

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

Shao-Rui Chen, M.D.,\* Hui-Lin Pan, M.D., Ph.D.†

**Background:** Spinal nitric oxide (NO) is important for the analgesic actions of morphine and cholinergic agents. Its role in the analgesic effect of  $\delta$ -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<sup>2</sup>, D-Pen<sup>5</sup>]-enkephalin (DPDPE), a  $\delta$ -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  $\mu$ g, dose-dependently increased the withdrawal threshold in response to the noxious pressure in normal and diabetic rats. However, the ED<sub>50</sub> 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.<sup>1,2</sup> 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.<sup>3,4</sup> Although the analgesic action of systemic and intrathecal morphine is diminished in neuropathic pain in the animal model of diabetes, administration of [D-Pen<sup>2</sup>,D-Pen<sup>5</sup>]-enkephalin (DPDPE), a  $\delta$ -opioid receptor agonist,

remains largely effective for diabetic neuropathic pain.<sup>5,6</sup> The  $\delta$ -opioid receptor agonists may possess potential clinical benefits compared with the  $\mu$ -opioid drugs used for analgesia. These advantages include greater relief of neuropathic pain,<sup>7</sup> reduced respiratory depression and constipation,<sup>8,9</sup> and a minimal potential for development of physical dependence.<sup>10</sup> Thus, the  $\delta$ -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  $\delta$ -opioid receptor agonists *in vivo* remain to be established.

Both the functional  $\mu$ - and  $\delta$ -opioid receptors are located in the superficial laminae of the spinal cord dorsal horn.<sup>11</sup> Activation of spinal  $\delta$ -opioid receptors is known to produce antinociception.<sup>12–14</sup> Like  $\mu$ -opioid receptors,  $\delta$ -opioid receptors are also coupled to heterotrimeric guanine nucleotide-binding proteins (G proteins) of the G<sub>i</sub> and G<sub>o</sub> family.<sup>15,16</sup> In the spinal cord dorsal horn, the immunoreactive neuronal nitric oxide (NO) synthase is predominantly present in the superficial laminae.<sup>17,18</sup> Several studies have shown that endogenous NO in the spinal cord is important for the analgesic action of intrathecal cholinergic agents and systemic morphine.<sup>19,20</sup> For example, intrathecal treatment with specific NO synthase inhibitors or NO scavengers attenuates the analgesic effects of intrathecal neostigmine and intravenous morphine.<sup>19,20</sup> Also, down-regulation of neuronal NO synthase 2 selectively blocks morphine analgesia in mice,<sup>21</sup> 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.<sup>22</sup> In a rat model of inflammatory pain, NO can potentiate the analgesic action produced by local application of DPDPE.<sup>23</sup> 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.

\* Research Associate, Department of Anesthesiology, † Associate Professor and Director of Basic Research in Anesthesiology, Departments of Anesthesiology and Neuroscience and Anatomy.

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Address reprint requests to Dr. Pan: Department of Anesthesiology, H187, Penn State University College of Medicine, 500 University Drive, Hershey, Pennsylvania 17033-0850. Address electronic mail to: hpan@psu.edu. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

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.<sup>24-26</sup> 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.<sup>20,24-26</sup>

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).<sup>26,27</sup> 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.<sup>26,27</sup> 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.<sup>28</sup> 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  $\mu$ g) 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.<sup>12,29,30</sup>

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  $\mu$ g 1-(2-trifluoromethylphenyl) imidazole<sup>31</sup> (TRIM, a selective neuronal NO synthase inhibitor), 30  $\mu$ g *N*<sup>G</sup>-monomethyl-L-arginine (NMMA, a nonspecific NO synthase inhibitor), 30  $\mu$ g 2-(4-carboxyphenyl)-4,4,5,5-tetramethyl-imidazoline-l-oxyl-3-oxide potassium<sup>32</sup> (carboxy-PTIO, a specific NO scavenger), or saline (vehicle control), followed in 15 min by intrathecal injection of DPDPE. Different doses (5  $\mu$ g for normal and 10  $\mu$ g 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.<sup>20,33</sup> 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  $\mu$ g TRIM or 30  $\mu$ g 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.<sup>33</sup> An additional six age-matched normal and seven diabetic rats received intrathecal injection of 30  $\mu$ g NMMA plus DPDPE (5  $\mu$ g for normal and 10  $\mu$ g for diabetic rats). Fifteen minutes after intrathecal injection of DPDPE and NMMA, 100  $\mu$ g L-arginine or 100  $\mu$ g 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  $\mu$ g 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  $\mu$ l followed by a 10- $\mu$ l 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  $\pm$  SEM. For calculation of ED<sub>50</sub>, data were converted to percentage of maximal possible effect (%MPE) based on the following formula:

$$\%MPE = \frac{(\text{response} - \text{baseline})}{(\text{cutoff} - \text{baseline})} \times 100\%$$

The ED<sub>50</sub> 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  $122.6 \pm 2.5$  g in all rats used for this study. The mechanical threshold decreased significantly ( $72.3 \pm 2.1$  g, *P* < 0.05) 3 weeks after streptozotocin injection and lasted for at least 6 weeks.

In normal rats, intrathecal injection of 2–10  $\mu$ g DPDPE dose-dependently increased the paw withdrawal threshold (fig. 1, top). Also, intrathecal injection of 5–20  $\mu$ g 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<sub>50</sub> value increasing about twofold, compared to that in normal rats. The ED<sub>50</sub>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)  $\mu$ g, respectively. Even normalized for the baseline difference, the ED<sub>50</sub> (12.28  $\mu$ g) 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  $\mu$ g, was not associated with evident motor dysfunction. All rats received a score of 2 after intrathecal injection of 5–20  $\mu$ g 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  $\mu$ g 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  $\mu$ g TRIM, 30  $\mu$ g NMMA, or 30  $\mu$ g PTIO largely eliminated the analgesic effect of 5  $\mu$ g

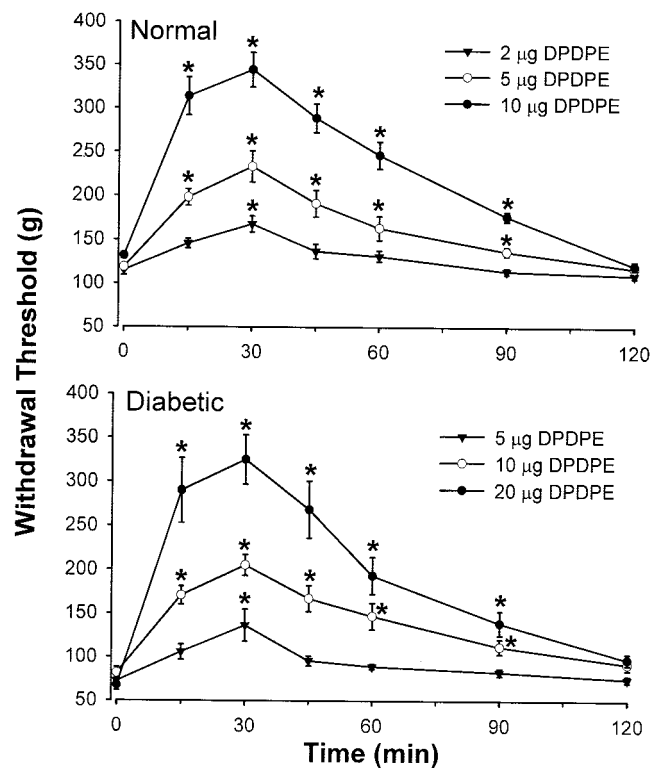


Fig. 1. (Top) Dose-dependent effect of 2 (*n* = 8), 5 (*n* = 8), and 10 (*n* = 7)  $\mu$ g intrathecal [D-Pen<sup>2</sup>, D-Pen<sup>5</sup>]-enkephalin (DPDPE) on the withdrawal response threshold in normal rats. (Bottom) Dose-dependent effect of 5 (*n* = 6), 10 (*n* = 6), and 20 (*n* = 8)  $\mu$ g intrathecal DPDPE on the withdrawal response threshold in diabetic rats. Data are presented as mean  $\pm$  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  $\mu$ g DPDPE significantly increased the withdrawal threshold, and such effect was comparable to that seen in normal rats following 5  $\mu$ g intrathecal DPDPE (fig. 2). Intrathecal pretreatment with 30  $\mu$ g TRIM, 30  $\mu$ g NMMA, or 30  $\mu$ g PTIO also diminished the antinociceptive effect of 10  $\mu$ g intrathecal DPDPE in diabetic rats (fig. 2, bottom). Neither 30  $\mu$ g NMMA nor 30  $\mu$ g 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  $\mu$ g NMMA plus DPDPE (5  $\mu$ g for normal and 10  $\mu$ g for diabetic rats), subsequent intrathecal administration of 100  $\mu$ g *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  $\mu$ g *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  $\mu$ g, alone had no effect on the baseline withdrawal threshold in normal and diabetic rats (data not shown). We did not observe any

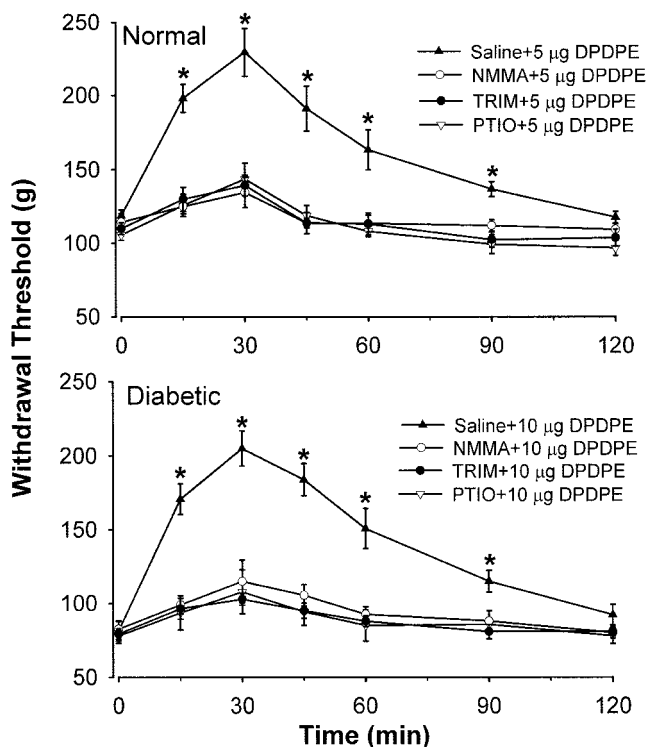


Fig. 2. (Top) Inhibition of the analgesic effect of 5  $\mu$ g intrathecal [D-Pen<sup>2</sup>, D-Pen<sup>5</sup>]-enkephalin (DPDPE) by intrathecal pretreatment with saline (n = 7), 30  $\mu$ g NMMA (n = 8), 30  $\mu$ g TRIM (n = 6), or 30  $\mu$ g carboxy-PTIO (n = 8) in normal rats. (Bottom) Attenuation of the effect of 10  $\mu$ g intrathecal DPDPE by intrathecal pretreatment with saline (n = 6), 30  $\mu$ g NMMA (n = 7), 30  $\mu$ g TRIM (n = 8), or 30  $\mu$ g carboxy-PTIO (n = 7) in diabetic rats. Data are presented as mean  $\pm$  SEM. \**P* < 0.05 versus baseline control in saline-treated group (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  $\delta$ -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<sub>50</sub> 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

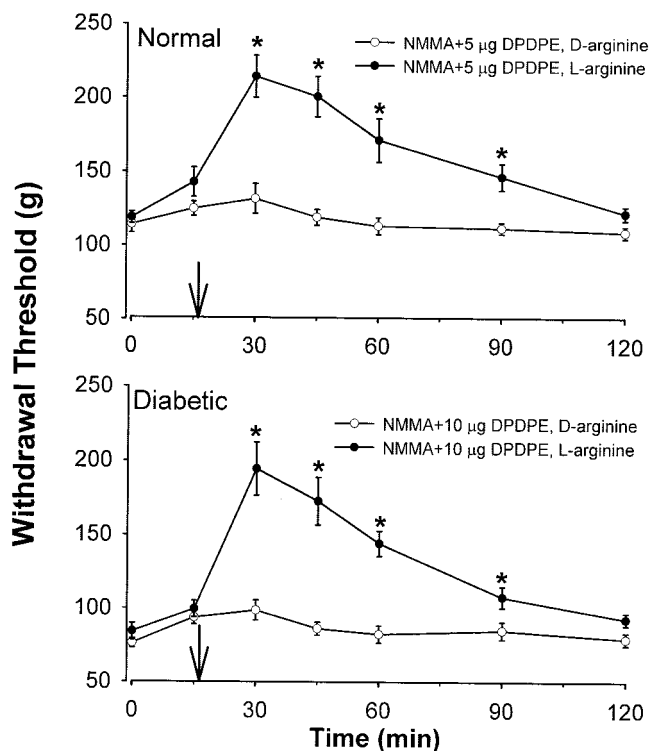


Fig. 3. (Top) Effect of L- and D-arginine on the inhibitory action of 30  $\mu$ g NMMA on the analgesic effect of 5  $\mu$ g intrathecal [D-Pen<sup>2</sup>, D-Pen<sup>5</sup>]-enkephalin (DPDPE) in six normal rats. (Bottom) Effect of L- and D-arginine on the inhibitory action of 30  $\mu$ g NMMA on the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

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.<sup>4,6,24,26</sup> We recently have found that the inhibitory effect of morphine on spinal dorsal horn neurons is substantially attenuated in diabetic rats.<sup>27</sup> These studies suggest that the number of  $\mu$ -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.<sup>5</sup> The functional  $\delta$ -opioid receptors are located predominantly in the superficial dorsal horn.<sup>11</sup> Activation of spinal  $\delta$ -opioid receptors can inhibit nociception at the level of the spinal cord in normal rats and mice.<sup>12-14</sup> The binding affinity of DPDPE for the  $\delta$ -opioid receptor is 175 times greater than that for the  $\mu$ -opioid receptor.<sup>34</sup> 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.<sup>29,35</sup> Thus, spinally administered  $\delta$ -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,<sup>12,29,30</sup> 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<sub>50</sub> 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  $\mu$ -opioid receptors is important for antinociceptive actions of  $\mu$ - as well as  $\delta$ -opioid agonists.<sup>36,37</sup> Furthermore, some recent studies suggest that the antinociceptive effect of DPDPE may be produced through a direct or indirect interaction with  $\mu$ -opioid receptors.<sup>38,39</sup> Using agonist-stimulated [<sup>35</sup>S]GTP $\gamma$ S receptor autoradiography, we recently have shown that the functional  $\mu$ - but not the  $\delta$ -opioid receptors are significantly reduced in the spinal cord dorsal horn in diabetic rats.<sup>40</sup> Thus, the attenuated effect of intrathecal DPDPE in the diabetic group likely is due to the reduction in functional  $\mu$ -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.<sup>17,18</sup> Recent studies have shown that spinal endogenous NO is an important mediator for the analgesic action of intravenous morphine and intrathecal neostigmine or clonidine.<sup>20,33</sup> The functional link between the analgesic action of  $\delta$ -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.<sup>23</sup> 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.<sup>19,20,33</sup> Since the actions of these agents are all mediated through G protein-coupled receptors (*i.e.*,  $\alpha_2$ -adrenergic,  $\mu$ -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,<sup>20,33</sup> 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.<sup>41</sup> On the other hand, intrathecal administration of NO synthase inhibitors attenuates hyperalgesia and allodynia caused by inflammation in rats.<sup>42</sup> 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.<sup>43</sup> 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  $\gamma$ -aminobutyric acid-containing neurons in the spinal cord,<sup>18</sup> suggesting a possible antinociceptive action of NO. Furthermore, application of NO synthase inhibitors increases the background discharge activity of dorsal horn neurons.<sup>44</sup> 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.<sup>45</sup> 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<sub>50</sub> 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  $\delta$ -opioid receptors in normal and diabetic neuropathic pain conditions.

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