Inhibition of Primate Spinothalamic Tract Neurons by Spinal Glycine and GABA Is Modulated by Guanosine 3',5'-Cyclic Monophosphate

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Lin, Qing, Jing Wu, Yuan Bo Peng, Minglei Cui, and William D. Willis. Inhibition of primate spinothalamic tract neurons by spinal glycine and GABA is modulated by guanosine 3',5'-cyclic monophosphate. J. Neurophysiol. 81: 1095–1103, 1999. Our recent work has suggested that the nitric oxide/guanosine 3',5'-cyclic monophosphate (NO/cGMP) signal transduction system contributes to central sensitization of spinothalamic tract (STT) neurons in part by influencing the descending inhibition of nociception resulting from stimulation in the periaqueductal gray. This study was designed to examine further whether activation of the NO/cGMP cascade reduces the inhibition of the activity of STT neurons mediated by spinal inhibitory amino acid (IAA) receptors. Responses of STT cells to noxious cutaneous stimuli were inhibited by iontophoresis of glycine and GABA agonists in anesthetized monkeys. Administration of 8-bromoguanosine-3',5'-cyclophosphosphate sodium (8-bromo-cGMP), a membrane-permeable analogue of cGMP, either by microdialysis or by iontophoresis reduced significantly the IAA-induced inhibition of wide dynamic range (WDR) STT cells in the deep layers of the dorsal horn. The reduction in inhibition lasted for up to 1–1.5 h after the cessation of drug infusion. In contrast, IAA-induced inhibition of WDR STT cells in the superficial dorsal horn and high-threshold (HT) cells in superficial or deep layers was not significantly changed during 8-bromo-cGMP infusion. Iontophoresis of 8-bromo-cGMP onto STT cells produced the same actions as produced by microdialysis of this agent, but the effect was not as long-lasting nor as potent. Finally, an attenuation of the IAA receptor–mediated inhibition of STT cells produced by iontophoretic release of a NO donor, 3-morpholinosydnonimine, could be blocked by pretreatment of the spinal cord with a guanylate cyclase inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one. These results suggest that an increased spinal cGMP level contributes to the sensitization of WDR STT neurons in the deep dorsal horn in part by down-regulating spinal IAA receptors. However, no evidence is provided in this study that the NO/cGMP cascade regulates IAA receptors on HT and superficial WDR neurons. Combined with the preceding studies, our data support the view that NO and cGMP function in the same signal transduction cascade and play an important role in central sensitization.

INTRODUCTION

A number of studies suggest that increased spinal levels of guanosine 3',5'-cyclic monophosphate (cGMP) contribute to the development of hyperalgesia and allodynia. An increased level of immunoreactive cGMP within the lumbar spinal enlargement was seen during hyperalgesia following intraplantar injection of carageenan (Garry et al. 1994b). Also, intrathecal injection of 8-bromoguanosine-3',5'-cyclophosphosphate sodium (8-bromo-cGMP), a membrane-permeable analogue of cGMP, produced hyperalgesia (Garry et al. 1994a; Garry and Davis 1997). We have recently shown that spinothalamic tract (STT) neurons can be sensitized when 8-bromo-cGMP is administered into the dorsal horn of the spinal cord (Lin et al. 1997).

As discussed in our preceding papers (Lin et al. 1999a,b), nitric oxide (NO) is well established as an intercellular regulator, and most of its effects appear to be mediated via cGMP (Schmidt et al. 1993). A soluble form of guanylate cyclase is a key enzyme linking NO with cGMP (Schmidt et al. 1993; Southam and Garthwaite 1991; Waldman and Murad 1987). In our experiments (Lin et al. 1999a), we found that an elevation of NO within the dorsal horn results in sensitization of STT cells to cutaneous mechanical stimuli. This action is accompanied by an attenuation of spinal inhibition of STT cells produced by activation of a descending inhibitory pathway from the periaqueductal gray (PAG) and of spinal glycine and GABA receptors (Lin et al. 1999b). NO is released within the spinal cord when STT cells are sensitized by intradermal injection of capsaicin (Lin et al. 1999a). We have also recently demonstrated that sensitization of STT neurons produced by intraspinal administration of 8-bromo-cGMP is accompanied by a reduction in PAG-induced inhibition of responses to peripheral mechanical stimuli (Lin et al. 1997). All these changes were seen consistently in deep wide dynamic range (WDR) STT cells. These data indicate strongly that the NO/cGMP cascade participates in the induction of central sensitization in the deep layers of the spinal cord dorsal horn in part by affecting spinal inhibition.

The present study asked whether an elevation in the cGMP level in the dorsal horn could also reduce the inhibition of primate STT neurons that is produced by the activation of spinal glycineric and GABAergic receptors. cGMP levels within the spinal cord were elevated by 8-bromo-cGMP delivered by microdialysis or iontophoresis. Inhibitory responses of STT cells to glycine and GABA agonists were tested using iontophoretic application of the inhibitory amino acids (IAAs). As predicted, 8-bromo-cGMP was found to reduce the inhibition produced by IAAs. The linkage between the effects of NO and cGMP production was then tested by administration of 3-morpholinosydnonimine (SIN-1), a NO donor, into the dorsal horn while releasing IAAs iontophoretically onto STT neurons. The reduction in inhibition by SIN-1 was antagonized by microdialysis of a guanylate cyclase inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ).
A preliminary report of these findings has been made (Lin et al. 1996c).

METHODS

Data were collected from 19 adult male monkeys (Macaca fascicularis, 1.9–2.5 kg). Animal preparation and maintenance were the same as described in detail in our two preceding papers (Lin et al. 1999a,b). Experimental approaches used in this study, such as placement of microdialysis fibers for drug administration and use of multibarrel electrodes for both extracellular recordings of STT neurons and drug delivery, have also been described (Lin et al. 1999b).

Drug administration

8-bromo-cGMP (from RBI) was administered in two ways. 8-bromo-cGMP was dissolved in artificial cerebrospinal fluid (ACSF) or normal saline to a concentration of 10 mM and infused into the spinal dorsal horn by microdialysis. The dose used in this study was comparable with that used in in vitro studies (Ito and Karachot 1992; Shibuki and Okada 1991) and has been shown to change the responses of STT cells to peripheral stimuli (Lin et al. 1997). 8-bromo-cGMP was also applied by iontophoresis. The concentration in the drug barrel was 15 mM (pH 7.2). IAA agonists, including glycine, GABA, and muscimol, were administered by iontophoresis in the same way as in our previous studies (Lin et al. 1994, 1996b,d). Briefly, effects of glycine, GABA, and muscimol on STT cells were tested by sequential iontophoretic release of these agents onto STT cells. Observations were made on the effects of glycine and GABA agonists on noxious stimulation-evoked activity by applying a sustained Pinch stimulus to the skin during drug administration. All agents were delivered iontophoretically using three 5- to 10-s-long graded current pulses. ODQ (Tocris) was delivered into the spinal cord at a concentration of 1 mM by microdialysis as previously described (Lin et al. 1997). ODQ has

![Fig. 1](http://jn.physiology.org/) Bar graph and rate histograms represent the changes in responses of a deep wide dynamic range (WDR) spinothalamic tract (STT) neuron (1,496 μm) to mechanical stimuli (A) and in the inhibition of Pinch-evoked activity produced by iontophoretic release of glycine, GABA, and muscimol (B) when the spinal dorsal horn was perfused with 8-bromo-guanosine-3′,5′-cyclophosphate sodium (8-bromo-cGMP) by microdialysis. Graded current pulses used for delivering drugs iontophoretically are indicated by upward- or downward-going square waves under each histogram. A Pinch stimulus was applied to the receptive field while agonists were released iontophoretically.
were again tested. GABA agonists were retested. The drug was then washed out with min, and the cell’s responses to iontophoretic release of glycine and recorded, 8-bromo-cGMP was infused into the dorsal horn for 30 – 60 cGMP on PAG inhibition (see Lin et al. 1997). After baseline inhibition of STT cells were examined on one group of STT neurons.

In this group, some cells were also used to test the effects of 8-bromo-cGMP on inhibition of STT cells according to their response patterns (Chung et al. 1986). The procedure for applying mechanical stimuli to the skin of the receptive field was identical to that in several studies by our group (Dougherty et al. 1992a; Dougherty and Willis 1991a,b; Lin et al. 1996a,b, 1997) and was described in detail in our preceding paper (Lin et al. 1999a). Recordings of responses to cutaneous mechanical stimuli were recorded to determine whether 8-bromo-cGMP sensitized STT cells.

The effects of microdialysis of 8-bromo-cGMP on IAA-elicited inhibition of STT cells induced by iontophoresis of SIN-1 were tested. SIN-1 was iontophoresed continuously (70–100 nA) during a period when a glycine or GABA agonist was delivered iontophoretically by three graded current pulses.

In some STT cells separate from the above two groups of cells, the effects of ODQ in reducing the IAA receptor–mediated inhibition of STT cells by iontophoresis of SIN-1 were tested. SIN-1 was iontophoresed continuously (70–100 nA) during a period when a glycine or GABA agonist was delivered iontophoretically by three graded current pulses while recording from the same cell. The effect of the first SIN-1 ejection was observed without ODQ infusion. After inhibition induced by IAAs was shown to recover, ODQ was infused into the dorsal horn for 30–60 min. The effect of the second SIN-1 ejection on IAA receptor–mediated inhibition was then tested during ODQ infusion.

Data were processed in the same way as in our two preceding papers (Lin et al. 1999a,b). The stored digital record of unit activity was retrieved and analyzed off-line. Frequency histograms were generated for all sensory- and drug-evoked events. The inhibitory effects of iontophoretic application of glycine and GABA agonists on Pinch-evoked activity were evaluated by calculating the total percentage of inhibition of evoked activity induced by three graded current pulses. A repeated measures ANOVA tested responses in each group. If significance was obtained, post hoc testing with paired t-tests assessed differences from the baseline levels. A value of \( P < 0.05 \) was considered significant. All values are given as the means ± SE.

FIG. 2. Bar graphs summarize the grouped data from deep WDR STT neurons for the effects of 8-bromo-cGMP administration on inhibition of Pinch responses elicited by iontophoretic release of glycine and GABA agonists. A: changes in inhibitory amino acid (IAA)–induced inhibition when 8-bromo-cGMP was infused into the dorsal horn by microdialysis (n = 7). B: changes in IAA-induced inhibition when 8-bromo-cGMP was applied onto STT cells iontophoretically (n = 8). \(* P < 0.05; \** P < 0.01, \) compared with the baseline level.

Experimental design

The responses of STT cells to cutaneous mechanical stimuli, which included Brush, Press, and Pinch stimuli, were recorded to classify STT cells according to their response patterns (Chung et al. 1986). The procedure for applying mechanical stimuli to the skin of the receptive field was identical to that in several studies by our group (Dougherty et al. 1992a; Dougherty and Willis 1991a,b; Lin et al. 1996a,b, 1997) and was described in detail in our preceding paper (Lin et al. 1999a). Recordings of responses to cutaneous mechanical stimuli were recorded to determine whether 8-bromo-cGMP sensitized STT cells.

The effects of microdialysis of 8-bromo-cGMP on IAA-elicited inhibition of STT cells were examined on one group of STT neurons. In this group, some cells were also used to test the effects of 8-bromo-cGMP on PAG inhibition (see Lin et al. 1997). After baseline inhibitions produced by iontophoretic application of IAA agonists were recorded, 8-bromo-cGMP was infused into the dorsal horn for 30–60 min, and the cell’s responses to iontophoretic release of glycine and GABA agonists were retested. The drug was then washed out with ACSF or normal saline for 30–60 min before the inhibitory responses were again tested.

In another group of STT cells, observations were made of the effects of iontophoretic application of 8-bromo-cGMP on inhibition of STT cells elicited by glycine and GABA agonists. After control responses were recorded, 8-bromo-cGMP was iontophoresed continuously (70–100 nA) during a period when a glycine or GABA agonist was delivered iontophoretically by three graded current pulses.

RESULTS

Recordings were made from a total of 31 STT neurons, including 28 WDR and 3 HT cells, in 19 animals. Cell depths ranged from 944 to 2,040 \( \mu \)m below the spinal cord dorsal surface, and thus the STT neurons were presumed to be in laminae I–V (see Lin et al. 1999a). Fourteen neurons (12 WDR cells and 2 HT cells) were tested with 8-bromo-cGMP administered by microdialysis. The responses of these cells to mechanical stimuli were presented in a paper on the effects of 8-bromo-cGMP (Lin et al. 1997). Thirteen neurons (12 WDR cells and 1 HT cell) were tested with 8-bromo-cGMP applied iontophoretically. The remaining four deep WDR cells were used to observe changes in the attenuation of IAA receptor–mediated inhibition produced by SIN-1 administration when guanylate cyclase was blocked. These STT cells were not reported in our previous work.

Effects of intraspinal administration of 8-bromo-cGMP by microdialysis on the inhibition of STT neurons produced by iontophoretic release of glycine and GABA agonists

We have reported recently that the responses of deep WDR STT cells to both weak and strong cutaneous mechanical stimuli were sensitized by spinally administered 8-bromo-cGMP and that the responses of superficial WDR cells and HT cells to cutaneous mechanical stimuli were reduced by the same dose of 8-bromo-cGMP (Lin et al. 1997). In the present study, we found that the inhibition of responses of deep WDR STT cells to mechanical noxious stimuli produced by glycine and GABA agonists was profoundly reduced when the spinal cord was perfused with 8-bromo-cGMP. The tests were made on deep WDR cells, in which responses to mechanical stimuli are enhanced by infusion of 8-bromo-cGMP within the spinal
Figure 1 is an example showing the changes in inhibition of a deep WDR STT cell (1,496 μm below the surface of the spinal cord) produced by glycine and GABA agonists during infusion of 8-bromo-cGMP at a concentration of 10 mM. The responses of this cell to both weak and strong mechanical stimuli were sensitized by 8-bromo-cGMP (Fig. 1A). An attenuation of IAA-induced inhibition occurred during 8-bromo-cGMP infusion (2nd row of Fig. 1B) and long outlasted the drug infusion period (3rd row of Fig. 1B). This long-lasting change was consistent with the change in the responses to mechanical stimuli (Fig. 1A). Similar results were obtained for most deep WDR cells tested. Blockade of glycine-induced inhibition was found in all seven cells and of GABA- and muscimol-induced inhibition in six of seven cells. Statistical analysis showed that all of these changes were significantly different from the baseline levels (Fig. 2A).

However, in most superficial WDR cells and HT cells, in which responses to mechanical stimuli were reduced by intraspinal infusion of 8-bromo-cGMP, inhibition of Pinch-evoked activity of these cells produced by iontophoresis of glycine and GABA agonists was not significantly changed when the spinal cord was perfused with 8-bromo-cGMP. A total of five superficial WDR cells and two HT cells were tested with 8-bromo-cGMP, and an attenuation of IAA-induced inhibition was only found in one cell. In some of these cells (4 for glycine-, 2 for GABA- and muscimol-induced inhibition), the inhibition was found to be potentiated during 8-bromo-cGMP administration.

**Effects of iontophoretic administration of 8-bromo-cGMP onto STT cells on the inhibition of STT neurons produced by iontophoretic release of glycine and GABA agonists**

In a total of 13 STT neurons, the effect of 8-bromo-cGMP applied by iontophoresis (70–100 nA) was tested on the inhibition of these cells produced by glycine and GABA agonists. 8-bromo-cGMP administered by iontophoresis also reduced the inhibition of Pinch-evoked activity produced by iontophoretic release of glycine and GABA agonists, and this effect was seen mainly in the deep WDR STT cells tested. However, 8-bromo-cGMP applied by iontophoresis did not produce an effect as long-lasting or as potent as that produced by 8-bromo-cGMP applied by microdialysis.

Figure 3 shows that the inhibition of the Pinch-evoked response of a deep WDR cell (1,404 μm) produced by IAA was reduced during continuous iontophoresis of 8-bromo-cGMP (100 nA). In a total of 8 deep WDR cells (1,374–1,700 μm), the effects of 8-bromo-cGMP on glycine-induced inhibition were examined, and an attenuation of inhibition was seen in seven of eight cells. Tests for the effects of 8-bromo-cGMP...
on GABA- and muscimol-induced inhibition were completed on seven cells, and attenuation of inhibition was obtained in six of seven cells. All of these changes were significantly different from the baseline levels (Fig. 2).

In the remaining five neurons, which included four superficial cells (944–1,276 μm) and one HT cell (1,600 μm), the effects of iontophoresis of 8-bromo-cGMP on GABA-induced inhibition were tested in all five cells, but a reduction in inhibition was seen in only one cell. Tests for the effects of iontophoresis of 8-bromo-cGMP on glycine- and muscimol-induced inhibition were completed in four cells. Reduction in inhibition produced by glycine was seen only in one cell, and no attenuation of the muscimol-induced inhibition was obtained. No statistically significant change in this group was observed during 8-bromo-cGMP application.

Figure 4 summarizes the effects of spinal administration of 8-bromo-cGMP microdiallytically and iontophoretically on IAA-induced inhibition of Pinch-evoked activities of different kinds of STT cells. ●, deep WDR cells; ●, superficial WDR cells; ▲, HT cells.

Effects of guanylate cyclase inhibitor on the reduction in IAA-induced inhibition produced by NO release

NO release within the spinal cord has been shown to attenuate the IAA-induced inhibition of deep WDR STT cells in our preceding paper (Lin et al. 1999b). Here we examined whether blockade of guanylate cyclase interfered with this effect of NO release. Observations were made on four deep WDR STT neurons. Figure 5 consists of rate histograms for a representative STT cell that shows the effects of iontophoretic release of SIN-1 on IAA-induced inhibition without and with intraspinal infusion of a guanylate cyclase inhibitor, ODQ. The top row shows the baseline recordings of inhibition of PINCH-evoked activity elicited by iontophoretic release of glycine and GABA agonists. A nearly complete blockade of inhibition produced by all three IAA agonists was seen while SIN-1 was being released by iontophoresis (2nd row). The inhibition recovered or was even increased around 30 min after terminating SIN-1 ejection (3rd row). ODQ was then infused into the dorsal horn for 30 min. IAA-induced inhibition showed no obvious change during ODQ application (4th row). A second ejection of SIN-1 by iontophoresis was made while testing the IAA-induced inhibition during ODQ infusion. The inhibition of Pinch-evoked activity was nearly unchanged, in contrast to its elimination during the initial ejection of SIN-1 (bottom row vs. 2nd row).

The grouped data (Fig. 6) show results similar to those obtained from this individual cell. There was no significant change in IAA-induced inhibition when the spinal cord was pretreated with ODQ (open bar vs. hatched bar in the right set of each group of bars), and ODQ prevented the blockade of inhibition induced by NO release by SIN-1 administration.

DISCUSSION

The current study provides further evidence for our hypothesis that activation of the NO/cGMP cascade may contribute to the development and maintenance of central sensitization of STT neurons in part by reducing the effectiveness of spinal inhibition. Our findings that an increase either in NO or cGMP level within the spinal cord can sensitize the responses of STT cells to peripheral mechanical stimuli and that this sensitization is accompanied by a reduction in spinal inhibition mediated by a descending inhibitory pathway from the PAG or spinal IAA receptors suggest that these effects produced by NO and cGMP involve the same mechanism. Furthermore, the finding that a reduction in IAA-induced inhibition produced by applying a NO releasing agent, SIN-1, can be prevented when guanylate cyclase is blocked provides evidence that NO and cGMP act through the same signal transduction cascade (Schmidt et al. 1993).
8-bromo-cGMP is a membrane-soluble analogue of cGMP and has been used to elevate intracellular cGMP levels (Hartell 1994a,b; Ito and Karachot 1990; Okada 1992). An increased cGMP within the lumbar spinal cord is associated with hyperalgesia produced by intraplantar injection of carrageenan (Garry et al. 1994b). cGMP formation is triggered by glutamate release (cf. Lerea et al. 1992; MacDermott et al. 1986; Manzoni et al. 1990; Mayer and Miller 1990; Otsuka and Yoshioka 1993; Schoep and Conn 1993; Watling 1992), which increases cGMP content in neurons through activation of NOS to produce NO from L-arginine (Bredt and Snyder 1989, 1990; Garthwaite and Balazs 1978; Garthwaite et al. 1988). This process involves Ca\(^{2+}\) influx through receptor-operated ion channels, such as N-methyl-D-aspartate (NMDA) and some non-NMDA receptors (Garthwaite and Balazs 1978; Garthwaite et al. 1988). This process involves Ca\(^{2+}\) influx through receptor-operated ion channels, such as N-methyl-D-aspartate (NMDA) and some non-NMDA receptors (Garthwaite et al. 1988; Lerea et al. 1992; MacDermott et al. 1986; Mayer and Miller 1990). We have discussed in previous papers (Lin et al. 1996a,b, 1997) that a prolonged central sensitization of spinal dorsal horn neurons is initiated by the activation of NMDA and neurokinin 1 (NK1) receptors due to the release of excitatory amino acids (EAAs) and substance P (SP) from primary afferent nociceptors following peripheral tissue injury or chemical irritation (Davies and Lodge 1987; Dougherty et al. 1992b, 1994, 1995; Dougherty and Willis 1992; Haley et al. 1990) and is maintained by triggering several second-messenger cascades, mainly through Ca\(^{2+}\)-dependent mechanisms (Garthwaite et al. 1988; Lerea et al. 1992; MacDermott et al. 1986; Mayer and Miller 1990). NMDA-induced hyperalgesia was blocked by intrathecal administration of an inhibitor of guanylate cyclase, methylene blue (Meller et al. 1992). Spinal administration of a guanylate cyclase inhibitor or cGMP-dependent protein kinase (PKG) inhibitor could block the sensitization of STT cells or allodynia and hyperalgesia after intradermal injection of capsaicin (Lin et al. 1997; Sluka and Willis 1997), which is associated with an increased release of EAAs and SP within the spinal cord due to selective activation of nociceptive primary afferent C-fibers (Baumann et al. 1991; Dougherty et al. 1992a, 1994; Gamse et al. 1979; Sorkin and McAdoo 1993). Therefore one of the routes by which the NO-cGMP cascade is triggered to mediate central sensitization of dorsal horn neurons is by activation of EAA and NK1 receptors during noxious stimulation.

On the other hand, we have observed in this study that inhibition of STT cells resulting from activation of spinal IAA receptors was attenuated when the responses of STT cells to mechanical stimuli were enhanced during spinal infusion of 8-bromo-cGMP. This and our other studies showing that administration of a protein kinase C (PKC) activator or NO donor reduced the effectiveness of IAA receptor–mediated inhibition of STT cells (Lin et al. 1996b, 1999b) suggest that spinal...
disinhibition could be responsible in part for central sensitization via certain second-messenger cascades. The duration of this effect on inhibition parallels the changes in responses to mechanical stimuli during spinal infusion of 8-bromo-cGMP. Even iontophoretic release of 8-bromo-cGMP produced a short-lasting attenuation of the inhibition produced by IA agonists. Thus these findings suggest a close association between central sensitization and spinal disinhibition.

PKG can be activated by the NO/cGMP cascade (Garthwaite et al. 1988; Schmidt et al. 1993) and, in turn, exerts its modulatory effects by phosphorylation of cell proteins (Butt et al. 1992; Hartell 1994a; Lincoln and Cornwell 1993). In other neural systems, protein kinases A and C were found to phosphorylate certain subunits of IAA receptors, decreasing inhibitory currents (Leidenheimer et al. 1991, 1992; Rapallino et al. 1993; Ragozzino and Eusebi 1993; Vaello et al. 1994). Recently, PKG-mediated phosphorylation of GABA<sub>A</sub> receptors has been demonstrated (Leidenheimer 1996; McDonald and Moss 1994; Wexler et al. 1998). With the use of whole cell voltage clamp, GABA<sub>A</sub> receptor–elicited currents recorded from dorsal root ganglion cells were decreased by increases in voltage clamp, GABAA receptor–elicited currents recorded by PAG and IAA receptors when either a NO releasing agent or a cGMP analogue was administered. We have discussed in our preceding papers (Lin et al. 1999a,b) possible reasons why the NO/cGMP cascade can produce heterogeneous cellular effects, even though our data cannot provide direct evidence about this. It is hard to assess the differences between effects on deep dorsal horn and superficial dorsal horn neurons without knowing the distance of recorded neurons from the source of SIN-1 or 8-bromo-cGMP when these are administered by microdialysis. To address this problem, one approach used was to apply SIN-1 or 8-bromo-cGMP by iontophoresis, in addition to delivering these agents by microdialysis. The drugs presumably did not spread very far since the iontophoresis currents were applied for only around 2 min at intensities of 70–100 nA. The results showed that drugs administered by iontophoresis produced similar effects to those produced when the drug was given by microdialysis, with the exception that microdialysis administration of drug produced a longer lasting effect. Because NO is a diffusible molecule, acting in a nonsynaptic manner, it can freely diffuse for a considerable distance to reach its target (Baringa 1991). Therefore our results support the view that different neurons may respond differently to NO release.

In conclusion, cGMP modulates the processing of STT neuronal nociceptive transmission in part by influencing the spinal inhibition mediated by glycine and GABA receptors. The modulation varies with the location of the STT cells on which cGMP acts. An increase in cGMP level within the spinal cord consistently sensitizes deep WDR STT neurons, and this sensitization is accompanied by an attenuation of spinal inhibition. Moreover, the current data support the view that cGMP is involved in the production of hyperalgesia and allodynia in the same way as NO, suggesting that NO and cGMP function in the same signal transduction cascade.

The authors thank K. Gondesen, G. Robak, and Drs. Elie Al-Chaer and Yi Feng for technical and collegial assistance in preparation of the experimental animals, and G. Gonzales for expert assistance with the illustrations. This work was supported by National Institute of Neurological Disorders and Stroke Grants NS-09743 and NS-11255. Present address of Y. B. Peng: PNMB/NIDR/NIH, Bldg. 49, Rm. 1WW14, 49 Convent Dr., Bethesda, MD 20892-4410. Address for reprint requests: W. D. Willis, Dept. of Anatomy and Neurosciences, Marine Biomedical Institute, The University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-1069.

Received 6 April 1998; accepted in final form November 1998.

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