, Ted Pella, Redding, CA). The rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and perfused through the heart with cold medium in which NaCl was replaced by an equimolar amount of sucrose. Ice-cold standard solution was used during slicing (see Solutions). After the slices were sectioned, they were placed in a holding chamber containing standard solution at 32–34°C for 60 min and then stored at room temperature until used. After a 1-h incubation period, one slice was transferred to a submersion-type recording chamber kept at room temperature (22–24°C). Solutions bathing the slices were bubbled continuously with a gas mixture of 95% O₂ and 5% CO₂ (pH 7.4). Glucose, and 0.4 ascorbic acid. Phosphate-buffered saline (PBS; pH 7.4, calcium-free, 140 mM NaCl, 5 mM KCl, 1.25 mM KH₂PO₄, 2.5 mM CaCl₂, 26 mM NaHCO₃, 80 mM Na₂HPO₄, 140 mM NaCl, and 2.5 mM KCl) was used to control pH (pH 7.1). Solutions bathing the slices were bubbled continuously with a gas mixture of 95% O₂ and 5% CO₂ (pH 7.4). Glucose, and 0.4 ascorbic acid. Phosphate-buffered saline (PBS; pH 7.4, calcium-free, 140 mM NaCl, 5 mM KCl, 1.25 mM KH₂PO₄, 2.5 mM CaCl₂, 26 mM NaHCO₃, 80 mM Na₂HPO₄, 140 mM NaCl, and 2.5 mM KCl) was used to control pH (pH 7.1).

Patch pipettes (3–5 MΩ) were pulled from thin-wall (1.5-mm outer diameter, 1.17-mm inner diameter) borosilicate glass (GC150F-7.5, Clark, Reading, UK) on a horizontal electrode puller (P-87, Sutter Instruments, Novato, CA). Whole cell recordings from SON neurons were made using visual control with an upright microscope (Axioskop, Zeiss, Oberkochen, Germany) equipped with Normaski IR-DIC optics and a water-immersion lens (×40). Neurons were filled with biocytin (12) and immunocytochemically identified as either OT or VP neurons (42). Whole cell recordings in voltage-clamp mode were obtained with an Axopatch 200B (Axon Instruments, Foster City, CA) patch-clamp amplifier. The series resistance at the onset of the recordings was on average 10.5 ± 0.6 MΩ and was monitored throughout the experiment. Traces were stored on a video-recorder device (Vetter, Rebersburg, PA). Data were digitized offline at 10 or 20 kHz and transferred to a personal computer. The analysis was restricted to miniature inhibitory postsynaptic currents (mIPSCs) with fast rise times (measured from 20% to 80% of the amplitude) ±0.4 ms, which were less likely to be attenuated by dendritic filtering. The detection threshold was set to −8 pA. Individual mIPSCs were aligned at the 50% crossing of the rising phase before averaging. To study the effect of SNP and L-arginine on the mean amplitude and frequency of GABA A mIPSCs, events and a water-immersion lens (×40). Neurons were filled with biocytin (12) and immunocytochemically identified as either OT or VP neurons (42). Whole cell recordings in voltage-clamp mode were obtained with an Axopatch 200B (Axon Instruments, Foster City, CA) patch-clamp amplifier. The series resistance at the onset of the recordings was on average 10.5 ± 0.6 MΩ and was monitored throughout the experiment. Traces were stored on a video-recorder device (Vetter, Rebersburg, PA). Data were digitized offline at 10 or 20 kHz and transferred to a personal computer. The analysis was restricted to miniature inhibitory postsynaptic currents (mIPSCs) with fast rise times (measured from 20% to 80% of the amplitude) ±0.4 ms, which are less likely to be attenuated by dendritic filtering. The detection threshold was set to −8 pA. Individual mIPSCs were aligned at the 50% crossing of the rising phase before averaging. To study the effect of SNP and L-arginine on the mean amplitude and frequency of GABA A mIPSCs, events and a water-immersion lens (×40). Neurons were filled with biocytin (12) and immunocytochemically identified as either OT or VP neurons (42).

Solutions. The standard solution contained (in mM) 126 NaCl, 2.5 KCl, 1.25 KH₂PO₄, 1 MgSO₄, 2 CaCl₂, 26 NaHCO₃, 10 glucose, and 0.4 ascorbic acid. Phosphate-buffered saline (PBS; pH 7.4) was used to control pH (pH 7.1). Glucose, and 0.4 ascorbic acid. Phosphate-buffered saline (PBS; pH 7.4, calcium-free, 140 mM NaCl, 5 mM KCl, 1.25 mM KH₂PO₄, 2.5 mM CaCl₂, 26 mM NaHCO₃, 80 mM Na₂HPO₄, 140 mM NaCl, and 2.5 mM KCl) was used to control pH (pH 7.1). Glucose, and 0.4 ascorbic acid. Phosphate-buffered saline (PBS; pH 7.4, calcium-free, 140 mM NaCl, 5 mM KCl, 1.25 mM KH₂PO₄, 2.5 mM CaCl₂, 26 mM NaHCO₃, 80 mM Na₂HPO₄, 140 mM NaCl, and 2.5 mM KCl) was used to control pH (pH 7.1).

In vivo recording and data analysis. Female Sprague-Dawley rats were anesthetized with urethane (1.25 g/kg ip), a femoral vein and the trachea were cannulated, and the pituitary stalk and right SON were removed transpharyngeally as described previously (20). A homemade U-shaped dialysis probe (molecular cut-off 6 kDa, Spectra/Por HC Hollow Fibers, Spectrum) was used to position the loop of the membrane flat onto the exposed ventral glial lamina of the SON after removal of the meninges (25). A glass micropipette (filled with 0.15 M NaCl, 20–40 MΩ) was introduced into the center of the loop of the dialysis probe to record the extracellular activity of single neurons in the SON. A bipolar stimulating electrode (SNEX-200X, Clarke) was placed on the pituitary stalk and set to deliver single matched biphasic pulses (1 ms, <1 ms peak to peak) for antidromic identification of SON neurons. OT neurons were distinguished from VP neurons by their firing pattern and by their opposite response to intravenous CCK (20 μg/kg, cholecystokinin-(26–35)-sulphated, Bachem, Saffron Walden, Essex, UK), i.e., transient excitation of OT neurons (35) and no effect or short-term inhibition of VP neurons (22). Artificial cerebrospinal fluid (aCSF; pH 7.2, composition in mM: 138 NaCl, 3.36 KCl, 9.52 NaHCO₃, 0.49 Na₂HPO₄, 2.16 urea, 1.26 CaCl₂, 1.18 MgCl₂) was dialyzed at 3 µl/min throughout the experiment. During recording, dialysis fluid was changed to aCSF containing the NO donor SNP (50 mM), the GABA A antagonist bicuculline methiodide (2 mM), or SNP combined with bicuculline (all drugs were purchased from Sigma). Drugs administered by microdialysis in this way penetrate only a short distance into the brain; the concentrations achieved 0.5–1 mm below the surface of the brain are ~3–4 orders of magnitude below the dialysate concentration over this duration of infusion (25). Two VP neurons were stimulated by intravenous infusion of 2 M NaCl (24 ml/min for 20 min). At the end of each experiment, the rats were killed by overdose of pentobarbital sodium anesthetic (60 mg/kg iv).

The firing rates of cells were recorded using Spike2 software (Cambridge Electronic Design, Cambridge, UK) and interfaced to a personal computer. The mean firing rate (spikes/s) and the activity quotient (proportion of time in which a cell is active) were analyzed for 10-min intervals before, during, and after drug treatment. Statistical analyses were completed using SigmaStat software package (Jandel Scientific, Erkraft, Germany). All data were normally distributed, and the responses to drug administration were analyzed by nonparametric Mann-Whitney test. All values were expressed as means ± SE, and differences were considered statistically significant at P ≤ 0.05.
RESULTS

*In vitro studies.* It was recently shown that NO presynaptically modulates GABA synaptic activity in the SON (31). To confirm these results and to further determine whether there is a cell-type specificity in NO-GABA interactions in the SON, we studied NO modulatory actions on GABA_A-mediated mIPSCs in immunochemically identified SON neurons. Figure 1 shows an example of GABA mIPSCs recorded from a VP neuron. GABA_A mIPSCs were recorded in the presence of TTX (0.5 μM) and were pharmacologically isolated with the di-α-amino-3-hydroxy-5-methylisoxazole-propionic acid and N-methyl-d-aspartate (NMDA) glutamate-receptor antagonists NBQX (10 μM) and (±)APV (100 μM), respectively. At a holding potential of −70 mV, GABA_A mIPSCs were observed as fast rising and decaying inward currents. Under these conditions, all synaptic events were blocked by the GABA_A-receptor antagonist bicuculline (20 μM). Bath application of the NO donor SNP (100 μM) or the NO precursor L-arginine (100 μM) markedly and reversibly increased the frequency and also the amplitude of GABA_A mIPSCs (see Fig. 1). Similar effects were observed in six other identified VP neurons. On average (Fig. 1C), NO increased the frequency and amplitude of mIPSCs by 128% and 50%, respectively (n = 7, P ≤ 0.02 for both variables, Wilcoxon’s rank test). On the other hand, the rise time (0.30 ± 0.04 and 0.35 ± 0.05 ms for control and NO, respectively) and decay time constants (9.8 ± 0.9 and 11.2 ± 1.1 ms for control and NO, respectively) were not affected (P > 0.05).

To study the time course of NO potentiation of GABAergic synaptic activity in SON neurons, we plotted the averaged amplitude and frequency of mIPSCs (30-s bins) as a function of time. Figure 2 shows a representative example obtained from an immunochemically identified OT neuron. Bath application of SNP (100 μM) or L-arginine (100 μM) for 5 min increased both the frequency and amplitude of mIPSCs. Similar results were obtained in eight other identified OT neurons. On average (Fig. 2E), NO stimulation increased the frequency and amplitude of mIPSCs by 71% and 43%, respectively (n = 9, P ≤ 0.02 for both variables, Wilcoxon’s rank test). On the other hand, the rise time (0.36 ± 0.04 and 0.32 ± 0.05 ms for control and NO, respectively) and decay time constants (10.1 ± 0.9 and 11.6 ± 1.2 ms for control and NO, respectively) were not affected (P > 0.05). Figure 2B shows GABA mIPSCs recorded before, during, and after SNP application in a representative OT neuron. As seen in Fig. 2B, the averaged mIPSC recorded during these periods was larger during SNP application. On the other hand, the rise and decay time courses of mIPSCs were not affected by SNP (results not shown). A frequency and amplitude distribution histogram for the same cell is shown in Fig. 2C. The occurrence of both small (<30 pA) as well as large (100–250 pA) mIPSCs was increased by SNP. Interestingly, SNP affected mIPSC amplitude and frequency with a different time course (Fig. 2D). The mean delay for evoked changes in

*Fig. 1. Effects of the nitric oxide (NO) donor sodium nitroprusside (SNP) on GABA_A miniature inhibitory postsynaptic currents (mIPSCs) in an identified vasopressin neuron. A: example of an immunochemically identified vasopressin neuron. The neuron was filled with biocytin and immunologically labeled for vasopressin (VP) and oxytocin (OT) neurophysin using double immunofluorescence. In A2, the recorded neuron was visualized with AMCA-conjugated avidin. In A3, the recorded neuron was visualized by tetramethylrhodamine-conjugated secondary antibody. In A4, the recorded neuron was negative for OT-neurophysin immunoreactivity (*), as visualized by fluorescein-conjugated secondary antibody. B: representative GABA_A mIPSCs recorded from the VP neuron shown in A before (B1), during (B2), and after (B3) bath application of SNP (100 μM; see METHODS for more details). Synaptic activity was recorded in the presence of 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo-

[f]quinoxaline-7-sulfonamide disodium (NBQX; 10 μM) and (±)-2-amino-5-phosphonovaleric acid ([±]APV; 100 μM) at a holding potential of −70 mV. Note the increased frequency of mIPSCs during SNP application. mIPSCs were blocked by bicuculline (20 μM; B4). C: on average, the frequency and amplitude of GABA_A mIPSCs were significantly increased by NO stimulation (*P < 0.02, n = 7, Wilcoxon’s rank test).
mIPSC amplitude (20% change from baseline) was 3.4 ± 0.8 min, whereas the mean delay for evoked changes in mIPSC frequency was 5.5 ± 1.1 min (n = 10). This observation suggests that different mechanisms might mediate pre- and postsynaptic modulatory actions of NO on GABAergic synaptic transmission in SON neurons. NO effects on GABA mIPSC amplitude and frequency were blocked by preincubating the slices in the specific nNOS inhibitor 7-nitroindazole (100 μM; n = 4). A tendency for a decreased frequency of GABA mIPSCs was observed during application of 7-nitroindazole alone, although the effect did not reach statistical significance (0.68 ± 0.2 and 0.51 ± 0.3 in control and 7-nitroindazole, respectively, P > 0.05).

In vivo studies. To determine whether the NO-GABA interactions within the SON shown in vitro play a significant role in mediating NO inhibitory actions on the firing activity of SON neurons in the whole animal, in vivo experiments were carried out on identified OT and VP neurons (1 neuron per rat). Neurons were characterized by frequency (spikes/s) and activity quotient and interburst frequency (spikes/s, phasic VP neurons), calculated for 10-min periods before, during, and after drug treatment. In each of six experiments, an identified OT neuron was tested during retrodialysis of 50 mM SNP onto the SON. SNP reduced the firing rate of every OT neuron tested from a mean of 4.26 ± 0.46 to 0.34 ± 0.23 spikes/s (n = 6, P = 0.002, Fig. 3). After recovery, in three of these neurons, the GABA_A antagonist bicuculline was dialyzed increasing the firing rate by 24.4% ± 10.6%. During the bicuculline infusion, SNP was then added to the dialysate. In a further three experiments, SNP was tested on neurons exposed directly to bicuculline without preexposure to SNP. In all six neurons, the SNP induced inhibition after bicuculline was significantly reduced (P ≤ 0.001), producing only an inhibition from 5.8 ± 1.1 to 4.1 ± 0.72 spikes/s (P ≤ 0.2 ns).

Nine VP cells were tested with SNP each in a separate experiment. All seven phasically active VP cells tested were inhibited by SNP, producing a decrease of the activity quotient from a mean of 0.84 ± 0.05 to 0.24 ± 0.09 (P = 0.002) with no significant changes in the intraburst frequency (from 13.0 ± 3.6 to 10.2 ± 2.9 spikes/s). After recovery, the GABA_A antagonist bicuculline was dialyzed onto all these neurons producing an increase in activity quotient and intraburst frequency of 16.4 ± 2.4% and 30.7 ± 11.6%, respectively. During the bicuculline infusion, SNP was then added to the dialysate in four of the tested neurons, producing a slight nonsignificant reduction of the activity quotient from 0.72 ± 0.16 to 0.62 ± 0.16 (P = 0.7 ns, Fig. 4). The intraburst frequency was not affected significantly by SNP (12.7 ± 3.9 control period; 13.2 ± 3.7 spikes/s in presence of SNP). In these four cells and in two additional continuously active VP neurons (data pooled), SNP reduced the mean firing rate from 8.24 ± 2.66 to 3.1 ± 1.65 spikes/s (67.4 ± 10.2% inhibition, P ≤ 0.003) after initial application. After application of bicuculline, SNP had much less marked effects produc-
ing inhibition from 9.6 ± 2.3 to 8.36 ± 2.14 spikes/s (13.2 ± 3.4% inhibition, not significant and \( P \leq 0.01 \) compared with SNP-induced inhibition before bicuculline administration). To exclude the possibility that the observed reduced inhibition was masked by the increase in excitability induced by bicuculline, two neurons were activated by intravenous infusion of 2 M NaCl resulting in an increase in the activity quotient and intraburst frequency by 16.3 ± 1.6% and 8.4 ± 0.6%, respectively. However, retrodialysis of SNP still strongly inhibited both neurons tested (42% and 75%, respectively; Fig. 4B).

DISCUSSION

By combining in vitro and in vivo electrophysiological recordings from identified SON OT and VP neurons, we demonstrated in the present study that 1) NO strongly inhibits the firing activity of OT and VP neurosecretory neurons and 2) that this inhibition is largely mediated by a potentiation of GABAergic synaptic inputs to these cells.

**NO increases the frequency and amplitude of GABA\(_A\)-mediated synaptic currents in both OT and VP neurons.** To study modulatory actions of NO on GABAergic synaptic activity in identified OT and VP neurons, we used the in vitro hypothalamic slice preparation. GABA\(_A\) mIPSCs (which reflect the activation of receptors at single synaptic sites) (33) were recorded from immunochemically identified OT and VP neurons. Bath application of the NO donor SNP and the NO precursor L-arginine increased both the frequency and amplitude of GABA mIPSCs. Pretreatment of the slices with the nNOS inhibitor 7-nitroindazol prevented the potentiation of mIPSCs. No differences in the actions of NO were observed between the two cell types. It is generally well accepted that neuromodulator-induced changes in the frequency of mIPSCs indicate a presynaptic locus of action, whereas changes in mPSC amplitude are believed to reflect a postsynaptic site of action (33). Thus our results suggest that NO modulates GABA signaling in SON neurons by acting both pre- and postsynaptically. Presynaptic modulatory actions of NO might involve an increased neurotransmitter release probability. The fact that NO efficiently modulated mIPSC amplitude without affecting decay kinetics suggests that NO postsynaptic actions might involve modulation of GABA\(_A\) single-channel conductance and/or open probability as opposed to receptor desensitization properties (for review, see Refs. 8 and 14). Future experiments will have to be carried out to further dissect the precise mechanisms by which NO modulates GABAergic synaptic activity in SON neurons. The fact that pre- and postsynaptic actions occurred at a different time scale suggests that different mechanisms might mediate NO actions at these two loci. In this regard, the guanylate cyclase-cGMP cascade is the best-known effector of NO in the brain (39). However, other mechanisms independent of the NO-cGMP pathway, such as ADP ribosylation of proteins, might also mediate NO actions in the SON (4). In a recent paper, Ozaki et al. (31) reported similar effects of NO on GABA mIPSCs obtained from unidentified neurons.
SON neurons. However, no effect on mIPSCs amplitude was observed in that study. Together, these observations support the idea that NO may act within the SON as a diffusible messenger responsible for the potentiation of GABAA mIPSCs in neurosecretory neurons. Similar modulatory actions of NO have been previously described in the hypothalamic PVN nucleus (2, 50), suggesting that feedback interaction between the NO and the GABA system might be a common mechanism for controlling the activity of hypothalamic neuroendocrine neurons.

GABAA receptors are involved in NO inhibition of the firing activity of magnocellular neurosecretory neurons. To determine whether the strong NO-GABA interactions shown to occur in vitro play a relevant role in controlling the firing activity of SON magnocellular neurons in the whole animal, we performed electrophysiological recordings in vivo from identified SON magnocellular neurons. We have previously shown that the spontaneous firing rate both of OT and of VP neurons was inhibited by retrodialysis of GABA_A antagonist bicuculline onto the SON. The VP cell was first inhibited by SNP and then activated by intravenous infusion of 2 M NaCl (26 µl/min). The second administration of SNP still had a strong inhibitory effect, indicating that the increase in activity induced by GABA_A antagonist is not responsible for the reduced SNP effect after bicuculline. C: example of a highly active neuron that was also firmly inhibited by SNP.

Fig. 4. Effects of the NO donor SNP on the activity of phasic VP neurons in vivo. The panels show the spontaneous activity of 3 phasic VP neurons (spikes/s). A: repeated SNP administration shows a reduced response of SNP after retrodialysis of GABA_A antagonist bicuculline onto the SON. B: the VP cell was first inhibited by SNP and then activated by intravenous infusion of 2 M NaCl (26 µl/min). The second administration of SNP still had a strong inhibitory effect, indicating that the increase in activity induced by GABA_A antagonist is not responsible for the reduced SNP effect after bicuculline. C: example of a highly active neuron that was also firmly inhibited by SNP.

GABA_A receptors are involved in NO inhibition of the firing activity of magnocellular neurosecretory neurons. To determine whether the strong NO-GABA interactions shown to occur in vitro play a relevant role in controlling the firing activity of SON magnocellular neurons in the whole animal, we performed electrophysiological recordings in vivo from identified SON magnocellular neurons. We have previously shown that the spontaneous firing rate both of OT and of VP neurons was inhibited by retrodialysis of SNP or the endogenous NO precursor L-arginine. In addition, dialysis infusion of the NOS inhibitor L-NAME increased the firing rates of both cell types as well as hormone secretion in response to hyperosmotic stimulation (40). The present results are also consistent with in vitro electrophysiological studies by others reporting inhibitory actions of NO on the neuronal activity both of phasic and of nonphasic firing neurons in the SON (e.g., Ref. 23). The reduction of this inhibitory action after administration of GABA_A antagonist shown here suggests that the effects are, at least in part, attributable to NO actions on GABAergic synaptic inputs to the SON.

At the concentration used in this study, bicuculline alone produced an increase in activity in most of the cells tested, indicating the presence of a tonic activity of GABAergic afferents to the SON. In a previous study, we showed that this excitation was not sustained but tended to decline after ~10 min of perfusion, possibly reflecting adaptation of the mechanisms underlying excitability in magnocellular neurons or reflecting the ability of these neurons to defend their firing rate against extrinsic perturbations (26). We excluded the possibility that the reduced effects of SNP after bicuculline were due to a general increase in firing activity by showing that highly active or osmotically stimulated neurons were still strongly inhibited by SNP.
Physiological relevance. GABAergic synaptic activity within the SON is amenable to neuromodulation by locally released factors. For example, presynaptic activation of metabotropic glutamate receptors modulates GABA release (37). Opioids, coreleased with VP and OT, act presynaptically within the SON to block CCK-evoked norepinephrine release (30). Furthermore, OT and VP, known to be locally released within the SON and PVN (see Ref. 24 for review), have both post- and presynaptic actions within the nuclei. Both peptides depress glutamate release within the SON (18), and VP increases GABAergic inhibition within the PVN (11). The results from this study together with recent work by Ozaki et al. (31) demonstrate that NO in the SON also acts as an important local modulator of GABAergic activity. Thus presynaptic modulation of neuronal activity may be a commonly used mechanism of negative feedback in hypothalamic nuclei.

Interactions between NO and excitatory neurotransmitter receptors systems have been previously shown. For example, neuronal NOS is activated after stimulation of glutamate NMDA receptors (17). Interestingly, activation of NMDA receptors in the PVN has recently been shown to increase inhibitory synaptic activity, probably through NO production (2). In addition, NO is known to modulate in a negative fashion NMDA receptors in SON neurons (6), constituting an alternative mechanism by which NO could affect neuronal excitability through modulation of synaptic activity.

From a physiological perspective, it is important to understand the mechanisms involved in the production and release of NO. In this sense, increases in intracellular Ca\(^{2+}\) levels are known to stimulate NO release. This can occur, for example, in response to NMDA-receptor activation (17). Alternatively, NO could be produced in an activity-dependent fashion, in response to rises in intracellular Ca\(^{2+}\) during repetitive action potential discharge. The fact that the expression of nNOS in OT and VP neurons is upregulated in conditions known to induce high-frequency discharge of action potentials in these cells suggests that NO actions within the SON may occur in an activity-dependent fashion.

Perspectives

The expression and activity of nNOS is dynamically modulated in response to specific physiological stimuli having a profound effect on the hypothalamoneurohypophysial system. For example, nNOS is upregulated during conditions known to stimulate VP hormone release, including dehydration and osmotic stimulation (16, 46). Thus NO within the SON may function as an important inhibitory feedback regulator during conditions of high neuronal activity, protecting neurons from excessive excitation. On the other hand, endogenous NO is downregulated at term pregnancy, a stage at which the OT system switches from active restraint of secretion to hypersecretion (40). It is now clear that a number of mechanisms actively contributes to this switch, including locally released dynorphin, acting via κ-receptors on OT terminal nerve endings (21). This opioid autoinhibition is upregulated in midpregnancy, but like the NO system, downregulated at term pregnancy. Thus downregulation of both dynorphin and NO systems contributes to the increased excitability of the OT system at term pregnancy, resulting in an increased capacity for greater hormonal release during parturition (21, 40).

Our present results indicate that an important mechanism by which NO modulates neuronal excitability in the hypothalamoneurohypophysial system is through potentiation of GABAergic synaptic activity. Interestingly, the GABAergic system itself in the SON undergoes plastic changes in an activity-dependent manner. For example, the number of GABA-containing synapses (10) and the constitution and properties of the postsynaptic GABA receptor itself change by the end of pregnancy (5, 9). Thus, depending on a particular physiological condition, NO modulation of GABA activity together with concerted plastic changes in each of the systems may result in either a positive or negative feedback compensatory mechanism regulating neuronal excitability and hormone release in the hypothalamoneurohypophysial system.

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