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Spinal Antinociceptive Effect of Epidural Nonsteroidal Antiinflammatory Drugs on Nitric Oxide–induced Hyperalgesia in Rats

Tatsuhiko Masue, M.D., Shuji Dohi, M.D.,† Toshio Asano, M.D.,‡ Hiroyuki Shimonaka, M.D.§*

Background: **Nonsteroidal antiinflammatory drugs (NSAIDs) suppress various hyperalgesia perhaps *via* inhibition of cyclooxygenase activity at the spinal cord. The present study aimed to examine whether epidural application of NSAIDs affects hyperalgesia induced by nitric oxide.**

Methods: **The authors studied the antinociceptive effects of epidurally administered NSAIDs in rats with a chronically indwelling epidural catheter by three hyperalgesic models, including nitric oxide-induced hyperalgesia by nitroglycerin (10 μg) or l-arginine (100 μg), and the biphasic response in the formalin test.**

Results: **Epidural, but not systemic, nitroglycerin induced hyperalgesia that was completely blocked by methylene blue but not by N^{ω}-nitro-L-arginine methyl ester (L-NAME). Epidural l-arginine, but not d-arginine, also induced hyperalgesia that was completely blocked by L-NAME. Epidural S(+)ibuprofen (100–1,000 μg) suppressed the nitroglycerin– and l-arginine–induced thermal hyperalgesia and also the second phase response in the formalin test. Neither systemic S(+)ibuprofen nor epidural R(-)ibuprofen suppressed the hyperalgesia. Epidural indomethacin (10–100 μg) or diclofenac (10–1,000 μg) dose-dependently suppressed nitroglycerin-induced thermal hyperalgesia. The order of potency for this suppression (ID_{50} in μg) was indomethacin ≒ diclofenac > S(+)ibuprofen ≫ R(−)ibuprofen.**

Conclusions: **The antinociceptive action of epidurally administered NSAIDs could be the result of suppression of spinal sensitization, perhaps induced with nitric oxide in the spinal cord. The ID_{50} values for epidural indomethacin, diclofenac, and S(+)ibuprofen were about 10 times higher than those reported in other studies for intrathecal NSAIDs in hyperalgesia models. (Key words: Cyclooxygenase inhibitors; NO donor; NO precursor; optical isomers; neuroplasticity.)**

NONSTEROIDAL antiinflammatory drugs (NSAIDs) have long been known to exert potent antiinflammatory, analgesic, and antipyretic actions at peripheral sites.[1] However, an increasing body of experimental evidence indicates that such drugs also have a powerful effect on experimental pain states that is independent of their antiinflammatory effects.[2] NSAIDs are believed to reduce enhanced nociceptive activity in the periphery by inhibiting the enzyme cyclooxygenase (COX); this inhibits the generation of the prostaglandins (PGs) that sensitize afferent nociceptors to the effects of substances such as substance P and bradykinins.[3] In addition to their inhibition of PGs synthesis in the periphery, a central action of NSAIDs has been suggested by experimental studies in which they have been demonstrated to act more potently on intrathecal administration than on systemic administration.[4–8] Malmberg and Yaksh[5] reported that intrathecal NSAIDs produced a significant analgesic effect, as evidenced by behavioral responses in animals in experiments undergoing the formalin test. Because they showed a significant dose-related suppression of the second phase response in the formalin test (which is believed to indicate a hyperalgesic state), but not of the first phase response, the results of their study would seem to suggest that the spinal action of NSAIDs results from a suppression of spinal sensitization.

Several studies have been carried out to test the spinal antinociceptive effects of NSAIDs in hyperalgesia models, including the formalin test[5,9,10] and the writhing

* Research Fellow, Department of Anesthesiology and Critical Care Medicine, Gifu University School of Medicine; Staff Anesthetist, Department of Anesthesia, Gifu Prefectural Hospital.

† Professor and Chair, Department of Anesthesiology and Critical Care Medicine, Gifu University School of Medicine.

‡ Instructor, Department of Anesthesiology and Critical Care Medicine, Gifu University School of Medicine.

§ Director, Department of Anesthesia, Gifu Prefectural Hospital; Clinical Associate Professor, Gifu University School of Medicine.

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Address reprint requests to Dr. Dohi: Department of Anesthesiology and Critical Care Medicine, Gifu University School of Medicine, 40 Tsukasamachi, Gifu City, Gifu 500-8705, Japan. Address electronic mail to: shu-dohi@cc.gifu-u.ac.jp

test,[7] as well in hyperalgesia models induced by the administration of agonists for the N-methyl-D-aspartate (NMDA) receptor and the neurokinin-1 tachykinin receptor.[6] Although nitric oxide (NO) is thought to play an important role in the production of hyperalgesia in such behavioral models,[11] no study has yet been performed to test the effects of NSAIDs on a hyperalgesia model directly related to NO. Systemic administration of l-arginine, an NO precursor, could antagonize a suppressive effect of NSAIDs such as acetaminophen,[12] diclofenac,[13] and S(+)ibuprofen[13] on NMDA-induced scratching, biting, and licking behavior. However, no study has been appeared in the literature whether spinal sensitization primarily induced by an NO donor or l-arginine is suppressed by NSAIDs. Nitroglycerin, as an NO donor, can release NO by nonenzymatic reaction in the presence of some reducing compounds such as cysteine,[14-16] and a few studies have described that nitroglycerin, given intracerebroventricularly and systemically, could induce activities related to its effect of NO release in the central nervous system.[17,18] In the present study, we examined (1) whether epidural administration of nitroglycerin (an NO donor) and l-arginine (an NO precursor) would cause nociceptive behavior in rats, (2) whether epidural administration of NSAIDs, which is not studied enough[19,20] compared with intrathecal administration, would suppress such NO-induced hyperalgesia, and (3) whether the antinociceptive effect of NSAIDs would be parallel to their potency of cyclooxygenase inhibition.

Materials and Methods

Animal Preparation

The experimental procedures employed in the present study were approved by the Gifu University Animal Care and Use Committee (protocol ACCM-940010). Adult male Sprague-Dawley rats, weighing 400-500 g, were anesthetized with intraperitoneal pentobarbital and implanted with a chronic indwelling epidural catheter. After a laminectomy of the lower thoracic spines, the yellow ligament was punctured at T11-T12 without damaging the dura mater. A polyethylene (PE-10) catheter was inserted caudally for a distance of 1.5 cm through the hole in the yellow ligament and exteriorized at the neck. After recovery from the surgery, each rat was housed in an individual cage. Four to 7 days later, 150 μl of 2% lidocaine was injected through the indwelling catheter, and analgesia was confirmed by the lack of response to a skin pinch. The tail-flick tests and the formalin test were performed 1-8 weeks after catheter implantation, with only rats with normal motor function used. Finally, 50 μl of methylene blue was injected through the catheter, and epidural staining was confirmed at autopsy.

Drugs

The following drugs were used for epidural administration in the present study: S(+)ibuprofen (molecular weight [MW] = 206.27; Research Biochemicals Incorporated, Natick, MA); R(-)ibuprofen (MW = 206.27; Research Biochemicals); indomethacin (MW = 357.8; Sigma Chemical Company, St. Louis, MO); diclofenac (MW = 318.1; Sigma); nitroglycerin (MW = 227.09; Nippon Chemical Company, Takasaki, Japan); l-arginine hydrochloride (MW = 210.7; Sigma); d-arginine hydrochloride (MW = 210.7; Sigma); methylene blue (MW = 373.9; Sigma); and N^{ω}-nitro-L-arginine methyl ester (L-NAME; MW = 269.7; Sigma). S(+)ibuprofen, R(-)ibuprofen, indomethacin, and diclofenac were dissolved in dimethylsulfoxide (DMSO). Nitroglycerin, l-arginine, d-arginine, methylene blue, and N^{ω}-nitro-L-arginine methyl ester were dissolved in physiologic saline solution (0.9% weight/volume).

Tail-flick Test

For assessment of the thermal-nociceptive response, a custom-made tail-flick analgesimeter (Natsume Co., Tokyo, Japan) was used. Tail-flick latencies (TF-L) were measured from the onset of a radiant heat stimulus applied to the tail as described previously.[21] Briefly, the light intensity was adjusted to yield mean baseline latencies of 4-6 s, and the cut-off time was set at 20 s to avoid damage to the tail. Before each drug trial, the baseline latency was measured. To study the suppression of hyperalgesia, "drug 1" (an antinociceptive drug) and "drug 2" (a hyperalgesic drug) were administered epidurally with a 10-min interval (table 1). Each epidural injection of a test drug was performed in a volume of 30 μl followed by 20 μl saline solution to flush out the catheter's dead space. Besides the epidural study, systemic effects of nitroglycerin and S(+)ibuprofen were studied by intramuscular nitroglycerin and S(+)ibuprofen. After administration of drugs, TF-L was measured at several time points, namely 5, 10, 20, 30, 40, 50, and 60 min after administration of drug 2.

Formalin Test

The formalin test was performed in a manner similar to that previously described by others.[22,23] The rat was first

Table 1. The Various Combinations of Drugs Administered through an Indwelling Epidural Catheter in the Tail Flick Test

Group	Drug 1	Drug 2
Control	Vehicle (DMSO)	Vehicle (saline)
NTG	Vehicle (DMSO)	NTG (10 μg)
S(+)ibp	S(+)ibp (1,000 μg)	Vehicle (saline)
S(+)ibp + NTG	S(+)ibp (10–1,000 μg)	NTG (10 μg)
R(−)ibp + NTG	R(−)ibp (100–1,000 μg)	NTG (10 μg)
Dic + NTG	Dic (3.16–1,000 μg)	NTG (10 μg)
Ind + NTG	Ind (1–100 μg)	NTG (10 μg)
L-NAME + NTG	L-NAME (1,000 μg)	NTG (10 μg)
MB + NTG	MB (100 μg)	NTG (10 μg)
L-arg	Vehicle (DMSO)	L-arg (100 μg)
D-arg	Vehicle (DMSO)	D-arg (100 μg)
S(+)ibp + L-arg	S(+)ibp (1,000 μg)	L-arg (100 μg)
L-NAME + L-arg	L-NAME (1,000 μg)	L-arg (100 μg)

DMSO = dimethylsulfoxide; NTG = nitroglycerin; S(+)ibp = S(+)ibuprofen; R(−)ibp = R(−)ibuprofen; Dic = diclofenac; Ind = indomethacin; L-NAME = N^{ω}-nitro-L-arginine methyl ester; MB = methylene blue; L-arg = L-arginine; D-arg = D-arginine.

In each rat drug 1 and drug 2 were administered through an indwelling epidural catheter with a 10-min interval.

transferred into a clear perspex box. Thirty minutes later, S(+)ibuprofen, R(-)ibuprofen, or vehicle (control) was administered epidurally. Ten minutes later, 50 μl of 5% formalin (prepared from a 37% formaldehyde solution by 1:19 dilution with physiologic saline solution) was injected subcutaneously into the dorsal surface of the left hindpaw using a 30-gauge needle. For 65 min after the injection, the response to the pain-like stimulus was recorded for each rat. The total time for which the paw was kept in a raised position during a given period was taken as an indicator of the response.

Behavioral Assessment

The rat's general behavior was observed after the administration of each drug tested. Motor functions were tested by examining the placing and stepping reflex and the righting reflex. Agitation behavior was assessed by observing behavior before and after the nociceptive stimulation.

Release of NO from Nitroglycerin In Vitro

To examine whether nitroglycerin given epidurally releases nitric oxide in the peri-spinal cord level, we have done the following *in vitro* experiments in the presence of the spinal cord tissue removed from rats. Adult male Sprague-Dawley rats were decapitated, and the spinal cords were removed. Cell suspension of the spinal cords was prepared through the homogenization and centrifugation. Final suspension of the spinal cord was prepared with the phosphate buffer saline (PBS). A reaction mixture, 1.8 ml, containing 1 or 0.1 mM nitroglycerin with the spinal cell suspension or 50 mM l-cysteine in the PBS was incubated at 37°C for 60 min. Each control experiment was performed without nitroglycerin, the spinal cell suspension, or l-cysteine. The measurement of nitrite content was performed as described previously[24,25] with some modifications: the nitrite in the samples was measured by fluorescence intensity at 460 nm when mixed with 2,3-diaminonaphthalene.

Statistical Analysis

For the tail-flick test, time-response data were calculated as the percentage changes in TF-L according to the formula:

$$\%\ \text{change in TF-L} = \frac{(\text{TF-L after administration of drugs}) - (\text{mean baseline latency})}{(\text{mean baseline latency})} \times 100$$

To enable an assessment to be made of the suppressant effects of NSAIDs on nitroglycerin-induced hyperalgesia, suppression ratios were calculated as:

$$\frac{\{(\%\ \text{change in TF-L in rats injected with an NSAID and nitroglycerin}) - (\%\ \text{change in TF-L in rats injected with nitroglycerin})\}}{\{(\%\ \text{change in TF-L in rats injected with vehicle}) - (\%\ \text{change in TF-L in rats injected with nitroglycerin})\}}$$

Dose-response curves for NSAIDs were obtained by taking the average of the suppression ratios at the observation points 10, 20, 30, 40, 50, and 60 min after administration of drug 2.

For the formalin test, time-response data are presented as the paw-raised time/min for the periods 0–5, 5–15, 15–25, 25–35, 35–45, 45–55, and 55–65 min after the injection of formalin.

The statistical significance of differences between treatment groups was assessed by means of an analysis of variance (ANOVA) for repeated measures. Significance was taken to be indicated by a P value of < 0.05.

Results

Hyperalgesia Models

Epidural administration of nitroglycerin (10 μg) shortened the TF-L within 5 min (thermal hyperalgesia). This

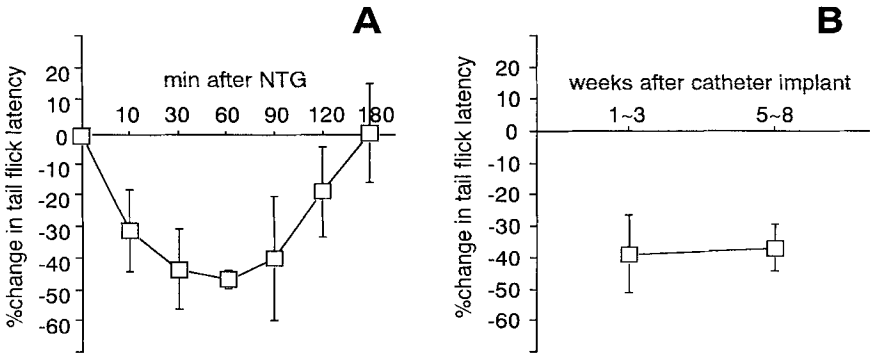

Fig. 1. Shortening of tail-flick latency caused by epidural administration of nitroglycerin (10 μg). (*A*) Time effect curve in the tail-flick test. Each symbol shows the mean ± SD from five animals. The tail-flick latency had returned to the control level by 180 min. (*B*) Comparison of the effect of nitroglycerin on the date when the tail-flick test was performed. Each symbol shows the mean ± SD of the data obtained at 10, 20, 30, 40, 50, and 60 min in 8–10 animals. There was not a statistically significant difference (P = 0.66) between the group of 1–3 weeks after catheter implantation and that of 5–8 weeks after catheter implantation (ANOVA for repeated measures).

effect was stable in the period of 10-90 min and returned to the control level in 180 min (fig. 1A). There was no significant difference in the nitroglycerin-induced hyperalgesia between the group tested 1-3 weeks after catheter implantation and that tested 5-8 weeks after catheter implantation (fig. 1B). Epidural nitroglycerin, 1 μg, also reduced the TF-L (to about the same extent as 10 μg). Nitroglycerin (1 and 10 μg) produced transient agitation behavior (slight biting and scratching), although this disappeared within 10 min in all rats. Both the hyperalgesia and agitation behavior were prevented by pretreatment with epidural methylene blue (100 μg) administered 10 min before the nitroglycerin, but not by pretreatment with epidural N^{ω}-nitro-L-arginine methyl ester (L-NAME, 1,000 μg) (fig. 2). Administration of nitroglycerin (10 μg) by the intramuscular route did not induce such hyperalgesia or agitation behavior (fig. 2).

Epidural administration of l-arginine (100 μg), but not of d-arginine (100 μg), shortened the TF-L within 20 min, producing a slowly developing thermal hyperalgesia without agitation behavior such as scratching or biting (fig. 2). The hyperalgesia was prevented by pretreatment with epidural L-NAME (1,000 μg) administered 10 min before the l-arginine (fig. 2).

Formalin injection into a rat's paw produced paw-lifting behavior with two distinct phases. The first phase of the response occurred over the period from 0-5 min

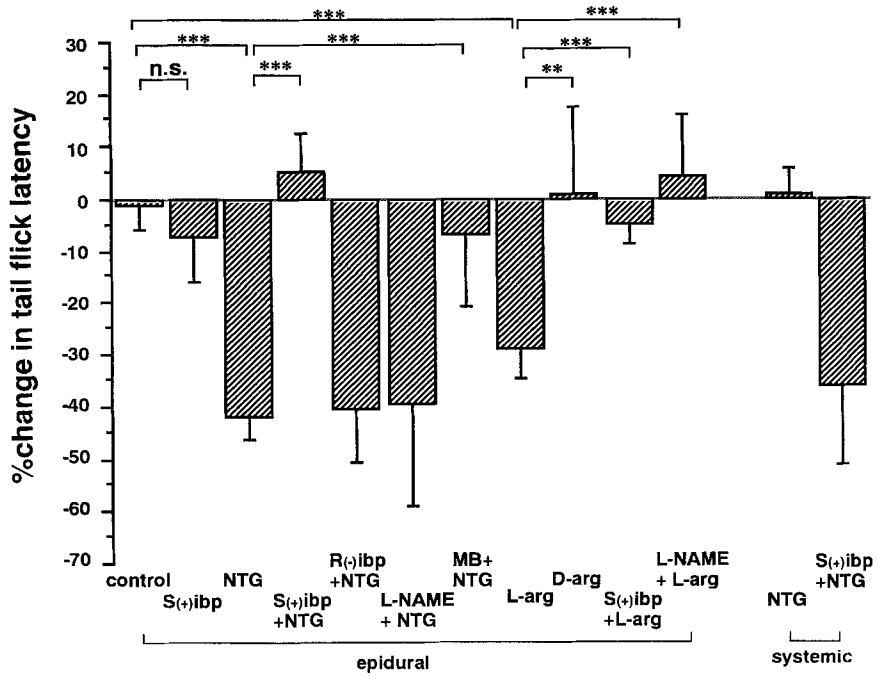

Fig. 2. Summary of the effects of drugs used in the tail- flick test. Each bar shows the mean ± SD of the data obtained at 10, 20, 30, 40, 50, and 60 min in five animals. For the groups of epidural administration, abbreviations are as in table 1, and doses used were as table 1, except: S(+)ibp + NTG, S(+)ibuprofen 1,000 μg + nitroglycerin 10 μg; R(−)ibp + NTG, R(−)ibuprofen 1,000 μg + nitroglycerin 10 μg. For the groups of systemic administration, NTG means intramuscular nitroglycerin 10 μg, and S(+)ibp + NTG means intramuscular S(+)ibuprofen 1,000 μg + epidural nitroglycerin 10 μg. Statistical significance was determined by ANOVA for repeated measures. **P < 0.01, ***P < 0.001 and NS indicates P > 0.05. There was a statistically significant difference (P < 0.05) between the following pairs of groups: control *versus* nitroglycerin; nitroglycerin *versus* S(+)ibuprofen + nitroglycerin; nitroglycerin *versus* methylene blue + nitroglycerin; control *versus* l-arginine; l-arginine *versus* d-arginine; l-arginine *versus* S(+)ibuprofen + l-arginine; and l-arginine *versus* L-NAME + l-arginine.

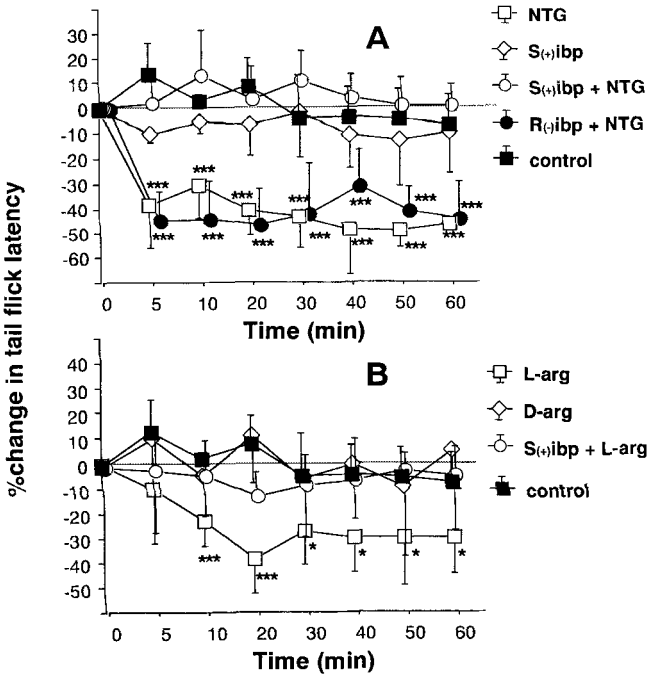

Fig. 3. (*A*, *B*) Time effect curves for various drugs in the tail flick test. Each symbol shows the mean ± SD from five animals. Abbreviations are as in table 1, and doses used were as in figure 2. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ *versus* control value at same time-point (ANOVA and Bonferroni-Dunn *post hoc* test). The groups administered NTG, and R(−)ibp + NTG had a statistically significant difference ($P < 0.0001$) with the control group in the data at 10–60 min (ANOVA for repeated measures).

after the injection, and the second phase occurred 15–65 min after the injection.

Effects of NSAIDs in Hyperalgesia Models

Nitroglycerin-induced and L-arginine–induced Hyperalgesia. Animals given epidural injections of S(+)ibuprofen (1,000 μg) and saline (vehicle) as drugs 1 and 2 did not show any change in TF-L (fig. 2; fig. 3A). Epidural nitroglycerin shortened the TF-L significantly, an effect that remained stable for 60 min. Pretreatment with epidural S(+)ibuprofen (1,000 μg) completely prevented this change, indicating suppression of the nitroglycerin-induced hyperalgesia (figs. 2 and 3A). Pretreatment with epidural S(+)ibuprofen (1,000 μg) also blocked the l-arginine–induced hyperalgesic state (figs. 2 and 3B). In contrast, epidural administration of R(-)ibuprofen (1,000 μg) did not block the hyperalgesia induced by either nitroglycerin (figs. 2 and 3A) or l-arginine (data not shown). As shown in figure 4, the suppression of the nitroglycerin-induced hyperalgesia that was induced by epidural S(+)ibuprofen (10–1,000 μg) occurred in a dose-dependent manner, as did the suppressions induced by epidural indomethacin (1–100 μg) and diclofenac (3.16–1,000 μg).

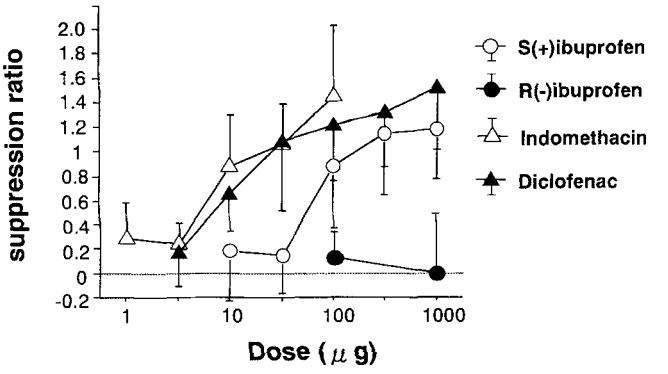

Fig. 4. Dose–response curves for the suppression by epidural NSAIDs of nitroglycerin-induced hyperalgesia in the tail- flick test. Each NSAID was epidurally administered 10 min before nitroglycerin. Ordinate shows "suppression ratio" (calculated as described in Materials and Methods). Each symbol shows mean ± SD of the data obtained at 10, 20, 30, 40, 50, and 60 min in five animals. The doses producing 50% inhibition of the nitroglycerin-induced hyperalgesia were as follows: 5.0 μg for indomethacin; 6.3 μg for diclofenac; 52 μg for S(+)ibuprofen; and > 1,000 μg for R(−)ibuprofen.

The rank order of the doses producing 50% inhibition of the nitroglycerin-induced hyperalgesia (ID_{50}) was indomethacin (5.0 μg) ≒ diclofenac (6.3 μg) > S(+)ibuprofen (52 mg) ≫ R(-)ibuprofen (> 1,000 μg) (fig. 4; table 2). The maximal suppression ratios obtained with S(+)ibuprofen, indomethacin, and diclofenac were in each case more than 1 (fig. 4; table 2). In rats injected with indomethacin (100 μg) followed by nitroglycerin and in rats injected with diclofenac (1,000 μg) followed by nitroglycerin, TF-Ls were significantly longer than in rats injected with DMSO and saline (control).

In contrast to its effect on epidural administration, S(+)ibuprofen (1,000 μg) injected by the systemic (in-

Table 2. Ability of Epidural NSAIDs to Suppress Hyperalgesia Induced by Epidural Nitroglycerin

Drug	ID_{50} (μg) and 95% Confidence Interval	Maximal Suppression Ratio ± SD
S(+)ibuprofen	52 (38–66)	1.18 ± 0.40
R(−)ibuprofen	>1,000	0.14 ± 0.21
Diclofenac	6.3 (5.7–7.6)	≧1.53 ± 0.50
Indomethacin	5.0 (2.8–7.3)	≧1.44 ± 0.58

ID_{50} values were calculated as the dose producing 50% inhibition of nitroglycerin-induced hyperalgesia. Maximal suppression ratio is shown as mean ± SD for five rats; it indicates the highest suppression ratio obtained with each drug.

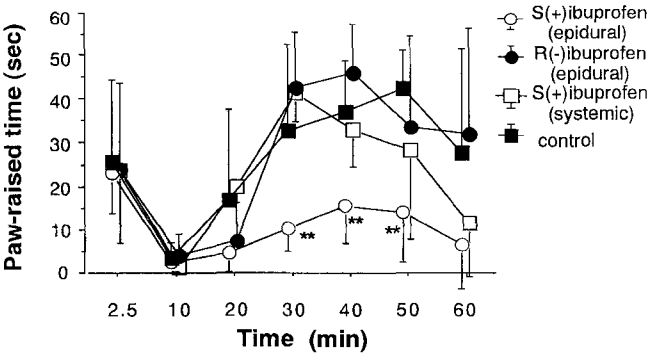

Fig. 5. Time–effect curves for S(+)ibuprofen and R(−)ibuprofen in the formalin test. S(+)ibuprofen means S(+)ibuprofen 1,000 μg and R(−)ibuprofen means R(−)ibuprofen 1,000 μg. Each drug was administered 10 min before the formalin injection. Each symbol shows mean ± SD of the total numbers of s for which the paw was kept raised per min in five to seven animals. The data points were obtained as described in Materials and Methods. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ *versus* control value at same time-point (ANOVA and Bonferroni–Dunn *post hoc* test). The group administered S(+)ibuprofen had a statistically significant difference ($P < 0.001$) with the control group in the data during 15–65 min (ANOVA for repeated measures).

tramuscular) route did not affect the shortening of the TF-L induced by epidural nitroglycerin (fig. 2).

Formalin Test. On epidural administration, S(+)ibuprofen (1,000 μg), but not R(-)ibuprofen (1,000 μg), suppressed the second phase paw-lifting behavior (fig. 5). In contrast, systemic (intramuscular) administration of S(+)ibuprofen (1,000 μg) did not cause any change in second phase paw-lifting behavior. Neither S(+)ibuprofen nor R(-)ibuprofen affected the first phase paw-lifting behavior.

Behavioral Assessment. Although transient agitation behavior associated with epidural nitroglycerin was observed (see above), no evidence of severe neurotoxicity (seizures or motor paresis) was seen. Epidural administration of S(+)ibuprofen, R(-)ibuprofen, indomethacin, or diclofenac did not elicit motor paresis, seizures, or any agitation behavior.

Release of NO from Nitroglycerin *In Vitro*. When nitroglycerin was given into the cell suspension of the spinal cord and l-cysteine, nitrite was detected in a dose-dependent fashion significantly more than when nitroglycerin was given in the PBS (table 3).

Discussion

The present results indicate that epidural administration of either nitroglycerin or l-arginine, but neither the systemic nitroglycerin nor epidural d-arginine, produces a significant shortening of TF-L that lasts for more than 60 min. This nitroglycerin-induced hyperalgesia was blocked by methylene blue but not by L-NAME, and the l-arginine-induced hyperalgesia was blocked by L-NAME. Epidural S(+)ibuprofen, indomethacin, and diclofenac, but not R(-)ibuprofen, suppressed thermal hyperalgesia. The rank-order of potency for the antinociceptive effects of the various NSAIDs on the nitroglycerin-induced thermal hyperalgesia agrees with those for inhibition of cyclooxygenase-2 (COX-2) activity observed *in vitro*.[26-28] Our results are also in accord with the previous finding that intrathecal indomethacin and S(+)ibuprofen, although epidural doses were 10 times or more higher than those used here, suppressed the second phase response in the formalin test[5] (table 4).

Nonsteroidal antiinflammatory drugs have been found to work centrally in animals.[4-8,29,30] Several reports indicate that the second phase response in the formalin test, which is believed to be a response to the production of a hyperalgesic state, can be blocked by the intrathecal NSAIDs, which are 100-1,000 times more potent than systemic administration.[5] The results obtained with epidural NSAIDs in the present study are in accord with those obtained by others using intrathecal NSAIDs.[4-8] It is suggested that NSAIDs could block the release of glutamate, aspartate, and substance P evoked by the subcutaneous injection of formalin in the spinal cord.[23] Because systemic S(+)ibuprofen did not affect the nitroglycerin-induced hyperalgesia and because epidural S(+)ibuprofen did not affect either the basal TF-L or the first phase response in the formalin test, it can be concluded that epidural S(+)ibuprofen suppresses the hyperalgesic state, but not acute noxious responses, through an action on antinociceptive processing at the spinal level.

Table 3. The Concentration of Nitrite in Reaction Mixtures in Which Nitroglycerin Was Incubated with Spinal Cell Suspension or L-Cysteine *In Vitro*

	Spinal Cell Suspension	L-Cysteine	PBS
NTG 1 mM	1.87 ± 0.29*	1.82 ± 0.71*	0.65 ± 0.35
NTG 0.1 mM	0.28 ± 0.09†	0.24 ± 0.06†	0.04 ± 0.07
PBS	0.00	0.00	0.00

NTG = nitroglycerin; PBS = phosphate buffer saline.

Data are mean ± SD of the concentration (μM) of nitrite in three or four experiments. The concentration of nitrite in the mixture of nitroglycerin and cell suspension of the spinal cord was higher than that of the control.

* $P < 0.01$ *versus* control (ANOVA and Bonferroni/Dunn post hoc test).

† $P < 0.05$ *versus* control (ANOVA and Bonferroni/Dunn post hoc test).

Table 4. Summary of Behavioral Studies Assessing Central Antinociceptive Effects of NSAIDs in Rats

Experimental Model and Route of Administration	Inhibitory Dose Resulting in a 50% Reduction of Nociceptive Responses (ID_{50}; μg)*				References
	Indomethacin	Diclofenac	S(+)ibuprofen	R(−)ibuprofen	
NTG-induced thermal hyperalgesia					
epi	5.0	6.3	52	>1,000	Masue *et al.*†
im			>1,000		Masue *et al.*†
NMDA-induced thermal hyperalgesia					
it			<5.6	>1,000	Malmberg and Yaksh 1992[6]
Second phase in the formalin test					
epi			<1,000	>1,000	Masue *et al.*†
im			>1,000		Masue *et al.*†
it	0.68		3.2	>56	Malmberg and Yaksh 1992[5]
ip	930				Malmberg and Yaksh 1992[5]
Writhing reflex in the writhing test					
it		3			Björkman 1995[7]
sc		300			Björkman 1995[7]

epi = epidural; im = intramuscular; it = intrathecal; ip = intraperitoneal; sc = subcutaneous.

* In the case of single-dose studies, data are preceded by > (more than 50% inhibition at the dose shown) or < (less than 50% inhibition at the dose shown).

† The present study.

Intrathecal L-NAME, an NO synthase inhibitor, has been observed to suppress the second phase response in the formalin test[11] and NMDA-induced hyperalgesia.[11] In the present study, we confirmed that nitroglycerin, an NO donor, is able to produce a hyperalgesic state when administered into the epidural space. Although this is the first demonstration that such hyperalgesia can be produced with nitroglycerin, in a previous study intrathecal sodium nitroprusside (SNP), another NO donor, has been reported to induce thermal hyperalgesia in the tail-flick test, an effect that could be antagonized with hemoglobin, an NO scavenger.[31] SNP is generally believed to evoke the release of immunoreactive calcitonin gene-related peptide (CGRP) and substance P from the dorsal horn.[32] However, SNP is not ideal for this purpose because intrathecal SNP in mice elicits severe neurotoxicity and excitatory responses such as intense biting and scratching accompanied by vocalization and seizures,[31] and sodium ferricyanide (the coproduct of SNP) also evokes the release of CGRP and substance P from the dorsal horn.[32] Nitroglycerin is strongly lipophilic with rather low molecular weight (MW, 227.09). Although the mechanisms in which nitroglycerin produces NO are not completely clear,[16] nitroglycerin could release NO with non-enzymatic reaction in the presence of some reducing agents such as cysteine *in vitro*.[14-16] We found that nitroglycerin induced NO in the presence of the rat's spinal cord similar to in the presence of l-cysteine *in vitro*. Nitroglycerin injected into the epidural space could reach the spinal cord by crossing the dural membranes and release some amount of NO, which would then act as a messenger in nociceptive transmission involving the NO-cGMP system. This sequence of events is likely to form the basis of the present nitroglycerin-induced hyperalgesia model because the hyperalgesic state induced by epidural nitroglycerin was completely blocked by methylene blue, a guanylate cyclase inhibitor. This idea is further supported by our finding that epidural l-arginine, a physiologic precursor of NO, although slower in onset than that induced by nitroglycerin, induced a similar thermal hyperalgesia, which was blocked by L-NAME.

It is possible that potential inflammation or vasodilation in the epidural space originated by nitroglycerin could stimulate peripheral nerve and induce spinal sensitization. Since in the present result, pretreatment with L-NAME did not affect the time course of nitroglycerin-induced hyperalgesic state, we can exclude the possibility that endogenous NO, even if released due to potential inflammation in the epidural space, could cause spinal sensitization in our rat experiments. Spinal sensitization induced by noxious stimuli to the peripheral site such as epidural space is mediated *via* releasing endogenous NO or activation of NO synthase.[11] NO *per se* seems to suppress, not to facilitate, peripheral nerve axonal conduction and thus nociceptive transmission.[33,34] Because we administered nitroglycerin after injecting NSAIDs or methylene blue, it is also possible that they could affect the release of NO from nitroglycerin. However, NO release from nitroglycerin is unlikely to be affected with

the presence of an NSAID *in vitro*.[35] However, without measuring NO in the spinal cord, we cannot provide a conclusive answer to an important question: namely, whether epidural injection of nitroglycerin and l-arginine does lead to an increase in NO (exogenously or endogenously, respectively).

The epidural administration of drugs, although applicable to clinical practice, creates some problems of access. Because most NSAIDs have the characteristic of intermediate hydrophobicity (n-octanol/water partition coefficients = 1-4),[36] they would be expected to diffuse through the dural membranes,[37] and thus act on the spinal cord directly, although considerable dilution would occur due to the presence of cerebrospinal fluid. Because NSAIDs, which have a molecular weight of around 250-300, penetrate the blood-brain barrier,[38-40] another possible route for epidurally injected NSAIDs would involve transport to the spinal cord *via* the radicular arteries after their absorption through the epidural vasculature, and a consequent supraspinal action.[41] In addition to their central action both on spinal and supraspinal levels, NSAIDs' peripheral action could also augment the spinal antinociceptive processing. The suppression of nociceptive processing at the spinal site might expand the peripheral suppression of NSAIDs on inflammatory as well as nociceptive processings probably *via* additional mechanisms independent of prostaglandin synthesis inhibition.[42] Thus when a higher dose of NSAIDs given in the epidural space, it is possible that epidural route of NSAIDs administration could provide an excellent analgesia *via* action of peripheral, spinal, and supraspinal sites.

We have shown that epidural NSAIDs produced antinociceptive action in NO-induced hyperalgesic state. An animal study also indicates that epidural, but not intravenous, S(+)ibuprofen, 10 mg, produces an antinociceptive effect without any evidence of motor dysfunction or neurotoxicity.[19] As the adverse effects (such as upper gastrointestinal bleeding, coagulopathy, hepatotoxicity, etc.) associated with systemic NSAIDs are dose-related,[43,44] epidural NSAIDs may provide a useful level of analgesia on a regional basis in patients with hyperalgesia without producing such untoward reactions. Recently, it is reported that two patients with cancer pain, when given accidentally NSAIDs into the epidural space, had excellent analgesia without any toxicity.[20] However, because NSAIDs administered epidurally may be in a high concentration close to the spinal cord, a neural structure that normally has a marginal flow, and because COX has recently been reported to play a role as a neurotransmitter in the central nervous system,[45] further studies will be needed to investigate their neurotoxicity and the relation between spinal cord blood flow characteristics and neuronal activity under various conditions.

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