Anesthesiology
83:336–343, 1995


# *Contrasting Actions of Intrathecal U50,488H, Morphine, or [D-Pen², D-Pen⁵] Enkephalin or Intravenous U50,488H on the Visceromotor Response to Colorectal Distension in the Rat*

*Y. Harada, M.D.,* K. Nishioka, M.D.,* L. M. Kitahata, M.D., Ph.D.,† K. Nakatani, M.D.,* J. G. Collins, Ph.D.‡*

***Background:*** **Visceral sensations are an important component of many clinical pain states. It is apparent that intrathecal pain relief may be more effective if appropriate combinations of drugs rather than a single agent can be used. The purpose of this study was to examine the relative contribution of opioid receptor subtypes to visceral antinociception using colorectal distension as a visceral pain model.**

***Methods:*** **The minimum colorectal distending pressure necessary to evoke a visceromotor response (contraction of abdominal musculature) was determined before and after the administration of opioid agonists for the μ (morphine), δ ([D-Pen², D-Pen⁵] enkephalin [DPDPE]), and κ (U50,488H) opioid receptors. In addition to the three drugs administered intrathecally, U50,488H was also administered intravenously.**

***Results:*** **Morphine and DPDPE produced a reversible increase in threshold for activation of the visceromotor response (50% maximum possible effect [MPE] at intrathecal doses of 2.2 and 16.4 μg, respectively). The maximum intrathecal dose of U50,488H (100 μg) produced only a 20% MPE. Intravenous U50,488H produced a 50% MPE at a dose of 2.6 mg/kg.**

***Conclusions:*** **The results suggest that spinal μ- and δ- but not κ-opioid receptors have a significant role in the modulation of visceral nociception induced by colorectal distension. In addition, the results indicate that activation of nonspinal κ receptors may mediate visceral antinociception. (Key words: Analgesics, opioid: [D-Pen², D-Pen⁵] enkephalin; morphine; U50,488H. Opioid receptor antagonists: naloxone; naltrindole. Pain, visceral: antinociception; colorectal distension.)**

DEEP pain associated with the viscera is different from somatic pain and is of clinical importance.[1] Visceral pain has been less well studied than somatic pain, and we therefore need a better understanding of the pharmacologic control of visceral pain. With use of colorectal distension (CRD) as a reliable model of visceral pain, investigators have begun to focus on visceral antinociception and the mechanisms of visceral pain produced by mechanical distension of a hollow viscus.[2–6]

Opioids are of obvious importance in the control of pain. In experimental studies using somatic stimuli, it has been well established that μ-opioid receptor agonists such as morphine inhibit both nociceptive behavioral reflexes and neuronal activity at the spinal cord level.[7–9] Using the CRD test in the rat, Ness and Gebhart[2,3] and Maves and Gebhart[6] demonstrated that intrathecally or intravenously administered morphine powerfully inhibited nociceptive cardiovascular and behavioral (visceromotor) reflexes and dorsal horn neuronal activity. To date, at least three opioid receptor subtypes (μ, δ, and κ) have been shown to be involved in mediating the processing of a variety of nociceptive information,[10–13] yet the antinociceptive effects of the three opioid subtype agonists on visceral nociception have not been fully appreciated.

Using relatively highly selective μ-opioid, δ-opioid, and highly selective κ-opioid agonists (morphine, DADLE, and U50,488H), respectively, Schmauss and Yaksh[10] reported that spinal μ- and κ- but not δ-opioid receptors were involved in modulating the writhing response of a rat to a chemically induced visceral stimulus. In contrast, Porreca *et al.*,[11] studying three agonists (DAGO, [D-Pen², D-Pen⁵] enkephalin [DPDPE], and U50,488H) with the highest degree of selectivity for μ-, δ-, and κ-opioid receptors, respectively, reported that spinal μ and δ receptors had a more important role than κ receptors in inhibiting the writhing response in the mouse. Therefore, an important question is what

* Postdoctoral Associate in Anesthesiology.

† Professor of Anesthesiology.

‡ Associate Professor of Anesthesiology.

Received from the Department of Anesthesiology, Yale University School of Medicine, New Haven, Connecticut. Submitted for publication April 28, 1994. Accepted for publication April 10, 1995. Supported by National Institutes of Health grant NS 09871.

Address reprint requests to Dr. Collins: Department of Anesthesiology, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510.





role the spinal δ- and κ-opioid a
pain modulation.[12]

Visceral pain and perhaps m
equate stimuli for the product
not well understood.[13] Althou
duced writhing test is related
producibility, reliability, and s
questionable.[14] On the other
reliable, reproducible, and use
visceral pain in the rat. In a
visceral pain sensation in huma
these stimuli are assumed to
likely that they do so by very
is possible that the short-durati
associated with CRD activates
ent from those activated by th
sociated with writhing tests.
moreceptors have been postu
for the response to some form
Chemical stimulation, because
the stimulus (30–60 min), n
mation that contributes to th
Short-term CRD does not appea

The current study was desig
ative contribution of the three
subtypes, especially δ and κ re
tinociception induced by the
mary μ agonist (morphine), a h
(DPDPE), and a highly selective

## Materials and Methods

### *Animals*

The protocol of this study w
Animal Care and Use Commi
conducted on adult male Sprag
300–400 g. Animals were hou
given free access to food and w
h light–dark cycle, with light
In all animals in this study
was implanted for drug admin
thane (1–2%) anesthesia, a
[PE]-10 tube, 8.5–9 cm long
a slit in the atlantooccipital m
arachnoid space, and the ti
placed near the lumbar enla
cord according to a method d
The rostral end of the cathete
other catheter (PE-50 tube, 3

role the spinal $\delta$- and $\kappa$-opioid agonists play in visceral pain modulation.[12]

Visceral pain and perhaps more importantly the adequate stimuli for the production of visceral pain are not well understood.[13] Although the chemically induced writhing test is related to visceral pain, the reproducibility, reliability, and specificity of this test are questionable.[14] On the other hand, the CRD test is a reliable, reproducible, and useful method for study of visceral pain in the rat.[2] In addition, CRD produces visceral pain sensation in humans.[15–18] Although all of these stimuli are assumed to elicit visceral pain it is likely that they do so by very different mechanisms. It is possible that the short-duration mechanical stimulus associated with CRD activates neuronal systems different from those activated by the chemical stimuli associated with writhing tests. In the skin, silent chemoreceptors have been postulated as an explanation for the response to some forms of noxious stimuli.[19] Chemical stimulation, because of the long duration of the stimulus (30–60 min), may also induce inflammation that contributes to the sensory experience. Short-term CRD does not appear to cause inflammation.

The current study was designed to examine the relative contribution of the three spinal opioid receptor subtypes, especially $\delta$ and $\kappa$ receptors, in visceral antinociception induced by the CRD test. We used a primary $\mu$ agonist (morphine), a highly selective $\delta$ agonist (DPDPE), and a highly selective $\kappa$ agonist (U50,488H).

## Materials and Methods

### *Animals*

The protocol of this study was approved by the Yale Animal Care and Use Committee. Experiments were conducted on adult male Sprague-Dawley rats weighing 300–400 g. Animals were housed in individual cages, given free access to food and water, and kept on a 12-h light–dark cycle, with light beginning at 6:00 AM.

In all animals in this study an intrathecal catheter was implanted for drug administration. During halothane (1–2%) anesthesia, a catheter (polyethylene [PE]–10 tube, 8.5–9 cm long) was inserted through a slit in the atlantooccipital membrane into the subarachnoid space, and the tip of the catheter was placed near the lumbar enlargement of the spinal cord according to a method described previously.[20] The rostral end of the catheter was connected to another catheter (PE-50 tube, 3 cm long) for drug administration. The catheter was anchored to the surrounding musculature to maintain its position. Total dead space of the whole catheter was 12 $\mu$l. At the termination of the surgery, animals were treated with 15,000–21,000 U intramuscular penicillin G to prevent infection. Animals were observed for at least 10 days before experimental use. Animals exhibiting neurologic impairments or infection as a result of the surgical procedure (10–15% of the animals) were excluded from experimental use. The location of the tip of the catheter was verified at the end of the terminal experiment by dye injection.

### *Analgesiometric Test*

In this study, visceromotor response (VMR) (a contraction of abdominal musculature) to CRD was used as a measure of visceral nociception. CRD was achieved by pressure-controlled air inflation of a latex distension balloon (5 cm long) as illustrated in figure 1. The distension balloon was connected to a pressure-controlled balloon inflator through a distension balloon catheter and was inflated continuously at a rate of 6 mmHg/s beginning at 0 mmHg until a clear VMR was evoked or until a maximum pressure of 80 mmHg was reached. The distending pressure was limited to 80 mmHg to

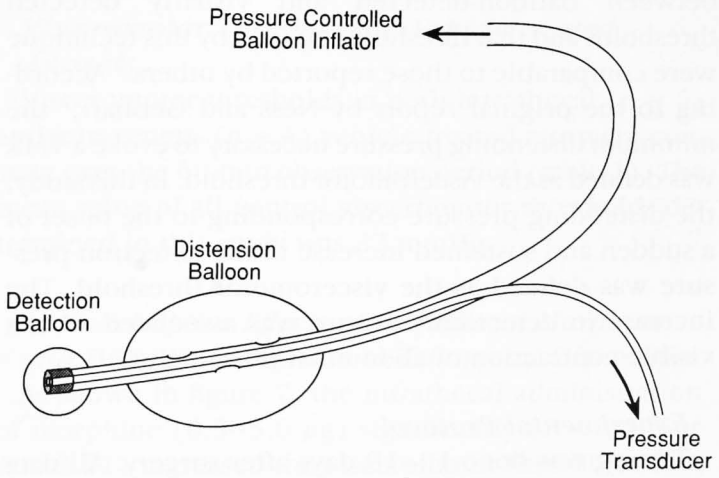


**Fig. 1. Method of detection of visceromotor response to colorectal distension. A distension balloon (5 cm long, flexible latex) was connected to a pressure-controlled balloon inflator that could inflate the distension balloon with air and that could control pressure within the distension balloon. A detection balloon (1.5 cm long, flexible latex) was located ahead of the distension balloon. The detection balloon was completely isolated from the pressure-controlled balloon inflator and far enough away (1.5 cm) from the rostral end of the distension balloon to avoid mechanical interference with the distension balloon. Pressures within the two balloons were recorded simultaneously.**

avoid tissue damage. The pressure control device was modeled after one described previously.[21] Pressure within the distension balloon was monitored continuously through an in-line pressure transducer.

In this study, a small latex balloon (1.5 cm long) was used to detect the VMR objectively. Figure 1 illustrates the device used for both the CRD and the detection of VMR. The detection balloon was attached distal to the distension balloon and was used to detect the increase in intraabdominal pressure when the VMR was evoked. The detection balloon was connected to a pressure transducer through a smaller catheter (PE-160 tube), and pressure within the detection balloon was continuously monitored along with the distending pressure on a chart recorder. The detection balloon was completely isolated from the pressure control device and mounted far enough away (1.5 cm) from the distension balloon so as not to interfere with or be affected by the distension balloon. The detection balloon was filled with 0.6 ml air after placement to ensure that it would reliably detect changes in intraluminal pressure. The distension balloon was not inflated before each test.

Because gastrointestinal distension can evoke reflex contraction and relaxation in other parts of the gastrointestinal tract, it was important that we validated the use of the detection balloon. In pilot studies§ we demonstrated that there was no significant difference between balloon-detected and visually detected thresholds and that thresholds defined by this technique were comparable to those reported by others.[2] According to the original report by Ness and Gebhart,[2] the minimum distending pressure necessary to evoke a VMR was defined as the visceromotor threshold. In this study, the distending pressure corresponding to the onset of a sudden and sustained increase in the detection pressure was defined as the visceromotor threshold. The increase in detection pressure was associated with a visible contraction of abdominal musculature.

*Experimental Protocol*

Testing was done 10–19 days after surgery. All data (visceromotor thresholds) were obtained from 87 awake rats. Fifty-nine of 87 rats were used again 3–5 days after the initial experiment but never received the same drug twice.

§ Harada Y, Nishioka, Kitahata, LM, Collins JG: Additional technique for detecting visceromotor response to colorectal distension in awake and lightly pentobarbital anesthetized rats (abstract). Society for Neuroscience Abstracts 17:1010, 1991.

In the case of intrathecal administration, the animal was lightly anesthetized with halothane for insertion of the distension and detection balloons. Both balloons, lightly coated with petroleum jelly, were inserted intraanally. The distension balloon was positioned in the descending colon and rectum such that the end of the balloon was 1–2 cm inside the anus. The detection balloon was positioned in the descending colon proximal to the distension balloon. Both balloons were kept in position by taping the balloon catheter to the base of the tail. After balloon insertion, the rats were allowed to recover from anesthesia for 10–20 min. For 20–60 min after full recovery, baseline visceromotor thresholds were repeatedly (5–8 times) measured every 5–10 min. The average of the last three values was defined as a control threshold. After baseline measurements, drugs were administered through the chronically implanted catheter. Postdrug thresholds were measured 5, 10, 15, 20, 30, and 45 min after administration. Each postdrug measurement was done only once at each time point. Five rats received intrathecal vehicle (physiologic saline 5 $\mu$l) for control trials and evaluation of the reliability of the detection balloon technique.

In the case of intravenous administration, a catheter (PE-50 tube) was inserted into the external jugular vein during halothane (1–2%) anesthesia, and both the distension and detection balloons were inserted intraanally as for intrathecal administration. After insertion, the rats were allowed to recover from anesthesia for 30–60 min. Within 20–60 min after full recovery, baseline visceromotor thresholds were measured repeatedly (five to eight times) every 5–10 min. The average of last three values was defined as a control threshold. Postdrug thresholds were measured 2.5, 5, 10, 15, and 20 min after administration. Each measurement was done only once at each time point. Four rats received intravenous vehicle (physiologic saline 0.5 ml) for control trials and evaluation of the reliability of the detection balloon method.

*Drugs*

The following drugs were used: morphine sulfate, DPDPE, U50,488H, naloxone hydrochloride, and naltrindole hydrochloride. All drugs except naltrindole were purchased from Sigma Chemical (St. Louis, MO). Naltrindole was purchased from Research Biochemicals (Natick, MA). Morphine, U50,488H, and naloxone were dissolved in sterile physiologic saline (0.9% sodium chloride). DPDPE and naltrindole were dissolved in distilled water. DPDPE was frozen in aliquots at





Table 1. Time Course of Visceromot
or Intravenous (iv) Vehicle

| | Control | 2.5 min |
|---|---|---|
| it (n = 5) | 22.6 ± 3.3 | — |
| iv (n = 4) | 22.0 ± 2.2 | 22.5 ± 1.7 |

Values (mmHg) are mean ± SD. Thresholds v

-80°C until use. The compou
after thawing.

Drugs were administered int
of 5 μl. The dead space 12 μ
cleared by a slow flush (30–60 s
Doses of agonists were as follow
2.5, and 5.0 μg; DPDPE, .5, 5
and U50,488H 5.0, 10.0 50.0
one (2.5 μg) was coadminister
μg) in four animals. Naltrindol
istered with morphine (.5 μ
with DPDPE (10 μg) in fi e an
U50,488H was also administ
volume of 0.5 ml by slow inj
riod). Doses were 0.5, 1 0, 2.
7 for each).

*Data Analysis*

To evaluate the time course a
the antinociception, the thresh

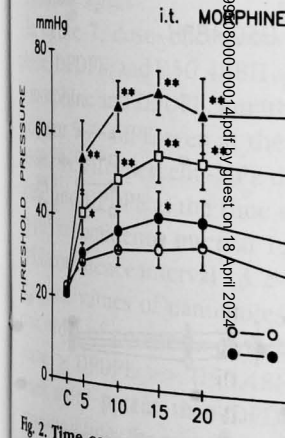


Fig. 2. Time course of visceromot
intrathecal morphine. Each point a
value and SEM in 6–11 animals. Co
dose were approximately 22 mmHg
thresholds in a dose-dependent m
change (*$P < 0.05$ and **$P < 0.01$ by
pared with those in vehicle-treat
sponding time point (data for vehi
Peak effect time was at approxima
tration. C = control.

**Table 1. Time Course of Visceromotor Thresholds in Awake Rats (n = 9) that Received Intrathecal (it) or Intravenous (iv) Vehicle**

| | Control | 2.5 min | 5 min | 10 min | 15 min | 20 min | 30 min | 45 min | 60 min |
|---|---|---|---|---|---|---|---|---|---|
| it (n = 5) | 22.6 ± 3.3 | — | 21.0 ± 3.1 | 20.8 ± 3.4 | 22.2 ± 2.3 | 21.8 ± 2.5 | 23.4 ± 4.2 | 21.6 ± 3.5 | 21.8 ± 2.1 |
| iv (n = 4) | 22.0 ± 2.2 | 22.5 ± 1.7 | 20.8 ± 2.8 | 21.5 ± 1.9 | 21.0 ± 2.9 | 21.3 ± 3.0 | 20.8 ± 2.2 | 22.8 ± 1.7 | — |

Values (mmHg) are mean ± SD. Thresholds were determined by a detection balloon technique.

−80°C until use. The compound was not used again after thawing.

Drugs were administered intrathecally in a volume of 5 μl. The dead space (12 μl) of the catheter was cleared by a slow flush (30–60 s) of physiologic saline. Doses of agonists were as follows: morphine, 0.5, 1.0, 2.5, and 5.0 μg; DPDPE, 2.5, 5.0, 10.0, and 25.0 μg; and U50,488H 5.0, 10.0, 50.0, and 100.0 μg. Naloxone (2.5 μg) was coadministered with morphine (2.5 μg) in four animals. Naltrindole (5 μg) was coadministered with morphine (2.5 μg) in four animals and with DPDPE (10 μg) in five animals.

U50,488H was also administered intravenously in a volume of 0.5 ml by slow injection (over a 30-s period). Doses were 0.5, 1.0, 2.5, and 5.0 mg/kg (n = 7 for each).

*Data Analysis*

To evaluate the time course and dose dependence of the antinociception, the thresholds after drug administration were compared with those of vehicle and the predrug thresholds by one-way and two-way analyses of variance for repeated measures. Antagonism by naloxone or naltrindole of morphine or DPDPE was analyzed by one-way analysis of variance. Fisher's least-significant difference test was used for *post hoc* comparisons of individual mean values. $P$ values $< 0.05$ were deemed statistically significant. To draw dose–effect lines for drugs, all threshold values were converted to percentage maximal possible effect (MPE) by the following equation: percentage MPE = 100 × (postdrug threshold − control threshold)/(80 − control threshold). By least-squares linear regression analysis, doses producing 50% MPE and its 95% confidence intervals were calculated.

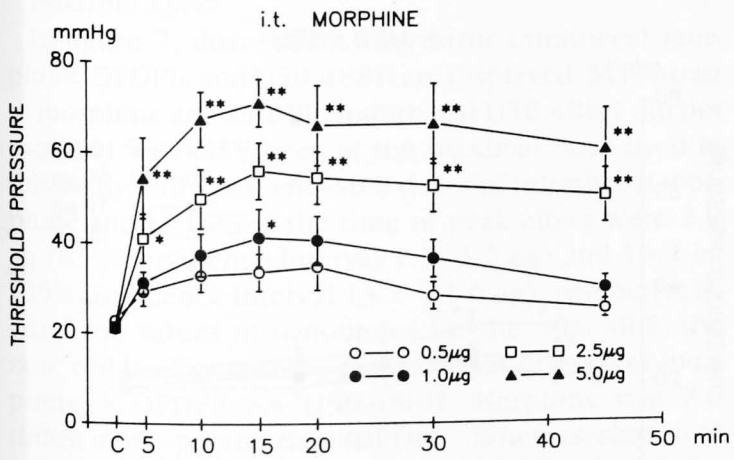


**Fig. 2. Time course of visceromotor threshold change after intrathecal morphine. Each point and bar represent the mean value and SEM in 6–11 animals. Control thresholds for each dose were approximately 22 mmHg. Morphine increased the thresholds in a dose-dependent manner with a significant change (*$P < 0.05$ and **$P < 0.01$ by analysis of variance) compared with those in vehicle-treated animals at the corresponding time point (data for vehicle are shown in table 1). Peak effect time was at approximately 15 min after administration. C = control.**

## Results

*Visceromotor Thresholds in Vehicle-treated Animals*

Visceromotor thresholds in both intrathecal (n = 5) and intravenous (n = 4) vehicle-treated rats were constant over the 60-min observation period (table 1). The mean value of all control visceromotor thresholds determined in this study was 22 mmHg.

*Antinociceptive Effects of Intrathecal Morphine and DPDPE in Response to Colorectal Distension*

As shown in figure 2, the intrathecal administration of morphine (0.5–5.0 μg) significantly ($P < 0.05$ or $P < 0.01$) increased the visceromotor thresholds in a dose-dependent manner. The peak effects were observed approximately 15 min after administration. At this time point, the thresholds (means ± SEM) for 0.5, 1.0, 25, and 5.0 μg morphine were 34 ± 5, 41 ± 6, 56 ± 6, and 71 ± 5 mmHg, respectively. Figure 3 shows the effects of 2.5–25 μg intrathecal DPDPE. The visceromotor thresholds increased significantly ($P < 0.05$ or $P < 0.01$) in a dose-dependent manner. The time for peak effect was approximately 15 min after admin-

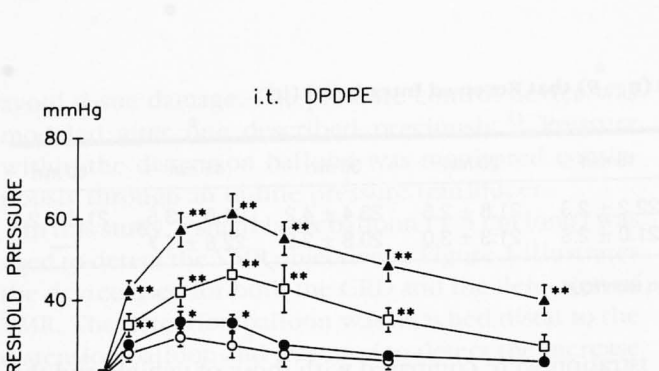


**Fig. 3. Time course of visceromotor threshold change after intrathecal [D-Pen², D-Pen⁵] enkephalin (DPDPE). Each point and bar represent the mean value and SEM in six to nine animals. Control thresholds for each dose were approximately 22 mmHg. DPDPE increased the thresholds in a dose-dependent manner with a significant change (*$P < 0.05$ and **$P < 0.01$ by analysis of variance) compared with those in vehicle-treated animals at the corresponding time point (data for vehicle are shown in table 1). Peak effect time was at approximately 15 min after administration. C = control.**

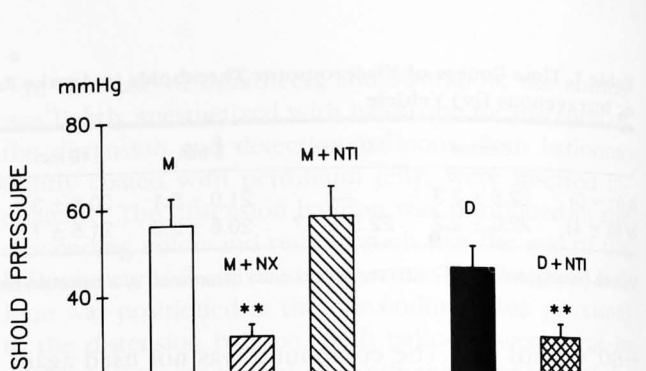


**Fig. 4. Histogram of visceromotor thresholds 15 min after the administration of intrathecal morphine (M) (2.5 μg) or [D-Pen², D-Pen⁵] enkephalin (D) (10 μg) with or without naloxone (NX) (2.5 μg) or naltrindole (NTI) (5 μg). Numbers of animals are shown under the x-axis. Each bar represents the SEM. By one-way analysis of variance, it was determined that the effect of morphine was antagonized by NX but not by NTI and that the effect of D was antagonized by NTI.**

istration. At this time point, the thresholds for 2.5, 5.0, 10, and 25 μg DPDPE were 28 ± 3, 34 ± 2, 46 ± 5, and 61 ± 5 mmHg, respectively. With the exception of the largest dose (25 μg), the thresholds returned to control levels within 45 min of administration.

As shown in figure 4, the antinociceptive effect of morphine (2.5 μg) was significantly ($P < 0.01$) antagonized by naloxone (2.5 μg) but not naltrindole (5 μg) at 15 min after administration. The antinociceptive effect of DPDPE (10 μg) was significantly ($P < 0.01$) antagonized by naltrindole (5 μg) at 15 min after administration.

### *Antinociceptive Effects of Intrathecal and Intravenous U50,488H in Response to Colorectal Distension*

In contrast to morphine and DPDPE, intrathecal U50,488H increased the visceromotor thresholds significantly ($P < 0.05$) at only 5 and 10 min after administration of 100 μg, a maximum dose that could be dissolved in 5 μl saline (fig. 5). The thresholds at each time point were 32 ± 3 and 33 ± 2 mmHg, respectively. Other doses of U50,488H had no significant effect on the threshold at any time. In contrast to intrathecal U50,488H, intravenous U50,488H (0.5–5.0 mg/kg) increased the visceromotor thresholds significantly ($P < 0.05$ or $P < 0.01$) in a dose-dependent manner (fig. 6). The time of peak effect was approximately 2.5 min after administration. At this time point, the thresholds for 0.5, 1.0, 2.5, and 5.0 mg/kg were 29 ± 3, 42 ± 3, 53 ± 7, and 65 ± 7 mmHg, respectively. The dose producing 50% MPE at 2.5 min after administration was 2.6 mg/kg (95% confidence interval 1.9–3.6 mg/

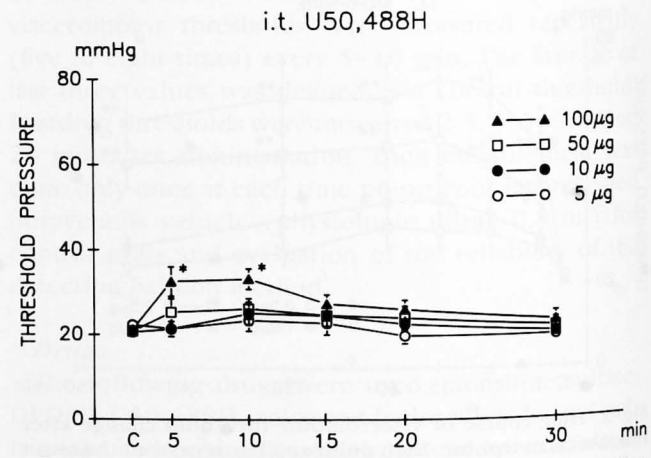


**Fig. 5. Time course of visceromotor threshold change after intrathecal U50,488H. Each point and bar represent the mean value and SEM in six to eight animals. Control thresholds for each dose were approximately 22 mmHg. U50,488H increased the thresholds significantly (*$P < 0.05$ by analysis of variance) only 5 and 10 min after 100 μg (a maximum dose that could be dissolved in 5 μl of saline) was administered. Other doses tested did not increase the threshold at any time. C = control.**





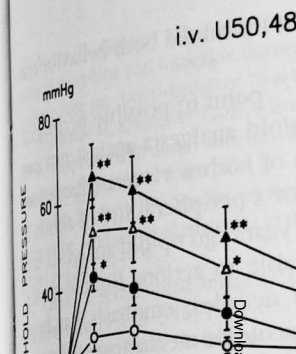


Fig. 6. Time course of viscero
intravenous U50,488H. Each point a
value and SEM in seven animals. C
dose were approximately 21 mmH
thresholds in a dose-dependent
change (*$P < 0.05$ and **$P < 0.01$ by
pared with those in vehicle-treat
sponding time point (data for vehi
Peak effect time was at approxima
istration. C = control.

kg). The thresholds returned t
min at all doses tested.

*Potencies of Intrathecal Mo*
*U50,488H for Producing 50*
*Possible Effect*

In figure 7, dose–effect curv
phine, DPDPE, and U50,488H a
to morphine and DPDPE, intrath
produce 50% MPE even at the
this study. Fifty percent–MPE d
phine and DPDPE at the time
μg (95% confidence interval 1.
(95% confidence interval 13.2–
From the values of nanomoles
rank order of potencies of ind
phine > DPDPE >> U50,488
times more potent than DPD
regression lines for morphine a
nificantly different, the regress
was not parallel with those for

## Discussion

In the current study, we have
trathecal μ (morphine) and

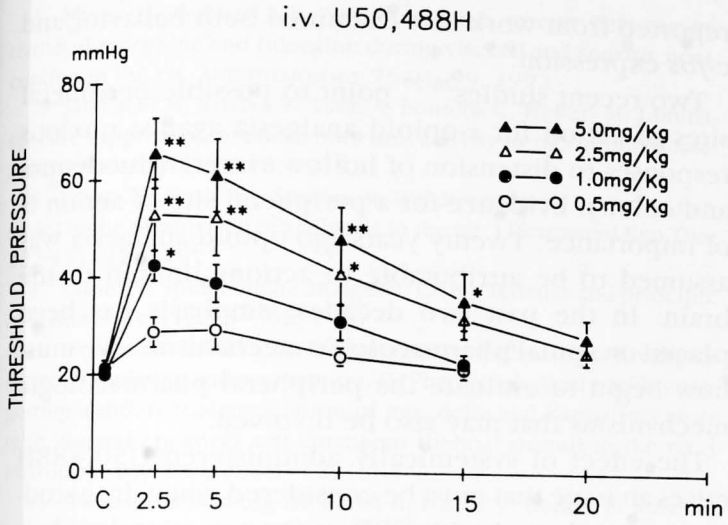


**Fig. 6. Time course of visceromotor threshold change after intravenous U50,488H. Each point and bar represent the mean value and SEM in seven animals. Control thresholds for each dose were approximately 21 mmHg. U50,488H increased the thresholds in a dose-dependent manner with a significant change (*$P < 0.05$ and **$P < 0.01$ by analysis of variance) compared with those in vehicle-treated animals at the corresponding time point (data for vehicle are shown in table 1). Peak effect time was at approximately 2.5 min after administration. C = control.**

kg). The thresholds returned to control levels by 20 min at all doses tested.

*Potencies of Intrathecal Morphine, DPDPE and U50,488H for Producing 50% Maximum Possible Effect*

In figure 7, dose–effect curves for intrathecal morphine, DPDPE, and U50,488H are displayed. In contrast to morphine and DPDPE, intrathecal U50,488H did not produce 50% MPE even at the maximal dose used in this study. Fifty percent–MPE doses of intrathecal morphine and DPDPE at the time of peak effect were 2.2 μg (95% confidence interval 1.5–3.2 μg) and 16.4 μg (95% confidence interval 13.2–21.6 μg), respectively. From the values of nanomoles for the 50% MPE, the rank order of potencies of individual drugs was morphine > DPDPE >> U50,488H. Morphine was 7.6 times more potent than DPDPE. Whereas slopes of regression lines for morphine and DPDPE were not significantly different, the regression line for U50,488H was not parallel with those for morphine and DPDPE.

## Discussion

In the current study, we have demonstrated that intrathecal μ (morphine) and δ (DPDPE) but not κ (U50,488H) opioid receptor agonists produce potent antinociceptive effects when tested against a visceral pain model that relies on mechanical distension of a hollow organ. κ-Opioid receptors seem to also be involved in attenuating a VMR to CRD but only when the agonists are administered systemically. These results are in agreement with a recent report of a similar study by Danzebrink and colleagues.[22]

The evidence that intrathecal morphine produces powerful visceral antinociception in the CRD test is consistent with previous reports.[2,6] Ness and Gebhart[2] reported that the median effective doses of intrathecal morphine for inhibiting cardiovascular responses in the rat were 6.2 μg (pressor response) and 5.2 μg (tachycardia response). Maves and Gebhart[6] reported that the 50% MPE dose of intrathecal morphine in inhibiting VMR was 1.5 μg in the rat. In the current study, the VMR was inhibited with a 50% MPE dose of 2.2 μg. That intrathecal morphine exerted visceral antinociceptive effects was also demonstrated using the writhing test in the rat.[10,13] These reports suggest that spinal μ-opioid receptors are likely to be involved in modulating visceral nociception.

Although morphine preferentially interacts with μ receptors, it has been reported that it may affect somatic and visceral[23] responses through interactions with δ receptors. In the current study, the antinociceptive effect of morphine was antagonized by a nonselective opioid receptor antagonist, naloxone, but not by a

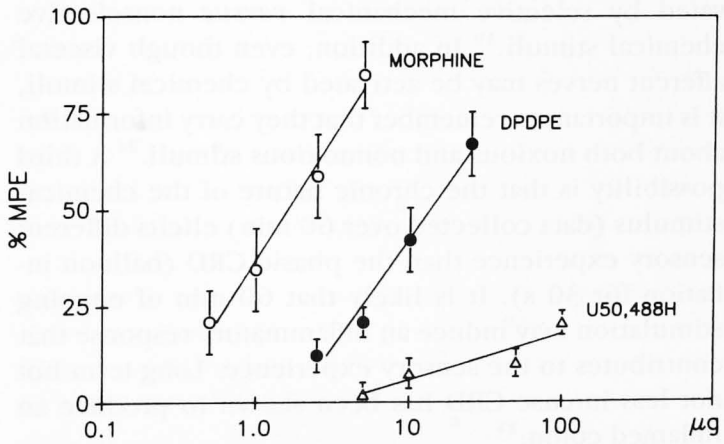


**Fig. 7. Dose–effect relations and regression lines for intrathecal morphine, [D-Pen², D-Pen⁵] enkephalin (DPDPE), and U50,488H. Each point and bar represent the mean value and SEM. The lines for morphine and DPDPE were derived from the data at 15 min and the line for U50,488H from the data at 10 min after administration (deemed the peak effect times). Doses are plotted in log scale. U50,488H produced a significant antinociceptive effect (20.6% MPE) at only 100 μg.**

highly selective δ receptor antagonist, naltrindole. Our results suggest that intrathecal morphine produces visceral antinociception against mechanical stimuli mainly through the μ- rather than δ-opioid receptors.

Our finding that intrathecal DPDPE is effective in the modulation of visceral nociceptive information is in agreement with experiments in mice reported by Porreca *et al.*[11] In addition, we demonstrated that the visceral antinociceptive effect of intrathecal DPDPE was antagonized by the selective δ receptor antagonist, naltrindole. Therefore, it is likely that spinal δ-opioid receptors mediate visceral antinociception induced by mechanical distension of hollow visceral.

Our results are not in complete agreement with the work of Schmauss and Yaksh.[10] They reported a significant reduction of writhing by U50,488H at doses tested in the current study. In their study 100 μg of U50,488H caused a 70% reduction in the cumulative writhing score. They also reported that δ agonists were without effect at the doses studied. The most obvious difference in the studies is the method for eliciting a noxious visceral stimulus. We propose that the differences in pharmacologic effect between the two studies is a result of differences between the stimuli. CRD provides a selective physiologic stimulus that activates mechanoreceptors in the muscle layer of the colon and rectum. In contrast, intraperitoneal injection of an irritant substance is thought to activate unknown receptors mainly on the serosal side of visceral structures but also those associated with nonvisceral structures. It is clear that different pathways are likely to be activated by selective mechanical *versus* nonselective chemical stimuli.[19] In addition, even though visceral afferent nerves may be activated by chemical stimuli, it is important to remember that they carry information about both noxious and nonnoxious stimuli.[24] A third possibility is that the chronic nature of the chemical stimulus (data collected over 60 min) elicits different sensory experience than the phasic CRD (balloon inflation for 30 s). It is likely that 60 min of ongoing stimulation may induce an inflammatory response that contributes to the sensory experience. Long-term but not less intense CRD has been shown to produce an inflamed colon.[24]

In this study, although we have not examined the direct antinociceptive effects of U50,488H at supraspinal sites, evidence that intravenous U50,488H is effective in inhibiting VMR to CRD suggests that the supraspinal or peripheral κ receptors can be involved in antinociception against CRD. Similar results have been reported from work that examined both behavior and c-*fos* expression.[25]

Two recent studies[26,27] point to possible peripheral sites of action for κ-opioid analgesia against noxious responses to distension of hollow viscera (duodenum and colon). Evidence for a peripheral site of action is of importance. Twenty years ago opioid analgesia was assumed to be attributable to actions limited to the brain. In the past two decades, emphasis has been placed on spinal pharmacologic mechanisms. We must now begin to evaluate the peripheral pharmacologic mechanisms that may also be involved.

The effect of systemically administered U50,488H raises an issue that must be considered when drug studies are conducted with CRD as the test stimulus. It is well established that opioids can influence gastrointestinal motility. We need to recognize that such an effect may influence responses to CRD in at least two different ways. If the resting tone of the smooth muscle is changed, then the resistance against which the stimulus is presented would be altered. We observed no such change in pressure records in the detecting balloon. A second possible change would be a change in the responsiveness of the smooth muscle or mechanoreceptors to mechanical stimulation. However, Diop and colleagues[26] reported no significant change in volume of air required to distend the colon, suggesting that the compliance of the colon was not altered.

It is important to keep in mind that all pain is not the same and that the pharmacologic control of pain may depend in part on the nature of the stimulus and the neurochemical events that are activated. A challenge we face is to define more precisely the nature of clinical pain and the neurotransmitter systems involved in communicating information about clinical pain.

## References

1. Ness TJ, Gebhart GF: Visceral pain: A review of experimental studies. Pain 41:167–234, 1990

2. Ness TJ, Gebhart GF: Colorectal distension as a noxious visceral stimulus: Physiologic and pharmacologic characterization of pseudoaffective reflexes in the rat. Brain Res 450:153–169, 1988

3. Ness TJ, Gebhart GF: Differential effects of morphine and clonidine on visceral and cutaneous spinal nociceptive transmission in the rat. J Neurophysiol 62:220–230, 1989

4. Ness TJ, Gebhart GF: Interactions between visceral and cutaneous nociception in the rat: II. Noxious visceral stimuli inhibit cutaneous nociceptive neurons and reflexes. J Neurophysiol 66:29–39, 1991b

5. Danzebrink RM, Gebhart GF: Evidence that 5-$HT_{-1}$ 5-$HT_2$ and 5-$HT_3$ receptor subtypes modulate responses to noxious colorectal distension in the rat. Brain Res 538:64–75, 1991





6. Maves TJ, Gebhart GF: Antinocice
rathecal morphine and lidocaine during
ception in the rat. ANESTHESIOLOGY 76:91
7. Kitahata LM, Kosaka Y, Taub A, Bo
specific suppression of dorsal horn unit a
ANESTHESIOLOGY 41:39–48, 1974
8. Yaksh TL, Rudy TA: Studies on the
cotics in the production of analgesia in th
202:411–428, 1977
9. Yaksh TL: Spinal opiate analgesia: C
of action. Pain 11:293–346, 1981
10. Schmauss C, Yaksh TL: In vivo stud
systems mediating antinociception: II. P
gesting a differential association of mu
with visceral chemical and cutaneous
Pharmacol Exp Ther 288:1–12, 1984
11. Porreca F, Mosberg HI, Hurst R,
mu, delta and kappa opioid receptors in
dation of gastrointestinal transit effect
the mouse. J Pharmacol Exp Ther 230:
12. Millan MJ: Multiple opioid syste
349, 1986
13. Lipkin M, Sleisenger M: Studies
ments of stimulus intensity and duratio
of pain in esophagus, ileum and colo
1957
14. Murray CW, Cowan A: Tonic pai
Differential modulation by three recept
J Pharmacol Exp Ther 257:335–341, 1
15. Bloomfield AL, Polland N
the gastrointestinal tract: II. Stomach,
Invest 10:453–473, 1931
16. Ritchie JA, Adrian GM, Trae
mentally induced colonic pain (abstra

6. Maves TJ, Gebhart GF: Antinociceptive synergy between intrathecal morphine and lidocaine during visceral and somatic nociception in the rat. ANESTHESIOLOGY 76:91–99, 1992

7. Kitahata LM, Kosaka Y, Taub A, Bonidos C, Hoffert M: Lamina-specific suppression of dorsal horn unit activity by morphine sulfate. ANESTHESIOLOGY 41:39–48, 1974

8. Yaksh TL, Rudy TA: Studies on the direct spinal action of narcotics in the production of analgesia in the rat. J Pharmacol Exp Ther 202:411–428, 1977

9. Yaksh TL: Spinal opiate analgesia: Characteristics and principle of action. Pain 11:293–346, 1981

10. Schmauss C, Yaksh TL: In vivo studies on spinal opiate receptor systems mediating antinociception: II. Pharmacological profiles suggesting a differential association of mu, delta and kappa receptors with visceral chemical and cutaneous thermal stimuli in the rat. J Pharmacol Exp Ther 288:1–12, 1984

11. Porreca F, Mosberg HI, Hurst R, Hruby V, Burks TF: Roles of mu, delta and kappa opioid receptors in spinal and supraspinal mediation of gastrointestinal transit effects and hot-plate analgesia in the mouse. J Pharmacol Exp Ther 230:341–348, 1984

12. Millan MJ: Multiple opioid systems and pain. Pain 27:303–349, 1986

13. Lipkin M, Sleisenger M: Studies of visceral pain: Measurements of stimulus intensity and duration associated with the onset of pain in esophagus, ileum and colon. J Clin Invest 37:28–34, 1957

14. Murray CW, Cowan A: Tonic pain perception in the mouse: Differential modulation by three receptor-selective opioid agonists. J Pharmacol Exp Ther 257:335–341, 1991

15. Bloomfield AL, Polland NS: Experimental referred pain from the gastrointestinal tract: II. Stomach, duodenum and colon. J Clin Invest 10:453–473, 1931

16. Ritchie JA, Adrian GM, Truelove SC: Observations on experimentally induced colonic pain (abstract). Gut 13:841, 1972

17. Ritchie J: Pain from distension of the pelvic colon by inflating a balloon in the irritable colon syndrome. Gut 14:125–132, 1973

18. Swarbrick ET, Hegarty JE, Bat L, Williams CB, Dawson AM: Site of pain from the irritable bowel. Lancet 2:443–446, 1980

19. LaMotte RH: Subpopulations of 'nocifensor neurons' contributing to pain and allodynia, itch and allokinesis. American Pain Society Journal 1:115–126, 1992

20. Yaksh TL, Rudy TA: Chronic catheterization of the spinal subarachnoid space. Physiol Behav 17:1031–1036, 1976

21. Anderson RH, Ness TJ, Gebhart GF: A distension control device useful for quantitative studies of hollow organ sensation. Physiol Behav 41:635–638, 1987

22. Danzebrink RM, Green SA, Gebhart CF: Spinal mu and delta, but not kappa, opioid receptor agonists attenuate responses to colorectal distension in the rat. Pain (in press)

23. Takemori AE, Portoghese PS: Evidence for the interaction of morphine with kappa and delta opioid receptors to induce analgesia in $\beta$-funaltrexamine-treated mice. J Pharmacol Exp Ther 243:91–94, 1987

24. Traub RJ, Pechman P, Iadarola MJ, Gebhart GF: Fos-like proteins in the lumbosacral spinal cord following noxious and non-noxious colorectal distension in the rat. Pain 49:393–403, 1992

25. Hammond DL, Presley R, Gogas KR, Basbaum AI: Morphine or U-50,488 suppresses fos protein-like immunoreactivity in the spinal cord and nucleus tractus solitarii evoked by a noxious visceral stimulus in the rat. J Comp Neurol 315:244–253, 1992

26. Diop L, Riviere PJM, Pascaud X, Dassaud M, Junien JL: Role of vagal afferents in the antinociception produced by morphine and U-50,488H in the colonic pain reflex in rats. Eur J Pharmacol 257: 181–187, 1994

27. Diop L, Riviere PJM, Pascaud X, Dassaud M, Junien JL: Peripheral kappa-opioid receptors mediate the antinociceptive effect of fetodozine on the duodenal pain reflex in rat. Eur J Pharmacol 271:65–71, 1994