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Spinal Nitric Oxide Contributes to the Analgesic Effect of Intrathecal [D-Pen^2,D-Pen^5]-Enkephalin in Normal and Diabetic Rats

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Background: **Spinal nitric oxide (NO) is important for the analgesic actions of morphine and cholinergic agents. Its role in the analgesic effect of δ-opioid receptor agonists is not known. In the present study, the authors determined the role of spinal endogenous NO in the antinociceptive effect of intrathecal [D-Pen^2, D-Pen^5]-enkephalin (DPDPE), a δ-opioid receptor agonist, in normal rats and a rat model of diabetic neuropathic pain.**

Methods: **Rats were rendered diabetic with streptozotocin (50 mg/kg, intraperitoneal). Intrathecal catheters were implanted in age-matched normal and diabetic rats. Nociceptive thresholds were determined by application of a noxious pressure stimulus to the hind paw. The dose-dependent effect of intrathecal DPDPE was first determined. The role of spinal NO in the analgesic effect of intrathecal DPDPE was then examined through intrathecal treatments with NO synthase inhibitors (NMMA and TRIM) and a specific NO scavenger (carboxy-PTIO).**

Results: **The diabetic rats developed a sustained mechanical hyperalgesia within 3 weeks after streptozotocin injection. Intrathecal DPDPE, 2–20 μg, dose-dependently increased the withdrawal threshold in response to the noxious pressure in normal and diabetic rats. However, the ED_{50} of DPDPE in diabetic rats was about twofold higher than that in normal rats. Intrathecal pretreatment with NMMA, TRIM, or carboxy-PTIO diminished the analgesic effect of DPDPE in both normal and diabetic rats. Furthermore, the inhibitory effect of NMMA on the action of intrathecal DPDPE was reversed by intrathecal L-arginine but not D-arginine.**

Conclusions: **Intrathecal DPDPE produces an antinociceptive effect in normal rats and a rat model of diabetic neuropathic pain. Spinal endogenous NO contributes importantly to the analgesic action of intrathecal DPDPE in both normal and diabetic neuropathic pain conditions.**

PAINFUL diabetic neuropathy is a common late complication of diabetes in patients.[1,2] Pain associated with diabetic neuropathy can occur either spontaneously or as a result of exposure to only mildly painful stimuli (hyperalgesia) or to stimuli not normally perceived as painful (allodynia). Diabetic neuropathic pain often is resistant to the classic analgesics, such as morphine.[3,4] Although the analgesic action of systemic and intrathecal morphine is diminished in neuropathic pain in the animal model of diabetes, administration of [D-Pen^2,D-Pen^5]-enkephalin (DPDPE), a δ-opioid receptor agonist, remains largely effective for diabetic neuropathic pain.[5,6] The δ-opioid receptor agonists may possess potential clinical benefits compared with the μ-opioid drugs used for analgesia. These advantages include greater relief of neuropathic pain,[7] reduced respiratory depression and constipation,[8,9] and a minimal potential for development of physical dependence.[10] Thus, the δ-opioid receptor is an attractive target for the development of new drugs to treat neuropathic pain. At the present time, the precise mechanisms of the analgesic action of δ-opioid receptor agonists *in vivo* remain to be established.

Both the functional μ- and δ-opioid receptors are located in the superficial laminae of the spinal cord dorsal horn.[11] Activation of spinal δ-opioid receptors is known to produce antinociception.[12–14] Like μ-opioid receptors, δ-opioid receptors are also coupled to heterotrimeric guanine nucleotide-binding proteins (G proteins) of the G_i and G_o family.[15,16] In the spinal cord dorsal horn, the immunoreactive neuronal nitric oxide (NO) synthase is predominantly present in the superficial laminae.[17,18] Several studies have shown that endogenous NO in the spinal cord is important for the analgesic action of intrathecal cholinergic agents and systemic morphine.[19,20] For example, intrathecal treatment with specific NO synthase inhibitors or NO scavengers attenuates the analgesic effects of intrathecal neostigmine and intravenous morphine.[19,20] Also, down-regulation of neuronal NO synthase 2 selectively blocks morphine analgesia in mice,[21] suggesting that NO is an important second messenger molecule in opioid analgesia. It has been suggested that NO may be involved in the signal transduction system of many G protein–coupled receptors.[22] In a rat model of inflammatory pain, NO can potentiate the analgesic action produced by local application of DPDPE.[23] However, the direct functional evidence is not yet available to support the role of endogenous NO in the spinal analgesic action of DPDPE in normal and neuropathic pain conditions. Therefore, in the present study, we tested a hypothesis that spinal endogenous NO contributes, at least in part, to the analgesic effect of intrathecal DPDPE in normal rats and a rat model of diabetic neuropathic pain.

Materials and Methods

Induction of Diabetes and Implantation of Intrathecal Catheters

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) initially weighing 220–250 g were used in this study.

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The surgical preparations and experimental protocols were approved by the Animal Care and Use Committee of the Penn State University College of Medicine (Hershey, Pennsylvania). Diabetes was induced by a single intraperitoneal injection of 50 mg/kg streptozotocin (Sigma Chemicals, St. Louis, MO) freshly dissolved in 0.9% sterile saline.[24–26] One week later, diabetes was confirmed in streptozotocin-injected rats by measuring plasma glucose concentrations (> 350 mg/dl) in blood samples obtained from the tail vein. The glucose level was assayed enzymatically using the Sigma diagnostic glucose reagents, and the colorimetric absorbance readings were performed using a spectrophotometer (SPECTRAmax Plus; Molecular Devices Co., Sunnyvale, CA). This experimental model of diabetic neuropathic pain has been described as a relevant model of chronic pain with alterations of pain sensitivity and poor response to morphine treatment.[20,24–26]

The age-matched normal and diabetic rats were anesthetized with 2% halothane in oxygen during surgical implantation of intrathecal catheters 2 to 3 weeks after streptozotocin injection. The catheters were inserted through an incision in the cisternal membrane and advanced 8.5 cm caudal so that the tip of each catheter was positioned at the lumbar spinal level. The intrathecal catheters were externalized to the back of the neck and sutured to the musculature and skin at the incision site. After a 5- to 7-day recovery following cannulation, the rats were used for the behavioral testing. All of the final pharmacologic experiments in diabetic rats were conducted between 4 and 6 weeks after streptozotocin injection.

Behavioral Testing of Nociception

Nociceptive mechanical thresholds, expressed in grams, were measured with the Randall-Selitto test using an Ugo Basil Analgesimeter (Varese, Italy).[26,27] The test was performed by applying a noxious pressure to the hind paw. By pressing a pedal that activated a motor, the force increased at a constant rate on a linear scale. When the animal displayed pain by withdrawal of the paw or vocalization, the pedal was immediately released, and the nociceptive pain threshold was read on a scale. The cutoff of 400 g was used to avoid potential tissue injury.[26,27] Both hind paws were tested in each rat, and the mean value was used as the withdrawal threshold in response to the noxious pressure. Motor function was evaluated by testing the animals' ability to stand and ambulate in a normal posture and to place and step with the hind paws.[28] We assessed the motor function by grading the ambulation behavior of rats as the following: 2 = normal; 1 = limping; 0 = paralyzed.

Experimental Protocols

In the first series of studies, we determined the dose–response effect of intrathecal DPDPE on nociception in age-matched normal and diabetic rats. After acclimation, baseline withdrawal thresholds in response to the pressure applied to the hind paw were determined. The animals were then given an intrathecal injection of DPDPE, and the mechanical threshold in response to the pressure stimulus was determined at 15, 30, 45, 60, and 120 min. The analgesic effect of intrathecal DPDPE (2–20 μg) was tested in eight normal and eight diabetic rats. Repeat intrathecal injections in the same animals were separated by at least 3 days. The above intrathecal doses of DPDPE were selected based on our pilot experiments and previous studies in rats.[12,29,30]

In the second series of experiments, we studied the role of spinal NO in the antinociceptive effect of intrathecal DPDPE in another eight normal and eight diabetic rats. Animals first received an intrathecal injection of 30 μg 1-(2-trifluoromethylphenyl) imidazole[31] (TRIM, a selective neuronal NO synthase inhibitor), 30 μg N^G-monomethyl-L-arginine (NMMA, a nonspecific NO synthase inhibitor), 30 μg 2-(4-carboxyphenyl)-4,4,5,5-tetramethyl-imidazoline-l-oxyl-3-oxide potassium[32] (carboxy-PTIO, a specific NO scavenger), or saline (vehicle control), followed in 15 min by intrathecal injection of DPDPE. Different doses (5 μg for normal and 10 μg for diabetic rats) of DPDPE were used in this protocol because these doses of intrathecal DPDPE produced a comparable effect (approximately 40% of maximal effect) in normal and diabetic rats (see Results). The doses of intrathecal NMMA, TRIM, and PTIO are effective to attenuate the analgesic actions of intrathecal clonidine and neostigmine in rats.[20,33] The withdrawal threshold in response to the pressure stimulus was then tested every 15–30 min for 120 min. To examine the influence of spinal endogenous NO on the nociceptive withdrawal threshold, 30 μg TRIM or 30 μg NMMA was injected alone intrathecally in eight normal and six diabetic rats.

To ensure the specificity of the NO synthase inhibitor used, we also determined whether the NO precursor, L-arginine, could reverse the inhibitory effect of NMMA on the analgesic action of intrathecal DPDPE, as we described previously.[33] An additional six age-matched normal and seven diabetic rats received intrathecal injection of 30 μg NMMA plus DPDPE (5 μg for normal and 10 μg for diabetic rats). Fifteen minutes after intrathecal injection of DPDPE and NMMA, 100 μg L-arginine or 100 μg D-arginine was administered intrathecally. The withdrawal response threshold was then tested every 15–30 min for 120 min. Also, the effect of intrathecal injection of 100 μg L-arginine alone on the paw withdrawal threshold was examined in six separate normal and diabetic rats. Drugs for intrathecal injections were dissolved in normal saline and administered in a volume of 5 μl followed by a 10-μl flush with normal saline. D- and L-arginine, DPDPE, carboxy-PTIO, NMMA, and TRIM were all purchased from RBI-Sigma (St. Louis, MO).

Data are presented as mean ± SEM. For calculation of ED_{50}, data were converted to percentage of maximal possible effect (%MPE) based on the following formula:

$$\%MPE = [(response - baseline)/(cutoff - baseline)] \times 100\%.$$

The ED_{50} values of DPDPE and their 95% confidence limits were calculated using GraphPad Prism (GraphPad Software, San Diego, CA). Paw withdrawal thresholds in response to the pressure stimulus before and 3 weeks after streptozotocin injection were compared using a paired Student *t* test. Effects of individual drugs on the paw withdrawal threshold were determined by repeated-measures analysis of variance followed by the Dunnett *post hoc* test. $P < 0.05$ was considered to be statistically significant.

Results

All the diabetic rats displayed polyuria, a reduced growth rate, and a marked increase in food and water intake. The paw withdrawal threshold in response to noxious pressure before streptozotocin treatment was 122.6 ± 2.5 g in all rats used for this study. The mechanical threshold decreased significantly (72.3 ± 2.1 g, $P < 0.05$) 3 weeks after streptozotocin injection and lasted for at least 6 weeks.

In normal rats, intrathecal injection of 2–10 μg DPDPE dose-dependently increased the paw withdrawal threshold (fig. 1, top). Also, intrathecal injection of 5–20 μg DPDPE significantly increased the withdrawal threshold to noxious pressure applied to the hind paw of diabetic animals in a dose-dependent manner (fig. 1, bottom). The analgesic effect of intrathecal DPDPE in both normal and diabetic rats reached maximum within 30 min and gradually returned to baseline within 2 h. However, the effect of DPDPE in diabetic rats decreased notably, with an ED_{50} value increasing about twofold, compared to that in normal rats. The ED_{50}s (95% confidence limits) of DPDPE in normal and diabetic rats were 6.15 (3.95–9.25) and 13.62 (9.14–19.69) μg, respectively. Even normalized for the baseline difference, the ED_{50} (12.28 μg) of DPDPE in diabetic rats was still much higher than that in normal rats. Intrathecal administration of DPDPE, at a dose of up to 20 μg, was not associated with evident motor dysfunction. All rats received a score of 2 after intrathecal injection of 5–20 μg DPDPE. Also, we observed no visible behavioral changes, such as sedation or agitation, in rats receiving the above doses of DPDPE.

In rats pretreated with intrathecal saline, intrathecal injection of 5 μg DPDPE significantly increased the withdrawal threshold, and the effect lasted for about 90 min in normal rats (fig. 2, top). On the other hand, intrathecal pretreatment with 30 μg TRIM, 30 μg NMMA, or 30 μg PTIO largely eliminated the analgesic effect of 5 μg

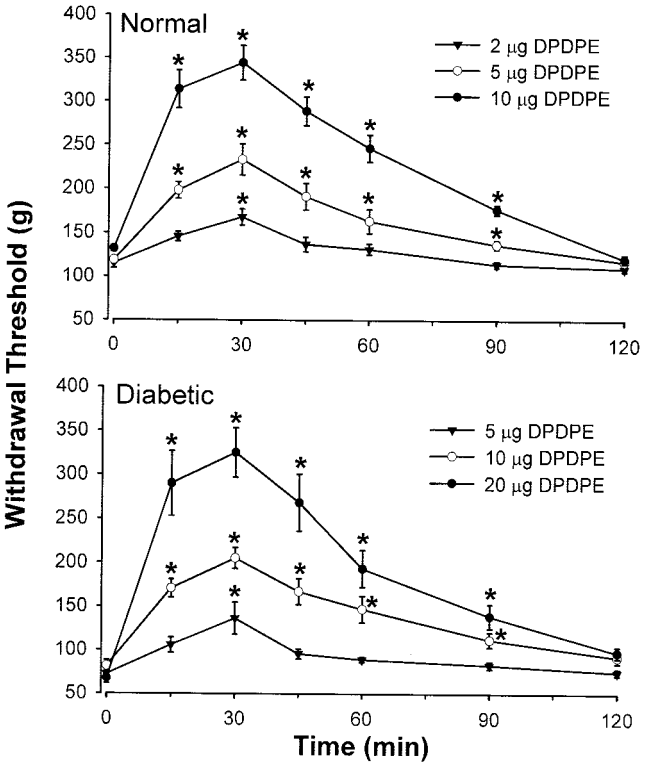

Fig. 1. (*Top*) Dose-dependent effect of 2 (n = 8), 5 (n = 8), and 10 (n = 7) μg intrathecal [D-Pen^2, D-Pen^5]-enkephalin (DPDPE) on the withdrawal response threshold in normal rats. (*Bottom*) Dose-dependent effect of 5 (n = 6), 10 (n = 6), and 20 (n = 8) μg intrathecal DPDPE on the withdrawal response threshold in diabetic rats. Data are presented as mean ± SEM. *$P < 0.05$ *versus* respective baseline control (time 0).

intrathecal DPDPE in normal rats (fig. 2, top). In diabetic rats, intrathecal injection of 10 μg DPDPE significantly increased the withdrawal threshold, and such effect was comparable to that seen in normal rats following 5 μg intrathecal DPDPE (fig. 2). Intrathecal pretreatment with 30 μg TRIM, 30 μg NMMA, or 30 μg PTIO also diminished the antinociceptive effect of 10 μg intrathecal DPDPE in diabetic rats (fig. 2, bottom). Neither 30 μg NMMA nor 30 μg TRIM injected alone significantly affected the baseline withdrawal threshold in both normal and diabetic rats (data not shown). There were no visible behavioral effects caused by intrathecal administration of NMMA or TRIM.

In animals intrathecally treated with 30 μg NMMA plus DPDPE (5 μg for normal and10 μg for diabetic rats), subsequent intrathecal administration of 100 μg L-arginine reversed the inhibitory effect of NMMA on the antinociceptive effect of DPDPE in six normal and seven diabetic rats (fig. 3). By contrast, 100 μg D-arginine did not alter significantly the inhibitory effect of NMMA on the effect of DPDPE in both normal and diabetic rats (fig. 3). Intrathecal L-arginine, 100 μg, alone had no effect on the baseline withdrawal threshold in normal and diabetic rats (data not shown). We did not observe any

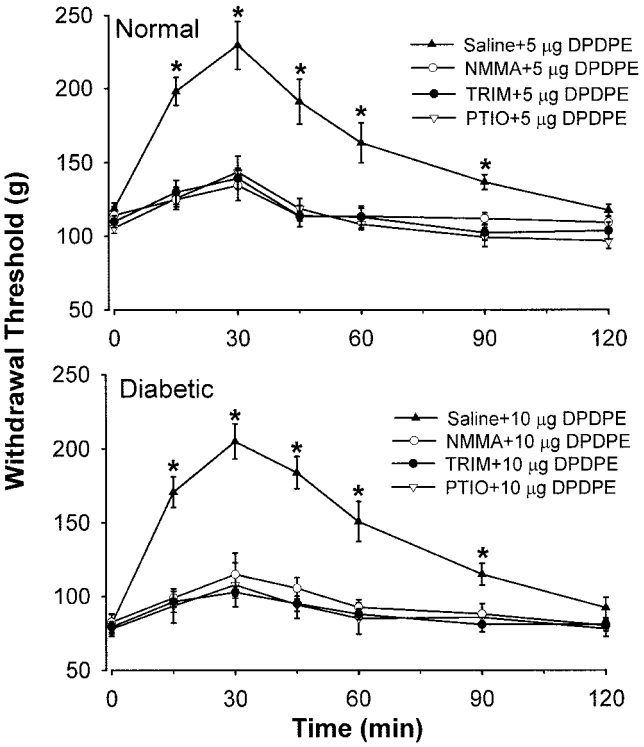

Fig. 2. (*Top*) Inhibition of the analgesic effect of 5 μg intrathecal [D-Pen^2, D-Pen^5]-enkephalin (DPDPE) by intrathecal pretreatment with saline (n = 7), 30 μg NMMA (n = 8), 30 μg TRIM (n = 6), or 30 μg carboxy-PTIO (n = 8) in normal rats. (*Bottom*) Attenuation of the effect of 10 μg intrathecal DPDPE by intrathecal pretreatment with saline (n = 6), 30 μg NMMA (n = 7), 30 μg TRIM (n = 8), or 30 μg carboxy-PTIO (n = 7) in diabetic rats. Data are presented as mean ± SEM. *$P < 0.05$ *versus* baseline control in saline-treated group (time 0).

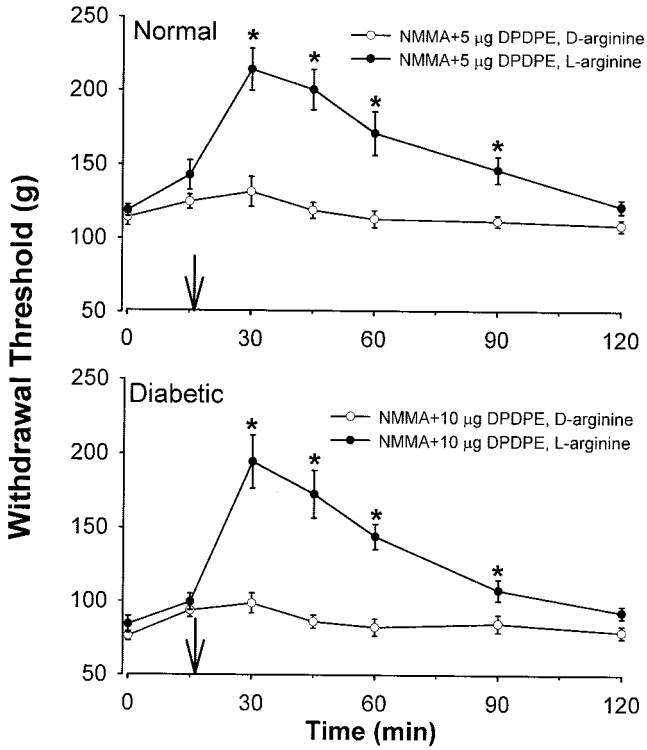

Fig. 3. (*Top*) Effect of L- and D-arginine on the inhibitory action of 30 μg NMMA on the analgesic effect of 5 μg intrathecal [D-Pen^2, D-Pen^5]-enkephalin (DPDPE) in six normal rats. (*Bottom*) Effect of L- and D-arginine on the inhibitory action of 30 μg NMMA on the analgesic effect of 10 μg intrathecal DPDPE in seven diabetic rats. In both groups, rats were pretreated with NMMA plus DPDPE. L-arginine (100 μg) or D-arginine (100 μg) was given intrathecally at time indicated by the arrow. Data are presented as mean ± SEM. *$P < 0.05$ compared to the withdrawal threshold measured before L-arginine treatment (time 0).

behavioral changes following intrathecal L- or D-arginine injections in all the rats tested.

Discussion

In the present study, we compared the antinociceptive effect of intrathecal administration of the δ-opioid receptor agonist, DPDPE, in normal rats and a rat model of diabetic neuropathic pain. The role of spinal NO in the analgesic effect of intrathecal DPDPE was also determined in normal and diabetic rats. We found that intrathecal DPDPE produced a profound antinociceptive effect in a dose-dependent manner in both normal and diabetic rats. However, the ED_{50} of DPDPE in diabetic rats was about twofold higher than that in normal rats. Furthermore, we demonstrated that pretreatment with two NO synthase inhibitors or a specific NO scavenger significantly attenuated the analgesic action of intrathecal DPDPE in both normal and diabetic rats. In addition, the inhibitory effect of NMMA on the analgesic effect of intrathecal DPDPE was effectively reversed by L-arginine but not D-arginine. Thus, our data suggest that intrathecal DPDPE produces an antinociceptive effect in normal and diabetic rats. Importantly, this study provides new information that endogenous NO in the spinal cord mediates the analgesic action of intrathecal DPDPE in both normal and diabetic rats.

Many animal and clinical studies have shown a diminished analgesic action of spinally administered morphine in diabetic neuropathic pain.[4,6,24,26] We recently have found that the inhibitory effect of morphine on spinal dorsal horn neurons is substantially attenuated in diabetic rats.[27] These studies suggest that the number of μ-opioid receptors or their signal transduction system may be altered in the spinal dorsal horn in diabetes. In diabetic as compared to normal animals, it also has been shown that morphine analgesia is reduced while the effect of DPDPE remains largely unchanged.[5] The functional δ-opioid receptors are located predominantly in the superficial dorsal horn.[11] Activation of spinal δ-opioid receptors can inhibit nociception at the level of the spinal cord in normal rats and mice.[12–14] The binding affinity of DPDPE for the δ-opioid receptor is 175 times greater than that for the μ-opioid receptor.[34] Previous studies also have shown that intrathecal DPDPE produces an effect on allodynia induced by ligation of the

sciatic nerve and spinal cord injury in rats.[29,35] Thus, spinally administered δ-opioid agonists may represent an alternative treatment for chronic pain in patients with diabetic neuropathy. In the present study, we demonstrated that intrathecal DPDPE produced an antihyperalgesic effect at a low dose and a profound analgesic effect at higher doses in this rat model of diabetic neuropathic pain. Although the analgesic action of intrathecal DPDPE has been demonstrated in normal animals,[12,29,30] the analgesic effect of intrathecal DPDPE in normal rats and rat models of neuropathic pain has not been compared directly using the same testing stimulus. Using the paw withdrawal threshold in response to the noxious pressure, we observed that the antinociceptive effect of intrathecal DPDPE appears to be reduced with an ED_{50} value increasing about twofold in diabetic rats. The reasons for the reduced effect of intrathecal DPDPE in diabetic rats are not clear. It is important to note that the "knockout" studies in mice have shown that the presence of μ-opioid receptors is important for antinociceptive actions of μ- as well as δ-opioid agonists.[36,37] Furthermore, some recent studies suggest that the antinociceptive effect of DPDPE may be produced through a direct or indirect interaction with μ-opioid receptors.[38,39] Using agonist-stimulated [^{35}S]GTPγS receptor autoradiography, we recently have shown that the functional μ- but not the δ-opioid receptors are significantly reduced in the spinal cord dorsal horn in diabetic rats.[40] Thus, the attenuated effect of intrathecal DPDPE in the diabetic group likely is due to the reduction in functional μ-opioid receptors in the spinal cord of diabetic rats.

The neuronal NO synthase is present in the dorsal horn of the spinal cord, especially in lamina I–III.[17,18] Recent studies have shown that spinal endogenous NO is an important mediator for the analgesic action of intravenous morphine and intrathecal neostigmine or clonidine.[20,33] The functional link between the analgesic action of δ-opioid receptors and NO has been implicated in a previous study. In a rat model of inflammatory pain caused by formalin injection, local application of an NO-releasing agent potentiates the analgesic action of topically applied DPDPE.[23] No functional evidence has been presented to support the role of spinal NO in the analgesic action of intrathecal DPDPE. In the present study, we found that pretreatment with a nonspecific NO synthase inhibitor (NMMA), a neuronal NO synthase inhibitor (TRIM), or a specific NO scavenger (carboxy-PTIO) consistently attenuated the analgesic effect of intrathecal DPDPE in both normal and diabetic rats. Furthermore, we demonstrated that intrathecal L-arginine but not D-arginine completely reversed the inhibitory effect of NMMA on the analgesic action of intrathecal DPDPE. Therefore, the present study provides strong evidence that spinal endogenous NO is essential for the analgesic effect of intrathecal DPDPE in normal rats and this rat model of diabetic neuropathic pain. Our previous studies have shown that NO in the spinal cord contributes to the analgesic action of clonidine, morphine, and neostigmine.[19,20,33] Since the actions of these agents are all mediated through G protein–coupled receptors (*i.e.*, α_2-adrenergic, μ-opioid, and muscarinic receptors), it suggests that NO may play an obligatory role in the analgesic action of activation of G protein–coupled receptors in the spinal cord. Data from the current study provide further evidence to support the view that NO is involved in the analgesic action produced by activation of receptors coupled to G proteins in the spinal cord.

We observed that intrathecal NMMA, TRIM, and L-arginine alone did not affect the hyperalgesic condition in the rat model of diabetic neuropathic pain. This observation is consistent with findings from previous studies,[20,33] which suggest that endogenous NO in the spinal cord is not responsible for the maintenance of neuropathic pain. It should be acknowledged that there is some evidence suggesting that spinal NO may be important in nociception. In this regard, epidural injection of L-arginine but not D-arginine produces a slowly developing thermal hyperalgesia in rats.[41] On the other hand, intrathecal administration of NO synthase inhibitors attenuates hyperalgesia and allodynia caused by inflammation in rats.[42] Also, spinal NO may play a role in early development of allodynia and hyperalgesia induced by nerve injury. It has been shown that pretreatment but not posttreatment with intrathecal NO inhibitors delays the development of thermal hyperalgesia induced by sciatic nerve constriction in rats.[43] This suggests that spinal NO may contribute to the early development of hyperalgesia. However, other studies have shown that NO in the spinal cord may play a role in antinociception. For example, the NO synthase is colocalized with inhibitory γ-aminobutyric acid–containing neurons in the spinal cord,[18] suggesting a possible antinociceptive action of NO. Furthermore, application of NO synthase inhibitors increases the background discharge activity of dorsal horn neurons.[44] At the present time, it is difficult to reconcile the different roles of spinal NO in nociception caused by inflammation and nerve injury and in antinociceptive actions produced by intrathecal DPDPE in diabetic rats. Different NO species formed in the spinal cord may be involved in the opposing actions of NO mentioned in the above studies. In this regard, we have shown that spinal NO interacts with L-cysteine to form S-nitrosothiols to produce an antiallodynic effect in a rat model of neuropathic pain.[45] Further studies on the interaction between various NO species and G protein–coupled receptors in the spinal cord should help to clarify this puzzling issue.

In summary, we found that intrathecal DPDPE produced a profound antinociceptive effect in normal rats and a rat model of diabetic neuropathic pain. However, the analgesic action of intrathecal DPDPE was reduced with an ED_{50} value increasing about twofold in diabetic

rats. Furthermore, removal of endogenous NO in the spinal cord diminished the analgesic action of intrathecal DPDPE in normal and diabetic rats. Data from the present study provide strong evidence that the spinal endogenous NO plays an important role in the analgesic effect produced by activation of spinal δ-opioid receptors in normal and diabetic neuropathic pain conditions.

References

1. Boulton AJ, Malik RA: Diabetic neuropathy. Med Clin North Am 1998; 82:909–29
2. Clark CM Jr, Lee DA: Prevention and treatment of the complications of diabetes mellitus. N Engl J Med 1995; 332:1210–7
3. Wright JM: Review of the symptomatic treatment of diabetic neuropathy. Pharmacotherapy 1994; 14:689–97
4. Arner S, Meyerson BA: Lack of analgesic effect of opioids on neuropathic and idiopathic forms of pain. Pain 1988; 33:11–23
5. Kamei J, Ohhashi Y, Aoki T, Kawasima N, Kasuya Y: Streptozotocin-induced diabetes selectively alters the potency of analgesia produced by mu-opioid agonists, but not by delta- and kappa-opioid agonists. Brain Res 1992; 571:199–203
6. Zurek JR, Nadeson R, Goodchild CS: Spinal and supraspinal components of opioid antinociception in streptozotocin induced diabetic neuropathy in rats. Pain 2001; 90:57–63
7. Dickenson AH: Plasticity: Implications for opioid and other pharmacological interventions in specific pain states. Behav Brain Sci 1997; 20:392–403
8. Cheng PY, Wu D, Decena J, Soong Y, McCabe S, Szeto HH: Opioid-induced stimulation of fetal respiratory activity by [D- Ala2]deltorphin I. Eur J Pharmacol 1993; 230:85–8
9. Sheldon RJ, Riviere PJ, Malarchik ME, Moseberg HI, Burks TF, Porreca F: Opioid regulation of mucosal ion transport in the mouse isolated jejunum. J Pharmacol Exp Ther 1990; 253:144–51
10. Cowan A, Zhu XZ, Mosberg HI, Omnaas JR, Porreca F: Direct dependence studies in rats with agents selective for different types of opioid receptor. J Pharmacol Exp Ther 1988; 246:950–5
11. Maher CE, Eisenach JC, Pan HL, Xiao R, Childers SR: Chronic intrathecal morphine administration produces homologous mu receptor/G-protein desensitization specifically in spinal cord. Brain Res 2001; 895:1–8
12. Drower EJ, Stapelfeld A, Rafferty MF, de Costa BR, Rice KC, Hammond DL: Selective antagonism by naltrindole of the antinociceptive effects of the delta opioid agonist cyclic[D-penicillamine2-D-penicillamine5]enkephalin in the rat. J Pharmacol Exp Ther 1991; 259:725–31
13. Dickenson AH, Sullivan AF, Knox R, Zajac JM, Roques BP: Opioid receptor subtypes in the rat spinal cord: electrophysiological studies with mu- and delta-opioid receptor agonists in the control of nociception. Brain Res 1987; 413:36–44
14. Heyman JS, Mulvaney SA, Mosberg HI, Porreca F: Opioid delta-receptor involvement in supraspinal and spinal antinociception in mice. Brain Res 1987; 420:100–8
15. Laugwitz KL, Offermanns S, Spicher K, Schultz G: mu and delta opioid receptors differentially couple to G protein subtypes in membranes of human neuroblastoma SH-SY5Y cells. Neuron 1993; 10:233–42
16. Offermanns S, Schultz G, Rosenthal W: Evidence for opioid receptor-mediated activation of the G-proteins, Go and Gi2, in membranes of neuroblastoma x glioma (NG108-15) hybrid cells. J Biol Chem 1991; 266:3365–8
17. Reuss MH, Reuss S: Nitric oxide synthase neurons in the rodent spinal cord: distribution, relation to substance P fibers, and effects of dorsal rhizotomy. J Chem Neuroanat 2001; 21:181–96
18. Valtschanoff JG, Weinberg RJ, Rustioni A, Schmidt HH: Nitric oxide synthase and GABA colocalize in lamina II of rat spinal cord. Neurosci Lett 1992; 148:6–10
19. Song HK, Pan HL, Eisenach JC: Spinal nitric oxide mediates antinociception from intravenous morphine. ANESTHESIOLOGY 1998; 89:215–21
20. Chen SR, Khan GM, Pan HL: Antiallodynic effect of intrathecal neostigmine is mediated by spinal nitric oxide in a rat model of diabetic neuropathic pain. ANESTHESIOLOGY 2001; 95:1007–12
21. Kolesnikov YA, Pan YX, Babey AM, Jain S, Wilson R, Pasternak GW: Functionally differentiating two neuronal nitric oxide synthase isoforms through antisense mapping: evidence for opposing NO actions on morphine analgesia and tolerance. Proc Natl Acad Sci U S A 1997; 94:8220–5
22. Christopoulos A, El-Fakahany EE: The generation of nitric oxide by G protein-coupled receptors. Life Sci 1999; 64:1–15
23. Nozaki-Taguchi N, Yamamoto T: Involvement of nitric oxide in peripheral antinociception mediated by kappa- and delta-opioid receptors. Anesth Analg 1998; 87:388–93
24. Calcutt NA, Chaplan SR: Spinal pharmacology of tactile allodynia in diabetic rats. Br J Pharmacol 1997; 122:1478–82
25. Courteix C, Eschalier A, Lavarenne J: Streptozocin-induced diabetic rats: behavioural evidence for a model of chronic pain. Pain 1993; 53:81–8
26. Courteix C, Bardin M, Chantelauze C, Lavarenne J, Eschalier A: Study of the sensitivity of the diabetes-induced pain model in rats to a range of analgesics. Pain 1994; 57:153–60
27. Chen SR, Pan HL: Hypersensitivity of spinothalamic tract neurons associated with diabetic neuropathic pain in rats. J Neurophysiol 2002; 87:2726–733
28. Chen SR, Eisenach JC, McCaslin PP, Pan HL: Synergistic effect between intrathecal non-NMDA antagonist and gabapentin on allodynia induced by spinal nerve ligation in rats. ANESTHESIOLOGY 2000; 92:500–6
29. Hao JX, Yu W, Wiesenfeld-Hallin Z, Xu XJ: Treatment of chronic allodynia in spinally injured rats: Effects of intrathecal selective opioid receptor agonists. Pain 1998; 75:209–17
30. Malmberg AB, Yaksh TL: Isobolographic and dose-response analyses of the interaction between intrathecal mu and delta agonists: effects of naltrindole and its benzofuran analog (NTB). J Pharmacol Exp Ther 1992; 263:264–75
31. Handy RL, Harb HL, Wallace P, Gaffen Z, Whitehead KJ, Moore PK: Inhibition of nitric oxide synthase by 1-(2-trifluoromethylphenyl) imidazole (TRIM) in vitro: Antinociceptive and cardiovascular effects. Br J Pharmacol 1996; 119:423–31
32. Kurihara T, Yoshioka K: The excitatory and inhibitory modulation of primary afferent fibre- evoked responses of ventral roots in the neonatal rat spinal cord exerted by nitric oxide. Br J Pharmacol 1996; 118:1743–53
33. Pan HL, Chen SR, Eisenach JC: Role of spinal NO in antiallodynic effect of intrathecal clonidine in neuropathic rats. ANESTHESIOLOGY1998; 89:1518–23
34. Mosberg HI, Hurst R, Hruby VJ, Gee K, Yamamura HI, Galligan JJ, Burks TF: Bis-penicillamine enkephalins possess highly improved specificity toward delta opioid receptors. Proc Natl Acad Sci U S A 1983; 80:5871–4
35. Mika J, Przewlocki R, Przewlocka B: The role of delta-opioid receptor subtypes in neuropathic pain. Eur J Pharmacol 2001; 415:31–7
36. Matthes HW, Maldonado R, Simonin F, Valverde O, Slowe S, Kitchen I, Befort K, Dierich A, Le Meur M, Dolle P, Tzavara E, Hanoune J, Roques BP, Kieffer BL: Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. Nature 1996; 383:819–23
37. Sora I, Funada M, Uhl GR: The mu-opioid receptor is necessary for [D-Pen2,D-Pen5]enkephalin-induced analgesia. Eur J Pharmacol 1997; 324:R1–2
38. Fraser GL, Pradhan AA, Clarke PB, Wahlestedt C: Supraspinal antinociceptive response to [D-Pen(2,5)]-enkephalin (DPDPE) is pharmacologically distinct from that to other delta-agonists in the rat. J Pharmacol Exp Ther 2000; 295:1135–41
39. He L, Lee NM: Delta opioid receptor enhancement of mu opioid receptor-induced antinociception in spinal cord. J Pharmacol Exp Ther 1998; 285:1181–6
40. Chen SR, Sweigart KL, Lakoski JM, Pan HL: Functional mu opioid receptors are reduced in the spinal cord dorsal horn of diabetic rats. ANESTHESIOLOGY 2002; 97:1602–8
41. Masue T, Dohi S, Asano T, Shimonaka H: Spinal antinociceptive effect of epidural nonsteroidal antiinflammatory drugs on nitric oxide-induced hyperalgesia in rats. ANESTHESIOLOGY 1999; 91:198–206
42. Meller ST, Cummings CP, Traub RJ, Gebhart GF: The role of nitric oxide in the development and maintenance of the hyperalgesia produced by intraplantar injection of carrageenan in the rat. Neuroscience 1994; 60:367–74
43. Yamamoto T, Shimoyama N: Role of nitric oxide in the development of thermal hyperesthesia induced by sciatic nerve constriction injury in the rat. ANESTHESIOLOGY 1995; 82:1266–73
44. Hoheisel U, Unger T, Mense S: A block of spinal nitric oxide synthesis leads to increased background activity predominantly in nociceptive dorsal horn neurones in the rat. Pain 2000; 88:249–57
45. Chen SR, Eisenach JC, Pan HL: Intrathecal S-nitroso-N-acetylpenicillamine and L-cysteine attenuate nerve injury-induced allodynia through noradrenergic activation in rats. Neuroscience 2000; 101:759–65