Modulation of Inhibitory Activity by Nitric Oxide in the Thalamus

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Yang S, Cox CL. Modulation of inhibitory activity by nitric oxide in the thalamus. J Neurophysiol 97: 3386–3395, 2007. First published March 21, 2007; doi:10.1152/jn.01270.2006. The dorsal lateral geniculate nucleus (dLGN) is essential for the transfer of visual information from the retina to visual cortex, and inhibitory mechanisms can play a critical role in regulating such information transfer. Nitric oxide (NO) is a neurotransmitter that is released in gaseous form and can alter neural activity without direct synaptic connections. Nitric oxide synthase (NOS), an essential enzyme for NO production, is localized in thalamic inhibitory neurons and cholinergic brain stem neurons that innervate the thalamus, although NO-mediated effects on thalamic inhibitory activity remain unknown. We investigated NO effects on inhibitory activity in dLGN using an in vitro slice preparation. The NO donor, SNAP, selectively potentiated the frequency, but not the amplitude, of spontaneous inhibitory postsynaptic currents (sIPSCs) in thalamocortical relay neurons. This increase also persisted in tetrodotoxin (TTX), consistent with an increase in GABA release from presynaptic terminals. The SNAP-mediated actions were attenuated both by the NO scavenger carboxy-PTIO but also by the guanylyl cyclase inhibitor ODQ. The endogenous NO precursor L-arginine produced actions similar to those of SNAP on sIPSC activity and these L-arginine–mediated actions were attenuated by the NOS inhibitor L-NAME acetate. The SNAP-mediated increase in sIPSC activity was observed in both dLGN and ventrobasal thalamic nucleus (VB) neurons. Considering the lack of interneurons in rodent VB, the NO-mediated actions likely involve an increase in the output of axon terminals of thalamic reticular nucleus neurons. Our results indicate that NO upregulates thalamic inhibitory activity and thus these actions likely influence sensory information transfer through thalamocortical circuits.

INTRODUCTION

Visual information is transferred from the retina to the visual cortex by the dorsal lateral geniculate nucleus (dLGN) of thalamus. This information transfer is a dynamic process that can be strongly influenced by thalamic inhibitory neurons, corticothalamic feedback neurons, and afferent innervation by various brain stem nuclei. Inhibitory innervation of thalamic relay neurons primarily arises from local circuit interneurons and thalamic reticular nucleus (TRN) neurons. Functionally, inhibitory mechanisms contribute to the discrimination of visual signals by enhancing surround inhibition of receptive fields (Lee et al. 1994; Livingstone and Hubel 1981) and to the spatial and temporal integration of ascending sensory signals (Berardi and Morrone 1984; Cox and Sherman 2000; Norton and Godwin 1992; Zhu and Uhlrich 1997). In addition to altering sensory information processing, inhibitory mechanisms play an important role in various intrathalamic rhythmic activities associated with different arousal states and pathophysiological conditions such as absence epilepsy (Cox et al. 1997a; Kim et al. 1997; McCormick 2002; Steriade et al. 1993; von Krosigk et al. 1993).

Nitric oxide (NO) has the unconventional characteristic of being a gaseous neurotransmitter (Boehning and Snyder 2003; Garthwaite et al. 1988). Unlike classical neurotransmitters, which are generally spatially restricted near the synapse, NO can behave hormonelike because it can freely move through membranes and influence neighboring neurons several hundred microns away (Garthwaite and Boulton 1995; Park et al. 1998). NO was previously found to produce a variety of actions in the nervous system by altering neuronal excitability and synaptic transmission through modulation of cGMP and S-nitrosylation (Ahern et al. 2002).

Although NO was found to produce a wide variety of actions in many brain regions, NO-mediated actions in the thalamus are predominantly excitatory (Cudeiro and Rivadulla 1999; Salt and Pape 1999). Nitric oxide synthase (NOS), the enzyme required for NO production, is localized within GABAergic dLGN interneurons and TRN neurons and within acetylcholine-containing neurons in mesopontine tegmental nuclei that innervate most thalamic nuclei (Carden et al. 2000; Erisir et al. 1997; Gabbett and Bacon 1994; McCartney et al. 2002, 2003). The NO-releasing compound SIN-1 selectively depolarizes thalamocortical relay neurons by shifting the activation curve of the hyperpolarization-activated mixed cation current I_h (Pape and Mager 1992). Previously NO was shown to selectively potentiate N-methyl-D-aspartate (NMDA)–dependent excitatory synaptic responses arising from cortico-geniculate afferents in vitro (Alexander et al. 2006). In vivo, the NO precursor l-arginine, the NO donor SIN-1, and cyclic-GMP analogue 8-Br-cGMP potentiate sensory-evoked responses (Do et al. 1994; Shaw and Salt 1997; Shaw et al. 1999). Within the visual system the NO donor SNAP selectively potentiates visual responses mediated by NMDA glutamatergic receptors (Cudeiro and Rivadulla 1999; Cudeiro et al. 1994). Furthermore, extracellular NO concentrations in thalamus are positively correlated with arousal levels, suggesting a putative role in regulating the excitability state of thalamic neurons (Marino and Cudeiro 2003; Williams et al. 1997). Despite the existing work indicating alteration in neuronal excitation and excitatory synaptic transmission, the role of NO in the regulation/modulation of inhibitory activity remains unexplored.

NOS is highly localized in γ-aminobutyric acid (GABA)–containing thalamic neurons as well as afferent cholinergic...
fibers. In addition, the increase in NO was previously associated with increased GABA release by presynaptic mechanisms in the paraventricular and supraoptic nucleus (Kraus and Prast 2002; Li et al. 2002, 2004; Ohkuma et al. 1998; Ozaki et al. 2000; Yu and Eldred 2005). Considering the thalamic NOS localization and NO-mediated influences on inhibitory activity elsewhere, we sought to test the putative role of NO on inhibitory activity within the thalamus. In addition to reports regarding excitatory actions of NO in thalamus, alterations in inhibitory activity could have a significant influence on accurate visual information transfer. Our results indicate that increasing NO levels potentiated inhibitory activity, presumably increasing GABA release from presynaptic terminals by a cGMP-dependent process. Such an action was further potentiated by the depolarization of TRN, thereby increasing the inhibitory tone in thalamocortical neurons.

Methods

Brain slice preparation

Sprague–Dawley rats (postnatal age: 10–16 days) were deeply anesthetized with sodium pentobarbital (55 mg/kg); the brains were quickly removed and placed into chilled (4°C), oxygenated (5% CO2–95% O2) slicing medium containing (in mM): 2.5 KCl, 1.25 NaH2PO4, 10.0 MgSO4, 0.5 CaCl2, 26.0 NaHCO3, 11.0 glucose, and 234.0 sucrose. Slices (300 µm thick) were cut using a vibrating tissue slicer in the coronal plane for dLGN recordings and in the horizontal plane for ventrobasal nucleus (VB) and TRN recordings. Slices were then transferred to a holding chamber containing oxygenated physiological saline that contained (in mM): 126.0 NaCl, 2.5 KCl, 1.25 NaH2PO4, 2.0 MgCl2, 2.0 CaCl2, 26.0 NaHCO3, and 10.0 glucose. Individual slices were then transferred to a recording chamber maintained at 32°C and oxygenated physiological saline was continuously superfused at a rate of 2.0 ml/min.

Intracellular recording procedures

Intracellular recordings were obtained using the whole cell configuration. Recording pipettes had tip resistances of 3–7 MΩ when filled with a solution containing (in mM): 117.0 Cs-glucuronate, 13.0 CsCl, 1.0 MgCl2, 0.07 CaCl2, 0.1 EGTA, 10.0 HEPES, 2.0 Na2-ATP, 0.4 Na-GTP, and 0.3% biocytin. The pH and osmolarity of the intracellular solution resulted in a junction potential of approximately 10 mV that was corrected in the voltage measures. A fixed-stage microscope (Axiskop2, Carl Zeiss) equipped with differential interference contrast optics and a 63 water-immersion objective was used to observe individual neurons within the slice. Inhibitory synaptic currents could be mediated by GABAergic fibers. In addition, the increase in NO was previously associated with increased GABA release by presynaptic mechanisms in the paraventricular and supraoptic nucleus (Kraus and Prast 2002; Li et al. 2002, 2004; Ohkuma et al. 1998; Ozaki et al. 2000; Yu and Eldred 2005). Considering the thalamic NOS localization and NO-mediated influences on inhibitory activity elsewhere, we sought to test the putative role of NO on inhibitory activity within the thalamus. In addition to reports regarding excitatory actions of NO in thalamus, alterations in inhibitory activity could have a significant influence on accurate visual information transfer. Our results indicate that increasing NO levels potentiated inhibitory activity, presumably increasing GABA release from presynaptic terminals by a cGMP-dependent process. Such an action was further potentiated by the depolarization of TRN, thereby increasing the inhibitory tone in thalamocortical neurons.

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GABAergic innervation of dLGN relay neurons arises from TRN neurons and local dLGN interneurons. We next tested whether SNAP altered the excitability of TRN neurons or dLGN interneurons. Current-clamp recordings were obtained from 15 TRN neurons that had an average resting membrane potential of $-79.0 \pm 3.8$ mV and apparent input resistance of $167.8 \pm 36.6$ MΩ. SNAP (500 μM) produced a long-lasting depolarization that averaged $3.6 \pm 1.2$ mV in 15 of 17 neurons tested (Fig. 2A). The depolarization recovered to baseline levels within 14 min. We next obtained current-clamp recordings from four dLGN interneurons. These neurons were differentiated by their unique intrinsic properties and post hoc from their distinct morphology (Govindaiah and Cox 2004; Pape and McCormick 1995; Williams et al. 1996). Interneurons had a lower resting membrane potential of $-64.3 \pm 5.0$ mV ($P < 0.01$, paired t-test) and a greater input resistance averaging $461.5 \pm 121.2$ MΩ ($P < 0.01$, paired t-test) compared with TRN neurons. In the interneurons, SNAP (500 μM) did not alter the membrane potential or input resistance (Fig. 2B, n = 4), indicating that the SNAP-mediated increase in sIPSC frequency in relay neurons could result from suprathreshold excitation of TRN neurons.

To determine if the increase in sIPSC activity results from suprathreshold excitation of TRN neurons, we tested whether the SNAP-mediated increase in sIPSC frequency in relay neurons was blocked in the presence of tetrodotoxin (TTX). In control conditions, SNAP (500 μM) increased sIPSC frequency with no apparent change in amplitude (Fig. 3, A and B). As illustrated in Fig. 3C, SNAP produced a significant decrease in interevent intervals ($P < 0.01$, KS test) with no change in sIPSC amplitude ($P > 0.1$, KS test). After the addition of TTX (0.5 μM), SNAP (500 μM) still produced a significant increase in sIPSC frequency (Fig. 3, A, B, and C, $P < 0.01$, KS test). In the overall population, in control conditions SNAP produced an increase in sIPSC frequency that averaged $145.2 \pm 9.9$% of control (predrug: $5.2 \pm 0.7$ Hz, SNAP: $7.0 \pm 0.9$ Hz, n = 7, $P < 0.01$, paired t-test). SNAP (500 μM) increased sIPSC frequency to $140.4 \pm 10.1$% of control (predrug: $6.5 \pm 0.8$ Hz, SNAP: $8.8 \pm 1.1$ Hz, n = 17, $P < 0.01$, paired t-test). The higher concentrations of SNAP tested (100, 500 μM) did not significantly differ from each other, but did significantly differ from 20 μM SNAP ($P < 0.01$, Tukey–Kramer multiple comparisons). In contrast to frequency, sIPSC amplitudes were not significantly altered at any SNAP concentration tested (Fig. 1D, $P > 0.1$, paired t-test).
baseline levels (Fig. 3, D and E, n = 10, P < 0.01, paired t-test) with no change in sIPSC amplitude. In the presence of TTX, SNAP produced an increase in IPSC frequency that averaged 140.5 ± 16.0% of baseline levels (Fig. 3, D and E, n = 10). The peak increase in IPSC frequency produced by SNAP in TTX did not differ significantly from that in control conditions (Fig. 3E, P > 0.1, t-test). These results indicate that the SNAP-mediated potentiation does not result from the suprathreshold excitation of TRN neurons or interneurons. The increase in sIPSC activity is consistent with a presynaptic action, likely arising from axonal terminals of either TRN neurons or interneurons, or from presynaptic dendrites of interneurons (Cox et al. 1998; Govindaiah and Cox 2004).

To evaluate the effects of endogenously synthesized NO on inhibitory activity, we next used the nitric oxide synthase (NOS) substrate L-arginine. In TTX (0.5 μM), L-arginine (1 mM, 2 min) produced an increase in IPSC frequency in 16 of 24 relay cells tested (Fig. 4, A and B). Overall, L-arginine produced a peak increase in sIPSC frequency that averaged 120.6 ± 3.3% of baseline levels (Fig. 4C, n = 24, P < 0.01, paired t-test). As with the SNAP-mediated actions, there was no alteration in IPSC amplitude (Fig. 4C). The inactive form of arginine, d-arginine, did not alter IPSC frequency (92.9 ± 7.0% of baseline level, n = 5, P > 0.1, paired t-test).

We next tested whether the specific NO scavenger PTIO could antagonize the SNAP-mediated potentiation of IPSC activity in relay neurons. First, we tested whether the SNAP-mediated increase in sIPSC activity was repeatable. The potentiation in sIPSC frequency produced by the first SNAP application (146.2 ± 11.8% of baseline level, n = 4) did not differ from the second SNAP application at a 20-min interval (149.5 ± 9.2%, P > 0.1, paired t-test). In a different subpopulation of relay neurons, the initial SNAP (500 μM) application produced a peak increase in IPSC frequency that averaged 152.5 ± 16.0% of control (n = 7). In PTIO (20 μM), the SNAP-mediated increase in mIPSC frequency was significantly decreased and did not differ from baseline levels (Fig. 5A, n = 7, P > 0.1, paired t-test). In addition, PTIO (20 μM) alone did not alter sIPSC activity (Fig. 5Aiii, P > 0.1, paired t-test).

![FIG. 3. SNAP-mediated increase in IPSCs persists in tetrodotoxin (TTX). A: histograms illustrating the frequency and amplitude of sIPSCs from a dLGN relay neuron. Histograms were constructed using 5-s bins. In control conditions, SNAP (500 μM) produced a robust increase in sIPSC frequency. In TTX (0.5 μM), the SNAP-mediated increase in sIPSC activity persists. B: representative traces from neuron in A that illustrate sIPSC activity in different experimental conditions. C: cumulative probability plots of amplitude and interevent intervals from neuron in A. Note that the clear increase in the interevent intervals even in both control and TTX conditions. D: summary plots of the population data (n = 10 cells) illustrating the action of SNAP on sIPSC amplitude and frequency. Data are presented as percentage of control levels in normal ACSF (filled squares) and after TTX (open circles). E: histogram plot illustrates the peak SNAP-mediated actions on sIPSC amplitude (filled bars) and frequency (open bars). **P < 0.01.](image-url)
Because L-arginine requires NOS to produce NO, we next tested the NOS inhibitor L-NMMA on the L-arginine–mediated actions. In control conditions (TTX, 0.5 μM), the initial application of L-arginine produced an increase in sIPSC frequency that averaged 146.6 ± 7.3% (n = 6) of baseline levels (Fig. 5B, Ai). In L-NMMA (100 μM), the subsequent application of L-arginine increased the sIPSC frequency (116.9 ± 10.1%), significantly less than the response to the initial L-arginine application (P < 0.01, paired t-test). L-NMMA alone did not alter sIPSC frequency (Fig. 5Bii). Furthermore, there were no alterations in sIPSC amplitude by L-arginine or L-NMMA.

NO was previously reported to engage multiple intracellular messenger systems including the guanylyl cyclase (GC)/cGMP pathway (Boehning and Snyder 2003; Bredt and Snyder 1989). We next tested whether the guanylyl cyclase inhibitor ODQ could attenuate the SNAP-mediated facilitation of inhibitory activity. In TTX (0.5 μM), SNAP (500 μM) produced a significant increase in sIPSC frequency that averaged 140.9 ± 10.8% of baseline levels (Fig. 6A, P < 0.01, paired t-test, n = 7). The subsequent SNAP application in ODQ failed to alter sIPSC frequency.
sIPSC frequency (Fig. 6A, 98.6 ± 7.5%, P > 0.1, paired t-test, n = 7). The addition of ODQ (100 μM) alone did not alter sIPSC activity (Fig. 6Ai, P > 0.1, paired t-test). These results suggest that NO exerts its effect through activation of soluble guanylyl cyclase (sGC) to increase the cGMP levels in thalamus.

To test whether a general increase in cGMP level mimics the SNAP-mediated action, we bath-applied the membrane permeable cyclic-GMP analogue, 8-bromo-cyclic-GMP (8Br-cGMP). In TTX (0.5 μM), 8Br-cGMP (50 μM, 2 min) did not significantly alter sIPSC frequency (Fig. 6Bi, 105.9 ± 13.0%, P > 0.1, paired t-test, n = 5). However, in the presence of a phosphodiesterase inhibitor, IBMX (100 μM), which inhibits cyclic nucleotide degradation (Li et al. 2002; Yawo 1999), 8Br-cGMP (50 μM) produced a significant potentiation in sIPSC frequency (Fig. 6Bii, and iv, 139.0 ± 11.4%, P < 0.01, paired t-test, n = 8), which did not reverse within 30 min after drug application. IBMX (100 μM) alone, before 8Br-cGMP application, did not significantly alter sIPSC frequency (98.0 ± 6.5%, n = 21, P > 0.1, paired t-test). Increasing the 8Br-cGMP concentration to 1 mM in the presence of IBMX produced a shorter-latency facilitation with a maximum effect similar to that of the lower 8Br-cGMP concentration (Fig. 6Biii, and iv, 135.0 ± 12.3%, n = 13, P < 0.01, paired t-test).

One of the targets for cGMP activity is the cGMP-stimulated protein kinase G (PKG) (Jaffrey and Snyder 1995) and this pathway is involved with increased GABA release in the paraventricular nucleus (Li et al. 2004). Furthermore, type II cyclic-GMP–dependent protein kinase was previously reported to be highly distributed in thalamus, so we next tested whether the membrane-permeable PKG inhibitor Rp-pCPT-cGMP could attenuate the 8Br-cGMP–mediated increase in sIPSC frequency (Bladen et al. 1996; El Hussein et al. 1999). In the presence of Rp-pCPT-cGMP (5 μM) and IBMX (100 μM), 8Br-cGMP (1 mM) still produced a significant increase in sIPSC frequency (126.9 ± 7.5%, n = 4, P < 0.01, paired t-test) and this increase did not differ from the 8Br-cGMP–mediated facilitation in control conditions (P > 0.05).

Our data strongly indicate that activation of the NO system enhances inhibitory activity by a presynaptic mechanism. Inhibitory innervation of dLGN relay neurons arises from three sources. The local circuit interneurons innervated relay neurons by presynaptic dendrites (named F2 terminals) and axon terminals (named F1 terminals) (Famiglietti Jr and Peters 1972; Storm-Mathisen 1984). In this preparation, sIPSCs arise from ventrobasal nucleus (VB), a structure that contains very few GABAergic local circuit interneurons in rodents and therefore lacks F2 terminals (Arcelli et al. 1997; Ottersen and Storm-Mathisen 1984). In this preparation, sIPSCs arise from axon terminals of TRN neurons (F1 terminals). As illustrated in Fig. 7A, in control conditions SNAP (500 μM) increased the sIPSC frequency with little apparent change in sIPSC amplitude (Fig. 7, A and B). After the addition of TTX (0.5 μM), the facilitation by SNAP persisted, but to a lesser extent than in control conditions (Fig. 7, A and B). This TTX-sensitive component within VB likely arises from suprabasal excitation of TRN neurons (e.g., see Fig. 2A) that remain synaptically intact in the horizontal thalamic slice. As indicated in the cumulative probability plots, there was a significant decrease in the interevent intervals in the presence of SNAP in control and TTX conditions (Fig. 7C, P < 0.01, KS test). Similarly, the population data indicate that SNAP significantly increased the sIPSC frequency an average of 133.5 ± 7.5% of control (Fig. 6, D and E, P < 0.01, paired t-test) and in TTX, SNAP produced a peak increase in sIPSC activity that averaged 118.6 ± 4.8%, which was significantly greater than baseline levels (Fig. 6, D and E, P < 0.05, paired t-test, n = 9). Notable was a statistical difference of the SNAP-mediated frequency potentiation in TTX between LGN and VB relay cells (140.5 ± 16.0 vs. 118.6 ± 4.8%, P < 0.01). Unlike dLGN neurons, SNAP produced a significant increase in sIPSC amplitude in the control conditions (Fig. 6C, P < 0.01, KS test). The population data support the increase of sIPSC amplitude that
averaged 109.0 ± 2.1% (Fig. 6, D and E, P < 0.01, paired t-test, n = 9), suggesting that a portion of increased sIPSCs resulted from suprathreshold depolarization of TRN neurons by SNAP.

DISCUSSION

In this study, we investigated the influence of NO on inhibitory synaptic activity within the thalamus based on previous anatomical studies indicating NOS localization in GABAergic thalamic neurons (Erisir et al. 1997; McCauley et al. 2002, 2003). To summarize, we found that the NO donor SNAP or the NO precursor l-arginine significantly increased spontaneous GABA-mediated inhibitory activity in thalamic relay neurons by enhancing the frequency—but not the amplitude—of the IPSCs. The specific NO scavenger carboxyl-PTIO attenuated SNAP-mediated potentiation and the NOS inhibitor, l-NMMA acetate, decreased the l-arginine-mediated potentiation. These NO-mediated actions appear to be involved in an increase of the cGMP level because the soluble guanylyl cyclase inhibitor ODQ attenuated the SNAP-mediated potentiation. Furthermore, the increase in IPSC frequency was mimicked by the cGMP analogue 8-Br-cGMP, in the presence of the phosphodiesterase inhibitor IBMX. The augmentation of inhibitory activity by SNAP was present in both dLGN and VB relay neurons, but was significantly less in VB neurons. The VB nucleus, unlike the dLGN, lacks local circuit interneurons and receives most of its inhibitory innervation from TRN neurons. Thus our data indicate that NO leads to an increase in GABA release from presynaptic terminals of TRN neurons and likely local circuit neurons as well, thereby increasing the inhibitory drive onto thalamic relay neurons.

cGMP dependency of presynaptic NO actions

Based on our results, the NO-mediated increase in inhibitory activity results from an increase in GABA release by presynaptic mechanisms. In our experiments, the sIPSC frequency was significantly increased by NO agonists; however, the sIPSC amplitude was unaltered. Furthermore, the increase in sIPSC frequency persisted in TTX, indicating suprathreshold excitation of presynaptic GABA-containing neurons (i.e., TRN neurons) was not required. Such a presynaptic mechanism is consistent with the NO actions reported in other brain regions.
including paraventricular nucleus of the hypothalamus, supraoptic neurons, nucleus accumbens, cultured neocortical neurons, and retinal neurons (Kraus and Prast 2002; Li et al. 2002; Ohkuma et al. 1998; Ozaki et al. 2000; Yu and Eldred 2005). Most NO-mediated actions described thus far in the CNS involve either cGMP or S-nitrosylation (Ahern et al. 2002) and our results appear dependent on the cGMP pathway. The cGMP analogue 8-Br-cGMP, in presence of the phosphodiesterase inhibitor IBMX, increased sIPSC frequency and the SNAP-mediated increase in sIPSC activity was inhibited by the guanyllyl cyclase inhibitor ODQ.

The increase in cGMP produced by NO can target cGMP-gated channels (Ingram and Williams 1996; Zagotta and Siegelbaum 1996), cGMP-dependent phosphodiesterases (Kraus and Prast 2002), and cGMP-dependent PKG (Jaffrey and Snyder 1995). Despite the presence of type II cyclic GMP-dependent PKG within thalamic neurons, the selective inhibitor Rp-pCPT-cGMP did not attenuate the SNAP-mediated actions (Bladen et al. 1996). Furthermore, the increase in inhibitory activity also persisted in the presence of the phosphodiesterase inhibitor IBMX. Assuming the lack of PKG and phosphodiesterase involvement, one possible mechanism is that cGMP directly activates cGMP-gated channels in the presynaptic terminal, leading to transmitter release. One candidate is the hyperpolarization-activated mixed-cation current I_h; the HCN channels that mediate I_h have binding sites for cGMP and cAMP (Zagotta et al. 2003). Moreover, I_h contributes to increased GABA release in presynaptic terminals of cerebellar basket cells (Southan et al. 2000). Therefore the increase in cGMP produced by NO could potentiate HCN channels in the axon terminals or dendrite of inhibitory neurons (TRN and dLGN interneuron), leading to the increase of GABA release. However, at this point, we cannot definitively exclude the role of PKG because our highest Rp-pCPT-cGMP concentration tested (5 μM) may not completely block all PKG-mediated activities, although a lower concentration (1 μM) was used to completely block SNAP-mediated inhibitory actions in a slice preparation of rat paraventricular nucleus (Li et al. 2004). Furthermore, the cGMP pathway also appears to be involved in the postsynaptic depolarization of dLGN thalamic relay neurons by NO and the facilitation of excitatory synaptic responses arising from corticogeniculate afferents (Alexander et al. 2006; Pape and Mager 1995). Therefore, it is important to note the negligible effects of 8-Br-cGMP reported from in vivo single-unit recordings in cat dLGN, suggesting potential diversity in NO-mediated actions in the thalamus (Cudeiro et al. 1994).

**Sources and target sites of NO in the thalamus**

One potential source of NO in the thalamus arises from acetylcholine-containing brain stem nuclei. Cholinergic neurons arising from the parabrachial region of the brain stem that innervate dLGN also contain NOS (Carden et al. 2000; Erisir et al. 1997). Activity of the parabrachial neurons is positively correlated with arousal levels and therefore increased levels of NO-mediated actions may be correlated with increasing arousal levels (Williams et al. 1997). In addition, these cholinergic neurons also innervate presynaptic GABA-containing dendrites of dLGN interneurons that form dendro dendritic synapses onto relay cell dendrites, often forming a characteristic “triadic” junction (F2 terminal) (Famiglietti Jr and Peters 1972; Guillery 1969; Hamos et al. 1985; Montero 1986; Ralston 1971). Thus NO may provide an effective output control of presynaptic dendrites of local interneurons (Cox and Sherman 2000; Govindaiah and Cox 2004). Another possible source of NO is from GABA-containing neurons in the thalamus. NOS and GABA are co-localized in a subpopulation of local inhibitory interneurons in the cat dLGN (Erisir et al. 1997; McCauley et al. 2003).

Considering the innervation of TRN by cholinergic neurons of the parabrachial region, the TRN is another site for NO-mediated actions. In addition, recent evidence indicates that NOS is also expressed in TRN of ferrets (McCauley et al. 2002). Our data indicate that increased NO activity leads to depolarization of TRN neurons (Fig. 2) and increased GABA release from presynaptic terminals of TRN neurons (Fig. 7). These experiments are the first electrophysiological observations suggesting that TRN neurons may be a target for endogenous NO. The activation of TRN neurons plays an important role in oscillations of thalamocortical systems shown during the sleep cycle (Cox et al. 1997b; McCormick 2002; Steriade et al. 1993; von Krosigk et al. 1993). Furthermore, NO is also an easy diffusible substance that can reach several hundred microns (Garthwaite and Boulton 1995; Park et al. 1998), presumably acting as a spatial signal simultaneously influencing a large neuronal population by local diffusion in thalamus. Therefore the widespread effects for functional control of neural networks without direct synaptic connections could be indicative of NO action in thalamus. These properties may endow the NO system with important functions in controlling sensory transfer and thalamic oscillatory activity in a global manner.

**Functional role of NO in the visual thalamus**

Considering GABA-mediated inhibition can have important influences on visual information processing in the thalamus, alterations in inhibitory activity by NO could significantly influence such processing. GABA-mediated inhibition was previously reported to be involved in accurate discrimination of incoming signals and enhancing sensitivity for local contrast information (Berardi and Morrone 1984; Govindaiah and Cox 2004; Holdefer et al. 1989; Livingstone and Hubel 1981; Norton and Godwin 1992; Sillito and Kemp 1983). GABA-mediated activity appears to regulate receptive field sensitivity (Holdefer et al. 1989; Norton et al. 1989). Lesions of TRN neurons increase receptive field size in somatosensory thalamic neurons, suggesting a surround antagonistic role of TRN neurons (Lee et al. 1994). The increment of contrast caused the increasing response of LGN target cells using extracellular single-unit recording (Kaplan et al. 1987). From this, we would predict that the inhibitory action of NO could play an important role on contrast information by regulating receptive field size and/or sensitivity.

Previous in vivo studies indicate that NO leads to an enhancement of sensory-evoked responses in presumed thalamic relay neurons (Cudeiro et al. 1994; Shaw et al. 1999). Although there is some controversy regarding the role of cGMP underlying the facilitation, Shaw et al. (1999) found a striking similarity between the actions of NO agonists and cGMP, whereas Cudeiro et al. (1994) found negligible actions of
cGMP activation. In vitro studies found postsynaptic depolarizations produced by NO agents in thalamic relay neurons, consistent with the excitatory effects of NO (Pape and Mager 1992). Our data provide a novel role of NO within the thalamus—that is, activation of NO by a cGMP-dependent pathway leads to a presynaptic enhancement of inhibitory activity that occurs independent of the action potential discharge of GABA-containing neurons. Depending on the spatial distribution of NO, such changes may provide a mechanism to sharpen the excitatory relay through the thalamus. Our working hypothesis is that with attentive states, increased output of NOS-containing cholinergic neurons would lead to increased NO synthesis and release, thereby leading to a direct postsynaptic depolarization decreasing the threshold for suprathreshold excitation through the thalamic relay and, at the same time, NO would lead to increased inhibitory activity that would in turn enhance surround antagonistic actions, ultimately increasing the signal-to-noise ratio and potentially sharpening receptive fields. Therefore we would speculate that NO may be crucial for sharpening visual transmission through potentiation of GABA release accompanied with the facilitation of sensory transmission.

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REFERENCES


