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Visceral Antinociceptive Effects of Spinal Clonidine Combined with Morphine, [D-Pen², D-Pen⁵] Enkephalin, or U50,488H

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Background: **Visceral pain is an important component of many clinical pain states. The perispinal administration of drug combinations rather than a single agent may reduce side effects while maximizing analgesic effectiveness. The purpose of this study was to examine the nature of interactions between an α_2-adrenergic agonist (clonidine) and a μ-opioid agonist (morphine), a δ-opioid agonist ([D-Pen², D-Pen⁵] enkephalin [DPDPE]), or a κ-opioid agonist (U50,488H).**

Methods: **Colorectal distension was used to elicit a nociceptive visceromotor response (contraction of abdominal musculature) in rats. The ability of intrathecally administered clonidine alone or in combination with morphine, DPDPE, or U50,488H to alter thresholds for the production of the visceromotor response was examined.**

Results: **Clonidine produced dose-dependent reduction in visceromotor response thresholds and, when combined with morphine or DPDPE, produced a synergistic reduction in the threshold. U50,488H, at the doses tested, showed no synergistic interaction with clonidine.**

Conclusions: **Spinal combinations of α_2-adrenergic and μ- or δ- but not κ-opioid agonists may be beneficial in the control of visceral pain. (Key words: Analgesics, α_2-adrenergic agonists: clonidine. Analgesics, opioid: [D-Pen², D-Pen⁵] enkephalin; morphine; U50,488H. Pain, visceral: colorectal distension.)**

DEEP pain associated with the viscera is different from somatic cutaneous pain.[1] Because of its clinical importance, there is a need for a better understanding of the pharmacologic control of visceral pain. So far, however, less attention has been devoted to visceral pain than somatic pain, probably in large part because of the lack of appropriate analgesiometric tests for visceral nociception. Colorectal distension (CRD) was originally characterized by Ness and Gebhart as a reliable and useful model of visceral pain in the awake rat.[2] CRD is a reproducible, minimally invasive, reliable noxious visceral stimulus.[2] In addition, it mimics visceral pain in humans.[3,4] Using the CRD test, investigators have begun to focus on visceral antinociception and mechanisms of visceral pain.[5–9] In these reports, it has been demonstrated that opioid (morphine) and α_2-adrenergic receptor agonists (*e.g.*, clonidine and ST-91) modulate visceral nociception at the level of the spinal cord.[2,6,7,9]

In somatic pain studies in animals, there is abundant evidence for synergistic-like interactions between spinally administered opioid and α_2-adrenergic agonists.[10–16] True synergism has been shown by isobolographic analysis.[17–19] Determining optimum drug combinations that, at minimal doses, produce powerful analgesia with less side effects is of great interest for management of pain.[20]

The purpose of this study was to examine by isobolographic analysis the nature of interactions between clonidine and morphine, clonidine and [D-Pen², D-Pen⁵] enkephalin (DPDPE), and clonidine and U50,488H for visceral antinociception with the CRD test. Portions of this study have been reported previously.§‖

Materials and Methods

The protocol of this study was approved by the Yale Animal Care and Use Committee. Experiments were

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§ Harada Y, Nishioka K, Kitahata LM, Collins JG: Significant synergism between intrathecal morphine and clonidine for visceral nociception (abstract). ANESTHESIOLOGY 75:A660, 1991.

‖ Harada Y, Nishioka K, Kitahata, LM, Collins, JG: Contrasting analgesic action of the intrathecal kappa agonist (U50,488H) in visceral pain processing as compared to morphine and clonidine (abstract). ANESTHESIOLOGY 75:A663, 1991.

conducted on adult male Sprague-Dawley rats weighing 280–360 g. An in-depth description of methods is contained in the accompanying article.[21] Under general anesthesia an intrathecal catheter was placed near the lumbar enlargement of the spinal cord according to a method described previously.[22]

Antinociceptive Test

CRD evokes reliable cardiovascular and behavioral (visceromotor) responses that are easily measured. These responses are useful measures of visceral nociception.[2] Inhibition of the responses by a drug is a valid and reliable indication of antinociceptive efficacy.[2] In this study, we used the visceromotor response (a contraction of abdominal musculature) as a measure of visceral nociception. CRD was achieved with pressure-controlled air inflation of a latex distension balloon (5 cm long). Visceromotor response was detected with a 1.5-cm–long detection balloon attached distal to the distension balloon and inflated with 6 ml air to ensure sensitivity to changes in intraluminal pressure.

According to previous reports,[2,5] the minimum distending pressure necessary to evoke a visceromotor response 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 balloon 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 10–18 days after surgery. All data (visceromotor thresholds) were obtained from 108 awake rats by the detection balloon method described above. (Information about the 50% effective doses [ED_{50}] of morphine, DPDPE, and U50,488H was obtained from 87 animals, as reported in the accompanying article.[21] Those 87 animals are included in the total of 108 animals described in this report.) Seventy-seven of 108 rats were used again 3–5 days after the initial experiment but never received the same drug twice. On the day of an experiment, the rats were lightly anesthetized with halothane for insertion of both distension and detection balloons. After balloon insertion, the rats were allowed to recover from anesthesia for 10–20 min. For 20–60 min after full recovery, baseline values of visceromotor thresholds were repeatedly (four to seven times) measured every 5–10 min. The average of the last three values was defined as a control threshold value. After baseline measurements, drugs were administered intrathecally through the chronically implanted catheter. Postdrug thresholds were measured 5, 10, 15, 20, 30, and 45 min after drug administration. Each postdrug measurement was done only once at each time point.

Drugs

Clonidine hydrochloride (Sigma Chemical, St. Louis, MO), morphine sulfate (Sigma), DPDPE (Research Biochemicals, Natick, MA), and U50,488H (Sigma) were used as α_2-adrenergic, μ-opioid, δ-opioid, and κ-opioid receptor agonists, respectively. Yohimbine hydrochloride (Sigma) and naloxone hydrochloride (Sigma) were used as α_2-adrenergic and opioid receptor antagonists, respectively. All compounds were dissolved in sterile physiologic saline, and 5 or 10 μl solution was administered intrathecally. Drugs were administered slowly (over a period of 30–60 s). The dead space (12 μl) of the catheter was cleared by a similarly slow flush of physiologic saline. Clonidine, as with the other agonists in the accompanying study,[21] was administered at four doses to derive dose–effect curves. Doses and volumes of drugs are summarized in table 1. Because the doses of 100 μg U50,488H or 20 μg yohimbine could not be dissolved in 5 μl saline, these compounds were dissolved in 10 μl saline.

To perform isobolographic analysis, clonidine and morphine were coadministered at a fixed dose ratio (2:1) as shown in table 1. This ratio was selected to be close to the actual ratio (2.7:1) of the ED_{50}s for clonidine and morphine when used alone (6.2 μg for clonidine and 2.3 μg for morphine). Clonidine and DPDPE were coadministered at a fixed dose ratio of 1:2.5 to be close to the actual ratio (1:2.65) of the ED_{50}s of clonidine and DPDPE when given alone (6.2 μg for clonidine and 16.4 for DPDPE). Clonidine and U50,488H were coadministered as shown in table 1. In this case, the isobolographic analysis was not performed because the 50% maximum possible effect (MPE) for U50,488H could not be acquired even when 100 μg (the maximum dose that could be dissolved in 10 μl saline) was administered. In addition, the dose–response curve for U50,488H and clonidine were not parallel. Yohimbine (20 μg) or naloxone (5 μg) was administered in some rats after the testing of clonidine (10 μg) or morphine (5 μg), respectively. All drug doses are presented as micrograms of the salt. Five rats received 5 μl intrathecal vehicle for control trials and

Table 1. Doses and Volumes of Drugs Administered Intrathecally

Drug	Dose (μg)				Volume (μl)	n
Clonidine (C)	1	2.5	5	10	5	46
Morphine (M)*	0.5	1	2.5	5	5	44
DPDPE (D)*	2.5	5.0	10	25	5	46
U50,488H (U)*	5	10	50	100	5	29
C + M	0.2 + 0.1		0.5 + 0.25		5	18
	1 + 0.5		2 + 1		5	17
C + D	0.4 + 1.0		1.0 + 2.5		5	15
	2.0 + 5.0		3.0 + 7.5		5	14
C + U	2.5 + 50		5 + 50		5	14
	12.5 + 100		5 + 100		10	12
Yohimbine		20			10	7
Naloxone		5			5	6

* From Harada *et al.*

evaluation of the reliability of the detection balloon technique.

Data Analysis and Isobologram Construction

The isobologram displays graphically a pharmacologic characterization of drug-to-drug interaction (supraadditive, additive, or subadditive) on x,y coordinates. It uses equieffective doses of individual and combined drugs. To calculate equieffective doses, all visceromotor thresholds were converted to percentage MPE by the following equation: percentage MPE = 100 × (postdrug threshold − control threshold)/(80 − control threshold). To construct an isobologram for the dose producing 50% MPE, using a least-squares regression analysis, the 50% MPE dose and its 95% confidence intervals (95% CIs) were calculated. For the combinations of clonidine and morphine and of clonidine and DPDPE, total doses of the combined drugs were used for a least-squares regression analysis. Component doses of clonidine and morphine and of clonidine and DPDPE for 50% MPE were derived from the combination dose ratio used in this study (clonidine:morphine = 2:1 and clonidine:DPDPE = 1:2.5). An isobologram was constructed by plotting the 50% MPE dose with its 95% CIs on the x,y coordinates (x for clonidine and y for morphine or DPDPE). If the experimentally determined isobole (a point representing x,y coordinates for the 50% MPE dose) fell significantly below the theoretically additive isobole, the interaction between clonidine and morphine or DPDPE was to be defined as supraadditive (synergistic). The theoretical isobole for the purely additive interaction was derived from an additive line and the combination dose ratio. The additive line was drawn by connecting the point indicating the 50% MPE dose on the x-axis (clonidine given alone) with that on the y-axis (morphine or DPDPE given alone). The 95% CIs for the theoretical additive isobole were similarly acquired by connecting the 95% CIs on the x-axis with that on the y-axis. Although the isobologram provides a convenient graphical display, it usually contributes little to the necessary statistical analysis.[23] For the statistical estimation of the difference between the experimental 50% MPE dose and the theoretically additive 50% MPE dose, potency ratio analysis was used, as described in the appendix.

Statistics

All values were expressed as the means ± SEM. One-way analysis of variance followed by Fisher's least-significant difference test as a *post hoc* test for multiple comparisons was used to compare the effect at different doses or at different times. A paired and unpaired Student's *t* test was used to analyze reversibility by yohimbine or naloxone. Dose–response curves were obtained using a least-squares linear regression analysis. The test for parallelism of dose–response curves and the potency ratio analysis were performed according to a method described previously.[24] *P* values < 0.05 were deemed statistically significant.

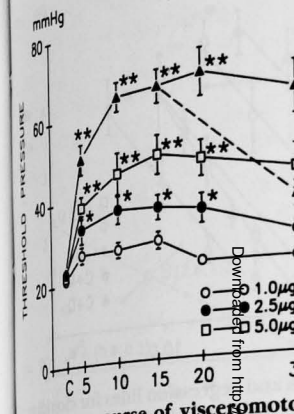

Fig. 1. Time course of visceromoto
intrathecal clonidine at four doses
resent the mean value and SEM in 10
for each dose was approximately 2
was between 10 and 20 min. Clonic
olds in a dose-dependent manner
(*$P < 0.05$ and $P < 0.01$ by analysis o
times. Intrathecal yohimbine (20 μg,
at 18 min after 10 μg clonidine, dec
nificantly at 30 and 45 min ($P < 0.$

Results

Reliability of the Detection Determining Visceromotor

The mean value of all control determined by the detection ba mmHg, a value comparable to originally reported by Ness an or electromyographic detectio rat.[2] In the animals that rece trathecally, the visceromotor constant during the 90-min ob

Effects of Intrathecal Clonic Visceromotor Threshold

As shown in figure 1, intrat cantly increased visceromotor pendent manner ($P < 0.05$). observed between 10 and 20 m when administered intratheca istration of clonidine (10 μg), c significantly at 30 and 45 mi with the thresholds in animals bine at 15, 30, and 45 min).

As reported in the accompany morphine increased the thresh 0.05) in a dose-dependent ma was approximately 15 min. N

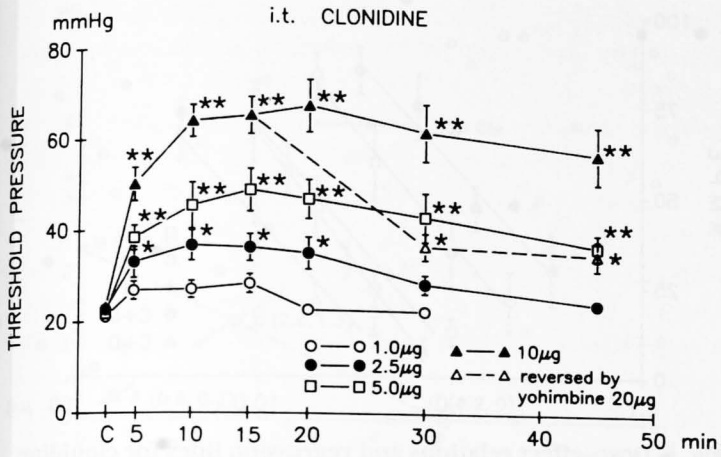

Fig. 1. Time course of visceromotor threshold change after intrathecal clonidine at four doses. Each point and bar represent the mean value and SEM in 10–13 rats. Control threshold for each dose was approximately 22 mmHg. Peak effect time was between 10 and 20 min. Clonidine increased the thresholds in a dose-dependent manner with a significant change (*$P < 0.05$ and $P < 0.01$ by analysis of variance) at the indicated times. Intrathecal yohimbine (20 μg, n = 7), when administered at 18 min after 10 μg clonidine, decreased the thresholds significantly at 30 and 45 min ($P < 0.05$).

Results

Reliability of the Detection Balloon for Determining Visceromotor Response

The mean value of all control visceromotor thresholds determined by the detection balloon method was 22.0 mmHg, a value comparable to the value (22.4 mmHg) originally reported by Ness and Gebhart using visual or electromyographic detection methods in the awake rat.[2] In the animals that received vehicle alone intrathecally, the visceromotor thresholds remained constant during the 90-min observation period.

Effects of Intrathecal Clonidine on the Visceromotor Threshold

As shown in figure 1, intrathecal clonidine significantly increased visceromotor thresholds in a dose-dependent manner ($P < 0.05$). The peak effects were observed between 10 and 20 min. Yohimbine (20 μg), when administered intrathecally 18 min after administration of clonidine (10 μg), decreased the thresholds significantly at 30 and 45 min ($P < 0.05$ compared with the thresholds in animals not treated with yohimbine at 15, 30, and 45 min).

As reported in the accompanying article,[21] intrathecal morphine increased the thresholds significantly ($P < 0.05$) in a dose-dependent manner. Peak effect time was approximately 15 min. Naloxone (5 μg), when administered intrathecally 18 min after morphine (5 μg), decreased the thresholds significantly ($P < 0.05$) at 30 and 45 min. Similarly, DPDPE increased the thresholds significantly ($P < 0.05$) in a dose-dependent manner. The peak effect occurred approximately 15 min after administration.

In contrast to clonidine, morphine, and DPDPE, as reported in the accompanying article,[21] U50,488H increased the thresholds significantly ($P < 0.05$) at only 5 and 10 min after administration of 100 μg, the maximum dose that could be dissolved in 10 μl saline. Other intrathecal doses of U50,488H had no significant effect on the visceromotor threshold at any time.

Antinociceptive Interactions after Intrathecal Coadministration of Clonidine and Morphine, Clonidine and DPDPE, or Clonidine and U50,488H

As shown in figure 2, combinations of clonidine and morphine and, in figure 3, clonidine and DPDPE increased the visceromotor thresholds in a dose-dependent manner with less of each drug compared with experiments in which the drugs were used alone. A regression line for the dose–effect relation of combined clonidine and morphine or clonidine and DPDPE at 15 min after administration was shifted leftward from the regression lines for both individual morphine and DPDPE doses at 15 min after administration (fig. 4). Dose–response functions for morphine and DPDPE alone shown in figure 4 and table 2 correspond to the values reported in the accompanying article.[21]

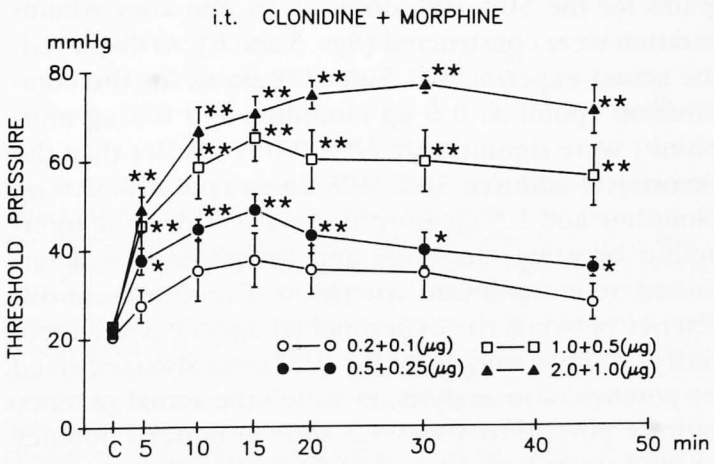

Fig. 2. Time course of visceromotor threshold change after intrathecal coadministration of clonidine and morphine in four combinations. Each point and bar represent the mean value and SEM in eight or nine rats. The ratio of combination doses was kept constant (clonidine:morphine = 2:1). *$P < 0.05$; **$P < 0.01$.

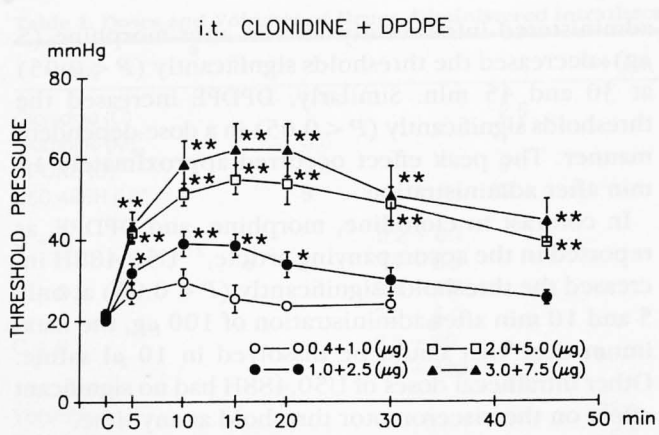

Fig. 3. Time course of visceromotor threshold change after intrathecal coadministration of clonidine and [D-Pen², D-Pen⁵] enkephalin (DPDPE) in four combinations. Each point and bar represent the mean value and SEM in seven or eight rats. The ratio of combination doses was kept constant (clonidine: DPDPE = 1:2.5). *$P < 0.05$; **$P < 0.01$.

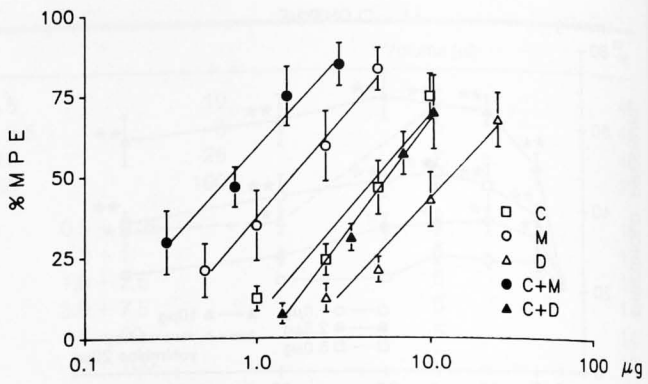

Fig. 4. Dose–effect relations and regression lines for clonidine, morphine, [D-Pen², D-Pen⁵] enkephalin (DPDPE), clonidine plus morphine, and clonidine plus DPDPE 15 min after administration (deemed as the peak effect times for drugs used alone). Each point and bar represent the mean value and SEM. Doses are plotted in log scale; for combinations, combined total doses are plotted on the x-axis. By least-squares regression analysis, doses for 50% maximum possible effect were calculated as follows: 6.2 μg for clonidine alone, 2.3 μg for morphine alone, 16.4 for DPDPE alone, 0.9 μg (0.6 μg clonidine plus 0.3 μg morphine) for the clonidine–morphine combination, and 6.8 μg (1.9 clonidine plus 4.9 DPDPE) for the clonidine–DPDPE combination.

Fifty percent–MPE doses for clonidine, morphine, and DPDPE used alone were 6.2, 2.3, and 16.4 μg, respectively. When drugs were combined, the total dose for 50% MPE was 0.9 μg (0.6 μg clonidine plus 0.3 μg morphine) and 6.8 μg (1.9 μg clonidine plus 4.9 μg DPDPE). These values (micrograms) are summarized in table 2 with nanomoles for 50% MPE and slopes of regression lines.

The leftward shift of the regression line suggested synergistic interactions between clonidine and morphine and between clonidine and DPDPE. To determine the nature of the interaction between drugs, isobolograms for the 50% MPE doses at 15 min after administration were constructed (figs. 5 and 6). As displayed, the actual experimental 50% MPE doses for the combination (point A: 0.6 μg clonidine and 0.3 μg morphine) were significantly ($P < 0.05$) smaller than the theoretical additive 50% MPE doses (point B: 2.6 μg clonidine and 1.3 μg morphine). Therefore, the interaction between clonidine and morphine was determined to be significant synergism. The significant difference between the experimental isobole A and theoretical additive isobole B ($P < 0.05$) was also confirmed by potency ratio analysis, in which the actual potency ratio of point B to A was 4.3 and the fiducial potency ratio of point B to A was 1.6. Thus, the difference between isobole A and B was determined again to be statistically significant (appendix). Likewise the interaction between DPDPE and clonidine was found to be synergistic (fig. 6).

A dose–effect regression line for U50,488H at 10 min after administration was not parallel with that for clonidine at 10 min after administration. U50,488H produced a less intense and shorter effect compared with that of clonidine. Therefore, a thorough isobolographic analysis was not conducted to evaluate the interaction

Table 2. Summary of 50% MPE Dose Values (μg or nM) and Slope Values of Regression Lines in figure 6

Drug	μg (95% CI)	nM (95% CI)	Slope
C	6.2 (5.3–7.4)	23.3 (19.9–27.8)	62.9
M	2.3 (1.6–3.2)	3.0 (2.1–4.2)	60.4
D	16.4 (13.2–21.5)	25.4 (20.4–33.3)	56.5
C + M	0.9 (0.6–1.4)	2.7 (1.8–4.1)	58.7
C in C + M	0.6 (0.4–0.9)	2.3 (1.5–3.4)	
M in C + M	0.3 (0.2–0.5)	0.4 (0.3–0.7)	
C + D	6.8 (5.8–8.2)	14.7 (12.3–17.7)	50.3
C in C + D	1.9 (1.6–2.3)	7.1 (6.0–8.6)	
D in C + D	4.9 (4.1–5.9)	7.6 (6.3–9.1)	

Values of clonidine (C), morphine (M), and clonidine (C) and DPDPE (D) and their combinations (C + M) were calculated from the data at 15 min after drug administration. From the values of nanomoles (nM) for 50% MPE, the rank order of potencies of individual drugs was morphine ≫ clonidine > DPDPE. When clonidine and morphine or clonidine and DPDPE were combined, nanomoles for the 50% MPE of each component were remarkably decreased from that of the respective drug used alone. Slopes of regression lines for C, M, D, C + M, and C + M were not significantly different.

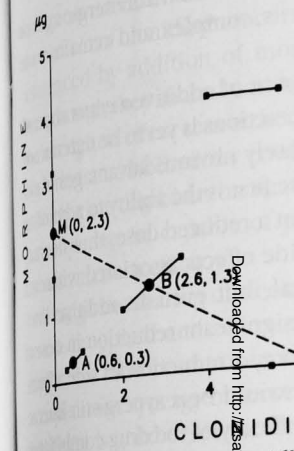

Fig. 5. Isobologram for dose providi
effect (MPE) with 95% confidence i
the time of peak effect (15 min) in
binations. The x,y coordinates rep
of doses of clonidine and morphin
and M represent 50% MPE dose of
used alone, respectively. Isobole A
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morphine). Isobole B represents th
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to produce the 50% MPE. As displa
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overlap each other. This finding i
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between clonidine and U50,48
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was combined with a subeffec
dose of U50,488H (50 or 100
7, combinations of clonidine an
tested showed no significant
compared with clonidine use
synergism between spinal clon
visceral antinociception.

Discussion

Visceral Antinociceptive Eff
Clonidine, Morphine, and U
The current study demonstrat
ministered clonidine produced
effects on visceral nociceptior
that intrathecal yohimbine reve
sults are consistent with the fin

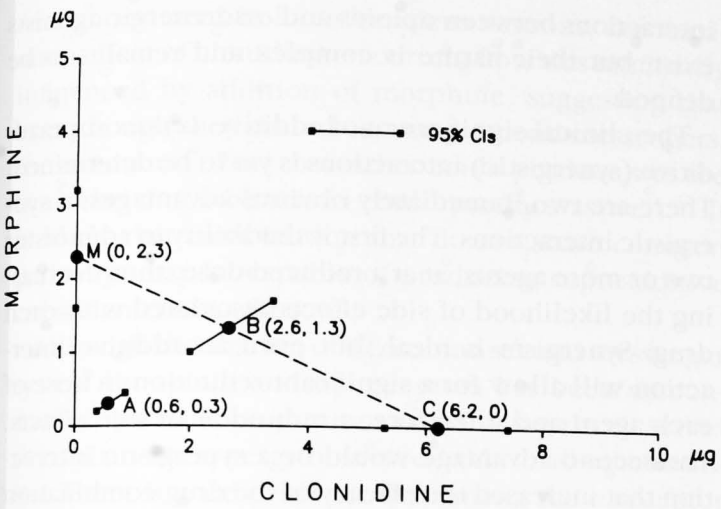

Fig. 5. Isobologram for dose providing 50% maximum possible effect (MPE) with 95% confidence intervals (CIs) (arrows) at the time of peak effect (15 min) in clonidine–morphine combinations. The x,y coordinates represent equieffective pairs of doses of clonidine and morphine, respectively. Isoboles C and M represent 50% MPE dose of clonidine and morphine used alone, respectively. Isobole A represents the actual experimental 50% MPE doses (0.6 μg for clonidine and 0.3 μg for morphine). Isobole B represents the theoretical additive 50% MPE doses of clonidine and morphine (2.6 μg for clonidine, 1.3 μg for morphine). If their interaction was purely additive, it would have required 2.6 μg clonidine plus 1.3 μg morphine to produce the 50% MPE. As displayed, isobole A fell below isobole B; in addition, 95% CIs of the two isoboles did not overlap each other. This finding indicates significant synergism between clonidine and morphine ($P < 0.05$).

between clonidine and U50,488H. Instead, to estimate (or predict) whether a synergistic-like interaction existed between clonidine and U50,488H, a mildly or moderately effective dose of clonidine (2.5 or 5 μg) was combined with a subeffective or mildly effective dose of U50,488H (50 or 100 μg). As shown in figure 7, combinations of clonidine and U50,488H at all doses tested showed no significant difference in effects as compared with clonidine used alone, indicating no synergism between spinal clonidine and U50,488H for visceral antinociception.

Discussion

Visceral Antinociceptive Effects of Spinal Clonidine, Morphine, and U50,488H

The current study demonstrated that intrathecally administered clonidine produced potent antinociceptive effects on visceral nociception induced by CRD and that intrathecal yohimbine reversed the effects. The results are consistent with the findings of Ness and Gebhart,[2,6] Danzebrink and Gebhart,[7] and Mares and Gebhart[9] that clonidine inhibits the behavioral and dorsal horn neuronal nociceptive responses to CRD at the spinal level. These results suggest that spinal α_2-adrenergic receptor systems are involved in visceral as well as somatic antinociception.

Synergism of Antinociceptive Interactions Between Clonidine and Morphine for Visceral Nociception

This study demonstrated a significant synergism between spinal clonidine and morphine and DPDPE for visceral antinociception. Although these results suggest a potential clinical significance of the combined spinal administration of α_2-adrenergic and μ- or δ-opioid agonists in visceral pain control and other animal studies have demonstrated supraadditive (synergistic) interactions between spinally administered clonidine and opioids,[19,25,26] we must exercise caution in assuming that synergism would be seen in humans. A recent clinical study[27] by Eisenach and colleagues in which iso-

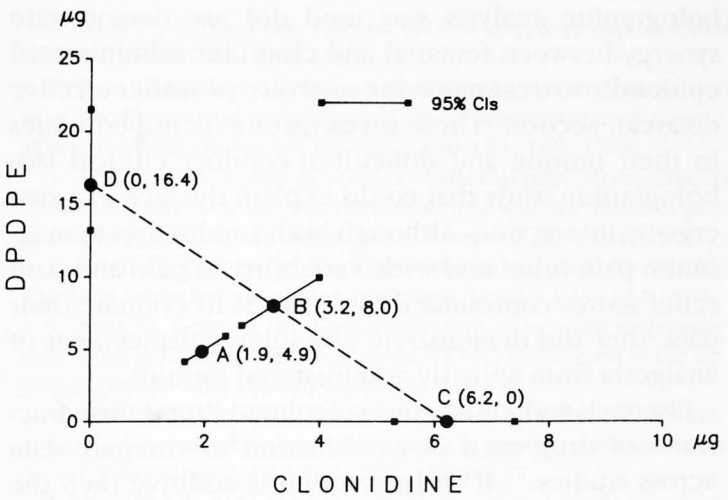

Fig. 6. Isobologram for dose providing 50% maximum possible effect (MPE) with 95% confidence intervals (CIs) (arrows) at the time of peak effect (15 min) in clonidine–[D-Pen², D-Pen⁵] enkephalin (DPDPE) combinations. The x,y coordinates represent equieffective pairs of doses of clonidine and DPDPE, respectively. DPDPE used alone, respectively. Isoboles C and D represent 50% MPE dose of clonidine. Isobole A represents the actual experimental 50% MPE doses (0.6 μg for clonidine and 0.3 μg for morphine). See also the legend to figure 7. Isobole B represents the theoretical additive 50% MPE doses of clonidine and DPDPE (3.2 g for clonidine and 8.0 μg for DPDPE). If their interaction were purely additive, it would have required 3.2 g clonidine plus 8.0 μg DPDPE to produce the 50% MPE. As displayed, isobole A fell below isobole B; in addition, 95% CIs of the two isoboles did not overlap each other. This finding indicates significant synergism between clonidine and DPDPE ($P < 0.05$).

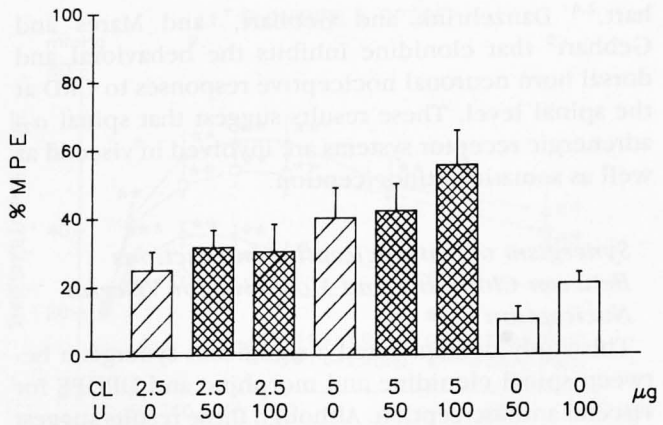

Fig. 7. Histogram of percentage maximum possible effect (MPE) for clonidine alone (diagonal line), clonidine plus U50,488H (cross-hatched line), and U50,488H alone (open box) 10 min after intrathecal administration. Each dose shown below the x-axis was administered in five to eight rats. By analysis of variance, combinations of clonidine and U50,488H at all doses tested showed no significant difference in effects as compared with the corresponding clonidine doses used alone (*e.g.*, 5 μg clonidine + 50 μg U50,488H, 5 μg clonidine + 100 μg U50,488H, or 5 μg clonidine alone). Cl = clonidine; U = U50,488H.

bolographic analysis was used did not demonstrate synergy between fentanyl and clonidine administered epidurally to treat moderate to severe pain after elective cesarean section. These investigators identified issues in their unique and difficult-to-conduct clinical isobolographic study that could explain the lack of a synergistic interaction. Although both a reduction in maximum pain relief and wide variability in pain and pain relief scores confounded their efforts to evaluate their data, they did demonstrate clonidine enhancement of analgesia from spinally administered opioids.

Eisenach and colleagues calculated "total dose fraction" of drug used in combination to compare data across studies.[27] If an interaction is additive then the total dose function would be 1 (*e.g.*, if the ED_{50} values of two drugs was determined and the combination of 0.25 of the ED_{50} of A and 0.75 of the ED_{50} of B produced 50% effect). If the dose function is less that 1, we can assume a synergistic interaction. In this study, the total dose fraction for clonidine and morphine was 0.22. For clonidine and DPDPE, it was 0.60. The morphine values compare favorably with numbers calculated by Ossipov *et al.* for data in mice[25] when clonidine was combined with opiates: morphine (0.04), meperidine (0.15), or fentanyl (0.05). The clonidine DPDPE value is closer to that in humans, where epidural clonidine and fentanyl produced a value of 0.52.[27] Important interactions between opioids and α adrenergic agonists exist, but their nature is complex and remains to be defined.

The clinical significance of additive *versus* supraadditive (synergistic) interactions is yet to be determined. There are two immediately obvious advantages of synergistic interactions. The first is the ability to administer two or more agents, at at a reduced dose, thus decreasing the likelihood of side effects associated with each drug. Synergism is ideal, but even an additive interaction will allow for a significant reduction in dose of each agent and, therefore, a reduction in side effects. The second advantage would be a synergistic interaction that increased the efficacy of the drug combination beyond that of the most efficacious drug in the combination. Because drugs capable of producing complete analgesia by the spinal route of administration were used in this study, we are unable to comment on altered efficacy except to state that the poor efficacy of U50,488H was not altered by clonidine. A greater appreciation of mechanisms of action by which synergistic interactions occur may help to identify possible combinations that do increase efficacy beyond that of the most efficacious agent in use.

The mechanisms by which clonidine and an opioid may synergistically interact are many. At the receptor level positive cooperative binding at either receptor could produced the observed effect. We are unaware of evidence for such an interaction.

In 1979 Sabol and Nirenberg[28] suggested that α receptors and opioid receptors may be functionally coupled to the same intracellular second messenger systems. As reviewed by Aghajanian and Wang,[29] it is now well established that α_2 and opiate agonists act through shared postreceptor effective mechanisms. It appears that in some neuronal cell types α_2 and opiate receptors have common actions mediated through inhibiting adenylate cyclase, an inhibitory guanosine triphosphate binding protein. Stimulation of either receptor type causes locus ceruleus neurons to be hyperpolarized by the opening of a common set of potassium channels. The clonidine action is likely to be produced by α_2 rather than imidazole receptor interaction because in the rat and bovine adrenal cells a separate second messenger system was activated by clonidine but only imidazole receptors were present. If the synergistic interaction of clonidine and an opioid is attributable to their sharing of a common second messenger system, it is unlikely that the combination would enhance efficacy beyond that for the drug with the highest efficacy.

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Appendix: Potency Ratio

Significance of the difference
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As demonstrated in locus ceruleus neurons, the maximum effect of clonidine on current flow was minimally influenced by addition of morphine, suggesting that even though they shared common second messengers, the maximum effect that either could produce was the limit that a combinations could produce as well.[30] It is likely that combination that do not share common pathways may be capable of producing supraadditive enhancement in efficacy.

Although the sharing of a common second messenger system by opioid and α_2 receptors has been demonstrated, we must be cautious in assuming that it serves to explain the synergy observed in this and other studies. There is good evidence that among the α receptor subtypes there is the opportunity to activate unique intracellular responses by multiple signal transduction pathways. In the current study, the synergistic effect may have occurred because of a systemic effect of clonidine activating other pain inhibitory systems.

Synergistic interactions between opioids and several analgesic drugs including lidocaine[31] and ketorolac[32] have been demonstrated. Clinically, this synergism is important because it allows a reduction in the amount of each agent and, thus, at least theoretically, reduces the probability of side effects associated with each agent. An understanding of the mechanism of action responsible for such interactions is of more than just academic interest. As demonstrated in opioid tolerance studies, although clonidine and morphine may share in some neurons a common second messenger system, rats rendered tolerant to opiates still respond to inhibitory effects of clonidine. Coombs *et al.*,[33] following experimentation in animals by Yaksh and Reddy,[34] demonstrated the potential value of this finding in providing analgesic rescue for a patient tolerant to opiate analgesia. As we continue to search for improved analgesic drug combinations, we will benefit from a better understanding of mechanisms responsible for them.

Appendix: Potency Ratio Analysis

Significance of the difference between the experimental 50% MPE dose and theoretical additive 50% MPE dose was determined using potency ratio (PR) analysis.[31] The PR for the experimental isobole with the theoretical isobole is defined as the ratio for each isobole. Namely, PR = 50% MPE_1 dose/50% MPE_2 dose, where the larger value of the two (experimental or theoretical value) was assigned as the 50% MPE_1 and the smaller as 50% MPE_2. The significance of difference between the two isoboles can be determined by the relation of PR and its fiducial PR (FPR). If PR is greater than FPR, the two isoboles (50% MPE values) are deemed to be significantly different from each other ($P < 0.05$). If PR is smaller than FPR, it means no significant difference between the two isoboles. The FPR was obtained from the nomogram[24] using F50% MPE_1 and F50% MPE_2, where F50% MPE = the fiducial limits of the 50% MPE value. The F50% MPE values were calculated from the following equations: 50% MPE × F50% MPE = upper 95% CI, and 50% MPE/F50% MPE = lower 95% CI. Using the PR and FPR, the interaction between clonidine and morphine was determined. When PR was larger than FPR, if the experimental isobole was below and to the left side of the theoretical additive isobole, the interaction was termed supraadditive or synergistic. If the experimental isobole was above and to the right side of the additive isobole, the interaction was termed subadditive or antagonistic. If the PR was less than FPR, the interaction was termed additive. As an example, the clonidine and morphine integration, in this study, PR was calculated as 4.3 and FPR was calculated as 1.6. Thus the interaction between clonidine and morphine was significantly ($P < 0.05$) determined as synergism.

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