

# Interaction Between Opiate Subtype and Alpha-2 Adrenergic Agonists in Suppression of Noxiously Evoked Activity of WDR Neurons in the Spinal Dorsal Horn

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Several studies have demonstrated synergistic antinociception following low-dose administration of morphine and alpha-2 adrenergic agonists at the spinal level. This study was carried out in order to identify the opiate subtypes that are likely to be involved in such synergistic suppression of noxiously evoked activity of wide-dynamic-range (WDR) neurons in the dorsal horn of the spinal cord. We also examined the effect of opiate antagonists and alpha-2 adrenergic antagonists on the suppression produced by opiate or alpha-2 adrenergic agonists. Extracellular activity of single WDR neurons in the spinal dorsal horn, which was evoked by a radiant heat stimulus (51° C), was recorded in decerebrate, spinally transected cats. Agonists were administered spinally and antagonists intravenously. In the synergism study, ineffective doses of the moderately selective mu agonist morphine (25 µg), the delta agonist DADL (20 µg), and the selective delta agonist DPDPE (30 µg), when combined with an ineffective dose of the alpha-2 adrenergic agonist clonidine (5 µg) produced significant synergistic suppression of noxiously evoked WDR neuronal activity. However, the ineffective or slightly effective dose of the selective mu agonist DAGO (1 or 1.5 µg, respectively) did not show any synergistic action with clonidine. Furthermore, the synergism between morphine and clonidine was reversed by the selective delta antagonist ICI 174,864. We interpret these results to indicate that opiates interact at spinal delta receptors to produce a synergistic suppression of evoked WDR neuronal activity in the presence of spinal clonidine. An alternative explanation is that ICI 174,864 may interact in some way with alpha-adrenergic systems. There was no statistically significant cross antagonism when naloxone or ICI 174,864 was used to reverse clonidine suppression or when the alpha-2 adrenergic antagonist yohimbine was used to reverse opiate suppression. (Key words: Analgesics, opiate: morphine; DAGO; DPDPE; DADL. Receptors, opiate: mu; delta. Spinal cord: WDR neurons. Sympathetic nervous system, alpha-2 agonists: clonidine.)

SPINAL OPIATE ANALGESIA, with its unique ability to produce analgesia without the loss of motor function or other sensory modalities, has played an important role in the control of acute and chronic pain. However, spinal

opiate analgesia is associated with side effects, including respiratory depression, development of tolerance and physical dependence, and urinary retention.<sup>1</sup> Respiratory depression is a life-threatening complication and limits the clinical use of spinal opiate therapy for pain management.

In addition to opiate analgesics, alpha-adrenergic agonists have been implicated in spinal analgesia.<sup>2-4</sup> Pharmacologic studies with intrathecal adrenergic agonists indicate that activation of spinal alpha-2 adrenoceptors inhibits behavioral responses to noxious stimuli<sup>3</sup> and produces a selective inhibition of dorsal horn neuronal responses to nociceptive stimulation.<sup>5-7</sup> However, the clinical utility of alpha-2 adrenergic analgesic effects may be limited by hypotension and bradycardia.<sup>8,9</sup>

Recent studies have demonstrated that concurrent low-dose intrathecal administration of opiates and alpha-2 adrenergic agonists produce a powerful antinociception,<sup>6,10-12</sup> extending earlier observations of systemic administration of these two classes of agonists.<sup>13,14</sup> Since decreasing the effective dosage of analgesics may lessen or eliminate the complications of spinal opiate or spinal alpha-adrenergic analgesia, these combined effects may be extremely useful for clinical pain management.

Selective agonists and antagonists have been developed recently for subclasses of opiate receptors,<sup>15-17</sup> allowing the identification of their likely involvement in the mediation of opiate effects. The current study examined the effects of combining the alpha-2 adrenergic agonist clonidine with the selective mu agonist DAGO, the less-selective mu agonist morphine, the selective delta agonist DPDPE, and the less-selective delta agonist DADL, administered directly onto the spinal cord. We also examined possible cross-antagonism between selective alpha-2 adrenergic and opiate antagonists.

## Materials and Methods

The protocol was approved by the Yale Animal Care and Use Committee, and institutional, state, and federal guidelines for the humane care and use of laboratory animals were observed during all aspects of this study. Experiments were carried out on 68 cats of both sexes and of weights of 3-4.5 kg.

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Received from the Department of Anesthesiology, Yale University School of Medicine, New Haven, Connecticut. Accepted for publication December 13, 1990. Supported by National Institutes of Health grant NS-09871. Presented in part at the 1989 IARS Meeting.

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### ANIMAL PREPARATION

Details of the animal preparation of this experiment have been given previously.<sup>4</sup> Under general anesthesia (halothane-nitrous oxide-oxygen), the trachea was intubated and the lungs were mechanically ventilated after intravenous administration of pancuronium bromide ( $0.2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ). End-tidal carbon dioxide concentrations were monitored and were maintained within normal limits. Catheterization of a jugular vein and a carotid artery were performed to administer intravenous fluids or drugs and to monitor arterial pressure. Decerebration was performed by making bilateral electrolytic lesions in the mid-brain reticular formation, after which general anesthesia was discontinued. The spinal cord transection was done at T11 or T12 to remove all descending influences. Thereafter, a laminectomy (L4-L6) was carried out, exposing the lumbar spinal cord. The dura was cut and reflected, and drug-free physiologic saline was placed on the spinal cord.

### ELECTROPHYSIOLOGIC RECORDING

A tungsten microelectrode was advanced by an hydraulic micromanipulator into the dorsal horn of the spinal cord while receptive fields on the ipsilateral hind paw were stimulated. When single neuron activity was isolated, the response profile of the neuron was determined. Wide-dynamic-range (WDR) neurons were classified based on a response that became greater as the intensity of stimulation increased (air puff, pinch with forceps, and noxious radiant heat [ $51^\circ \text{C}$ ]). For drug studies, activity of WDR neurons evoked by noxious radiant heat ( $51^\circ \text{C}$ , 8 s focused on the center of receptive fields) was recorded.

### DRUG ADMINISTRATION

All opiates for intrathecal administration were prepared fresh in physiologic saline. The following drugs were used: DAGO ( $\mu$  agonist) and DPDPE ( $\delta$  agonist) (Peninsula Laboratories, Belmont, CA), morphine sulfate ( $\mu$  agonist) (Mallinckrodt, St. Louis, MO), DADL ( $\delta$  agonist) and ICI 174,864, ( $\delta$  antagonist) (Cambridge Research Biochemicals, Valley Stream, NY), clonidine hydrochloride ( $\alpha$ -2 agonist) (Boehringer Ingelheim, Ridgefield, CT), naloxone hydrochloride (Manati, Puerto Rico) and yohimbine hydrochloride, ( $\alpha$ -2 antagonist) (Sigma Chemical, St. Louis, MO).

Data from another study in our laboratory<sup>18</sup> indicated that  $1 \mu\text{g}$  DAGO,  $5 \mu\text{g}$  clonidine,  $20 \mu\text{g}$  DADL,  $25 \mu\text{g}$  morphine, or  $30 \mu\text{g}$  DPDPE were ineffective in producing suppression of noxiously evoked activity of WDR neurons. To examine possible synergistic actions of these drugs, each ineffective dose of an opiate was combined with an ineffective dose of clonidine ( $5 \mu\text{g}$ ). In addition, the slightly

effective dose of DAGO ( $1.5 \mu\text{g}$ ) was combined with the ineffective dose of clonidine. After the control studies, the combined drugs in 0.5 ml physiologic saline were applied gently to the spinal cord. After administration, noxiously evoked activity of single WDR neurons was recorded every 3 min for 30 min. In the 11 animals that were administered the combination of morphine and clonidine,  $100 \mu\text{g}$  naloxone or  $200 \mu\text{g}$  ICI 174,864 was administered intravenously 31 min after administration of the combined drugs to evaluate the reversibility of the observed neuronal suppression. In other animals reversal by intravenous naloxone  $100 \mu\text{g}$  or yohimbine 3 mg was examined.

In the studies designed to evaluate cross antagonism,  $100 \mu\text{g}$  naloxone or  $200 \mu\text{g}$  ICI 174,864 was administered intravenously 31 min after the intrathecal administration of clonidine ( $30 \mu\text{g}$ ), or 3 mg yohimbine was administered intravenously 31 min after the intrathecal administration of a suppressive dose of DAGO ( $10 \mu\text{g}$ ), morphine ( $200 \mu\text{g}$ ), or DPDPE ( $200 \mu\text{g}$ ).

### DATA ANALYSIS

Activity of isolated single WDR neurons, the digital output of the amplitude discriminator, and the analogue skin temperature were digitized (CED 1401, Cambridge Electronic Design) and stored in computer format (IBM-AT personal computer). The integrated neuronal activity and the analogue skin temperature were recorded on a polygraph. Collected data were analyzed off-line using the computer program Spike 2 (Cambridge Electronic Design). The variables were expressed as percent suppression or as percent of control values. For the data from the synergism study, differences of suppression between opiate only and the combination with clonidine were evaluated by Student's *t* test for unpaired data. Student's *t* test for paired data was used to assess statistical significance of reversibility of antagonists. Differences were considered significant for two-tailed *P* values of  $<0.05$ .

### Results

All neurons studied in this experiment ( $n = 68$ ) were classified as WDR neurons. The depth of the neurons recorded was  $1,751 \pm 685 \mu\text{m}$  (mean  $\pm$  SD) below the surface of the lumbar spinal cord.

### POTENTIATION STUDY

Figure 1 demonstrates the effects of the combination of an ineffective dose of intrathecally administered morphine ( $25 \mu\text{g}$ ) and an ineffective dose of clonidine ( $5 \mu\text{g}$ ). In contrast to the ineffectiveness of either drug alone, the combination produced a synergistic suppressive effect on

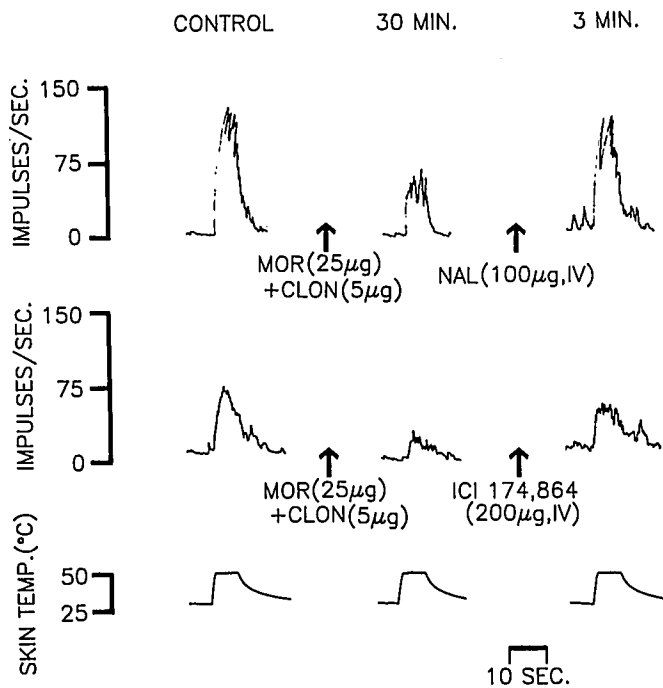


FIG. 1. Effects of the combination of morphine (MOR) 25 µg and clonidine (CLON) 5 µg on the evoked activity of single WDR neurons. Upper and middle traces represent the noxiously evoked activity expressed as impulses per second following the application of 51° C radiant heat for 8 s. Lower traces represent the skin temperature recorded at the center of the receptive field of the neuron. *Upper traces:* Morphine 25 µg combined with clonidine 5 µg produced potent suppression of evoked activity (41% of control at 30 min). Intravenously administered naloxone 100 µg reversed the suppression to 90% of control. *Middle traces:* The combination of morphine and clonidine produced potent suppression of activity of this neuron as well (47% of control) and intravenously administered ICI 174,864 200 µg produced reversal to 94% of control.

the noxiously evoked activity of the two WDR neurons shown in figure 1. This synergism was partially reversed by both intravenous naloxone and intravenous ICI 174,864. Figure 2 shows the effects of ineffectively administered DADL or DPDPE combined with an ineffective dose of clonidine on individual neurons. These combinations also suppressed the noxiously evoked activity, and the suppression was reversed by intravenous naloxone or yohimbine. In contrast to the synergistic interaction between morphine, DADL, or DPDPE and clonidine, there was a lack of synergism between an ineffective dose of DAGO (1 µg) and clonidine (fig. 3). Moreover, a slightly higher dose of DAGO (1.5 µg), which is known to suppress noxiously evoked activity, also failed to produce any synergistic suppressive effects with clonidine on the noxiously evoked activity of this individual WDR neuron.

Figure 4 summarizes the results of these synergism studies. It is apparent that ineffective doses of morphine,

DPDPE, or DADL combined with an ineffective dose of clonidine produced significant synergistic suppressive effects of noxiously evoked activity of WDR neurons. The combination of DADL and clonidine showed the most potent synergism among all the combinations. However, it is also apparent that the ineffective dose or slightly effective dose of DAGO did not produce any potentiation of the suppressive effect of clonidine.

As shown in figure 1, intravenously administered ICI 174,864 as well as naloxone effectively reversed the synergistic suppressive effect of morphine combined with clonidine. A summary of the effect of opiate antagonists on the synergistic suppression produced by the combination of morphine and clonidine are summarized in figure 5, in which it is shown that the selective delta antagonist ICI 174,864 as well as the nonselective antagonist naloxone significantly reverses the effects of the drug combinations.

#### ANTAGONISM STUDY

Intravenous naloxone reversed the effects of suppressive doses of DAGO (10 µg), morphine (200 µg), and DPDPE (200 µg) administered intrathecally, and intra-

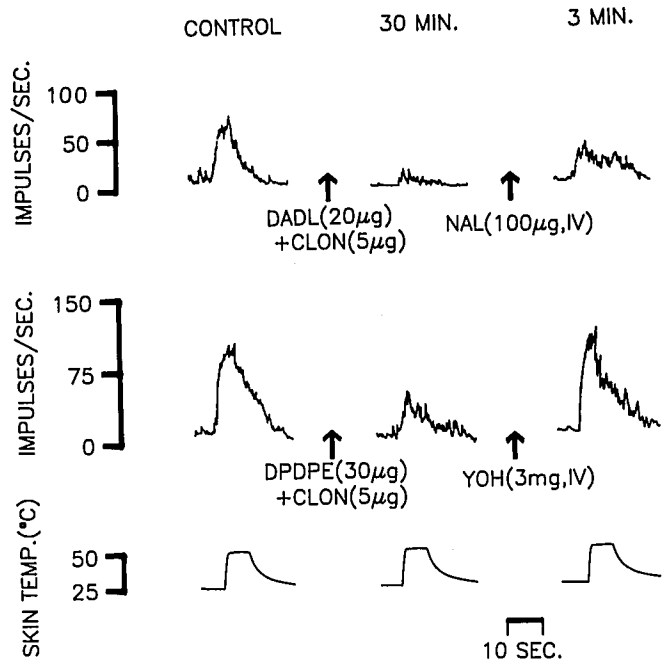


FIG. 2. Effects of the combination of DADL and clonidine (CLON) or the combination of DPDPE and clonidine on the evoked activity of single WDR neurons. *Upper traces:* DADL 20 µg combined with clonidine 5 µg suppressed the evoked activity of a single WDR neuron (17% of control 30 min after administration) and intravenously administered naloxone 100 µg reversed this suppression to 62% of control. *Middle traces:* DPDPE 30 µg combined with clonidine 5 µg suppressed the activity (42% of control) and intravenously administered yohimbine (YOH 3 mg) reversed this suppression to 88% of control.

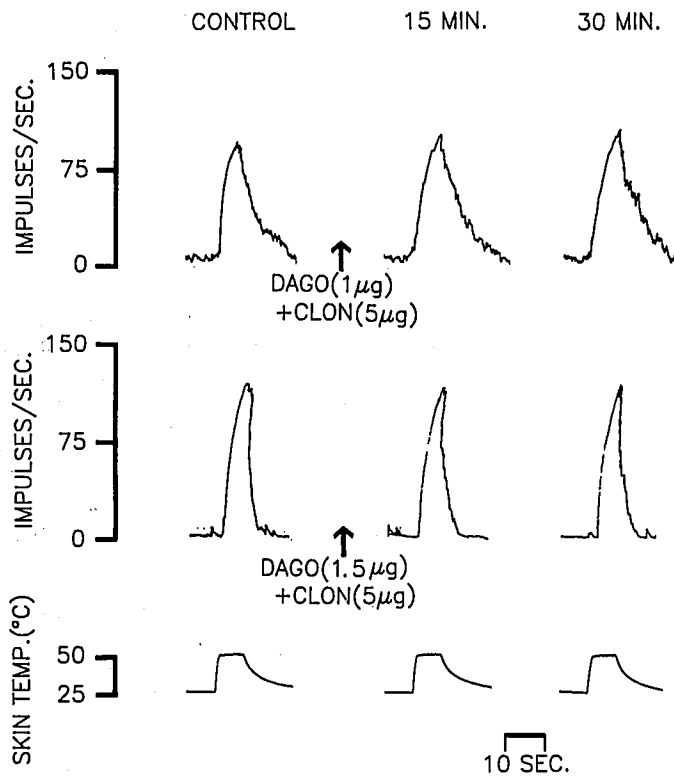


FIG. 3. Effects of the combination of DAGO and clonidine (CLON) on the noxiously evoked activity of the single WDR neurons. *Upper traces:* Ineffective dose of DAGO (1 µg) combined with clonidine 5 µg did not produce any suppression. *Middle traces:* Effective dose of DAGO (1.5 µg) combined with clonidine 5 µg also did not show any synergistic suppressive effect on the evoked activity of a single WDR neuron.

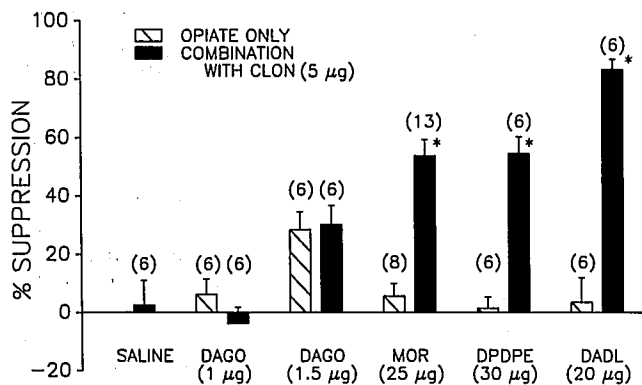


FIG. 4. The suppressive effect of the combinations of opiate and clonidine on the mean evoked activity of the WDR neurons. Ordinate represents percent suppression from the control mean evoked activity. Data represent the values at 30 min after administration of the combinations. Hatched bars indicate the percent suppression when only opiates were administered. Filled bars indicate percent suppression with drug combinations. Hatched bar data from reference 18. \**P* < 0.05 compared with opiate alone. The numbers in parentheses indicate the number of neurons studied. MOR = morphine.

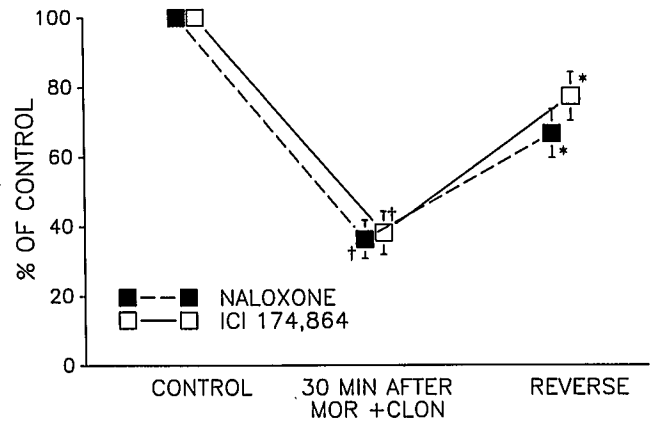


FIG. 5. Reversal effects of intravenously administered naloxone (100 µg) and ICI 174,864 (200 µg) on the WDR neuronal suppression of the combination of morphine (MOR) 25 µg and clonidine (CLON) 5 µg administered intrathecally. Data represent the values at 30 min after the administration of the combined drugs and the values at 3 min after intravenous administration of antagonists. Naloxone (n = 6) significantly reversed the suppression of the combined drugs, from 36 to 67% of control. ICI 174,864 (n = 5) also significantly reversed the suppression, from 38 to 77% of control. \**P* < 0.05 compared with the suppressed values by the combination of morphine and clonidine 30 min after administration. †*P* < 0.05 compared with control values.

venous ICI 174,864 reversed the effects of a suppressive dose of DPDPE (250 µg), as expected (data not shown). Of the nine WDR neurons that were suppressed by intrathecally administered clonidine (30 µg), four apparently were reversed by 100 µg intravenously administered naloxone (reversal greater than 20%). Figure 6 demonstrates a typical WDR neuron that was suppressed by clonidine and thereafter reversed by naloxone (reversal from 34 to

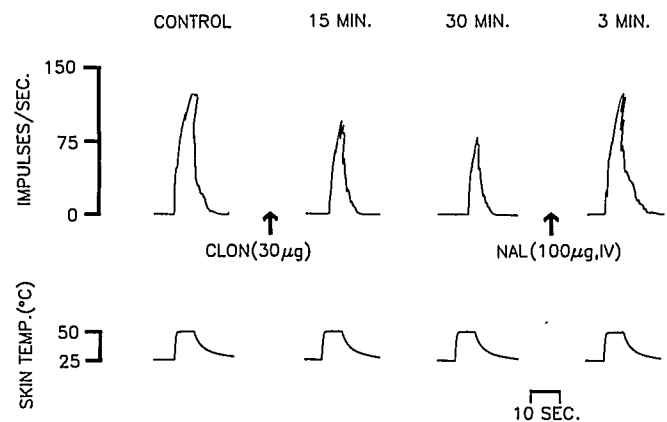


FIG. 6. Effect of intravenously administered naloxone (NAL) on the suppression of intrathecally administered clonidine (CLON). *Upper traces:* Clonidine 30 µg suppressed the evoked activity of a single WDR neuron of 34% of control 30 min after administration, and intravenously administered naloxone (100 µg) reversed this suppression to 85% of control. *Lower traces:* Skin temperature recorded at the center of the receptive field of the neuron.

86% of control). Two of the remaining five WDR neurons that were suppressed by clonidine were reversed by 200  $\mu\text{g}$  intravenously administered ICI 174,864 (reversal greater than 20%). However, although the activity of individual neurons was reversed, neither naloxone nor ICI 174,864 produced a statistically significant reversal of the mean suppression of intrathecally administered clonidine (fig. 7).

The ability to reverse the neuronal suppressive effects of opiates by the intravenously administered alpha-2 adrenergic antagonist yohimbine (3 mg) is shown in figure 8. There were no significant reversal effects of yohimbine on the mean suppression of DAGO, morphine, or DPDPE. However, of eight WDR neurons that were suppressed by DPDPE, three apparently were reversed by intravenously administered yohimbine. An example of noxiously evoked activity of an individual WDR neuron that was suppressed by DPDPE and reversed by yohimbine is shown in figure 9.

### Discussion

Studies in animals have suggested that systemically administered morphine produces its antinociceptive effects through a mechanism involving synergism of spinal and supraspinal opiate systems.<sup>19-21</sup> Descending monoaminergic pathways, in which noradrenergic as well as serotonergic descending systems can be strongly implicated,<sup>22-24</sup> participate in the production of antinociception by systemically administered opiates. Several lines of evidence indicate a synergistic interaction between opiate and adrenergic agonists in the spinal cord.<sup>4,6,10-12,25</sup> The important consideration arising from the apparent syn-

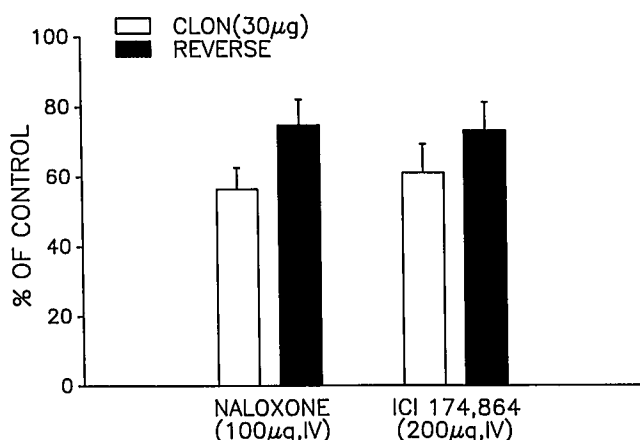


FIG. 7. Reversal effects of naloxone and ICI 174,864 on the mean neuronal suppression of clonidine (CLON). Neither intravenous naloxone (100  $\mu\text{g}$ ,  $n = 9$ ) nor ICI 174,864 (200  $\mu\text{g}$ ,  $n = 5$ ) significantly produced reversal effects on the suppression of intrathecally administered clonidine (30  $\mu\text{g}$ ).

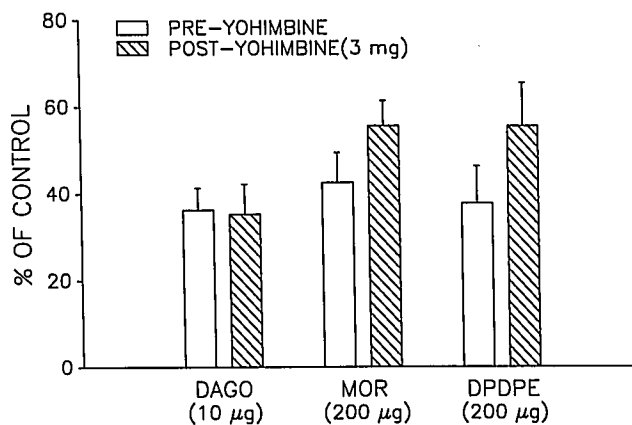


FIG. 8. Effects of yohimbine on the neuronal suppression of opiate subtypes. The neuronal suppressive effects of DAGO 10  $\mu\text{g}$  ( $n = 4$ ), morphine (MOR) 200  $\mu\text{g}$  ( $n = 5$ ) or DPDPE 200  $\mu\text{g}$  ( $n = 8$ ) were not significantly reversed by yohimbine 3 mg administered intravenously.

ergy demonstrated in this study is that significant antinociceptive effects can be achieved with doses of either agonist, which alone produce minimal effects. We have chosen to describe the observed interaction as synergistic, but our data do not include dose-response studies, which would determine precisely whether the observed interaction is simply additive or is synergistic.

With regard to the synergistic interactions of opiate and alpha-2 adrenergic agonists at the spinal level, two relevant problems are the identification of the opiate receptor subtypes mediating the synergism and the determination of the best combination of known opiate and alpha-2 adrenergic receptor agonists that at the lowest possible dose can produce significant suppression of noxiously evoked activity of WDR neurons.<sup>26</sup> Our study, al-

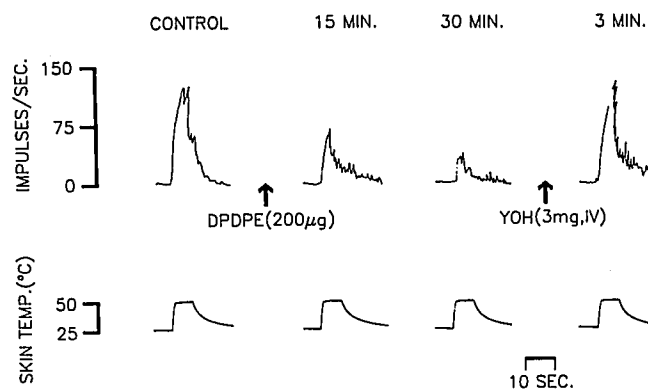


FIG. 9. Effect of intravenous yohimbine (YOH) on the suppression produced by DPDPE. DPDPE 200  $\mu\text{g}$  suppressed the evoked activity to 25% of control at 30 min after administration, and intravenously administered yohimbine (3 mg) reversed this suppression to 90% of control.

though limited by our use of single doses of agonists and antagonists, demonstrates that the intrathecally administered selective delta agonist DPDPE, the less-selective delta agonist DADL, and the less-selective mu agonist morphine are capable of producing a synergistic suppression of noxiously evoked WDR neuronal activity when combined with intrathecally administered clonidine. However, the combinations of either an ineffective or a slightly effective dose of the selective mu agonist DAGO with clonidine has shown no similar synergistic effect. Furthermore, as shown in the current finding that the synergistic suppression of the combination of morphine and clonidine is reversed by the intravenously administered nonselective antagonist naloxone or the selective delta antagonist ICI 174,864, the synergism between morphine and clonidine involves mediation through delta opiate receptors. Recent reports that the antinociception of spinal morphine may be mediated at delta receptors<sup>27,28</sup> are in agreement with the current results. Opiates appear to interact at spinal delta receptors to produce a synergistic interaction with spinal clonidine. These results suggest that opiate agonists with delta receptor affinity may be more appropriately combined with alpha-2 adrenergic agonists in order to produce a synergistic effect in blocking spinal pain transmission.

An alternative explanation for the ability of ICI 174,864 to reverse the observed synergism is that it may exert an effect on the alpha-adrenergic systems responsible for the observed suppression. That clonidine suppression of two WDR neurons was reversed by ICI 174,864 suggests that possible interaction.

One approach in determining the spinal interaction between opiates and alpha-2 adrenergic agents is to examine the existence of cross antagonism between these two systems. The ability of opiate antagonists to attenuate the antinociception of spinal alpha-2 adrenergic agonists or the alpha-2 adrenergic antagonist's ability to attenuate the spinal opiate-induced antinociception has been examined in several studies, but results are inconsistent.<sup>10-12,25,29-31</sup> In the current study, the intravenously administered nonselective opiate antagonist naloxone significantly reversed the effect of intrathecally administered DAGO, morphine, or DPDPE, and the selective delta antagonist ICI 174,864 reversed only the effect of DPDPE, as would be expected. The alpha-2 adrenergic antagonist yohimbine was without statistically significant effect in this regard. In contrast, yohimbine, but not naloxone, significantly reversed the effect of intrathecally administered clonidine. Thus, there was statistically no cross-antagonist reactivity in the current study. These results are in agreement with several previous reports demonstrating the efficacy of intrathecal opiate receptor or alpha-adrenoceptor antagonists to selectively antagonize

the antinociceptive effects produced by intrathecal application of the respective agonists.<sup>10,11,25,29,31</sup>

However, our data have also indicated that some neurons suppressed by spinal clonidine are apparently reversed by naloxone (shown in fig. 6) or ICI 174,864 and furthermore that a few neurons suppressed by DPDPE are reversed by yohimbine (shown in fig. 9). Loomis *et al.*<sup>30</sup> have demonstrated in the tail-flick assay that naloxone antagonizes the antinociceptive effects of intrathecal norepinephrine. Some authors<sup>20,30,32,33</sup> suggest the possibility that tonic descending fibers (monoaminergic neurons) do make synaptic connections with enkephalin-containing spinal interneurons. An intrathecal alpha-adrenergic agonist may activate alpha adrenoceptors on opiate-containing interneurons to increase the release of opiate peptides. If descending inhibition depends on enkephalinergic interneurons, opiate antagonists would be expected to antagonize the effects evoked by intrathecally administered clonidine.

With regard to the ability of yohimbine to antagonize the opiate-induced antinociception, although most studies do not support this antagonism,<sup>25,31</sup> Ossipov *et al.*<sup>12</sup> have demonstrated that intrathecally administered yohimbine attenuates the antinociceptive effect of intrathecal morphine. They have proposed that morphine activates opiate receptors that subsequently cause the activation of alpha-2 adrenergic receptor-mediated mechanisms, inhibiting nociceptive input at the spinal level.

Although alpha-adrenergic agents are known to have vasoconstrictor activity, we did not observe obvious blanching of vessels on the surface of the cord and therefore concluded that it is unlikely that changes in spinal cord blood flow are the mechanism of the synergistic effects observed in this study. In contrast to the delta-acting agonists, the selective mu agonist DAGO did not produce synergism with clonidine, and synergistic effects seen with the other opiates were reversible by either the opiate antagonist or the alpha-2 adrenergic antagonist (fig. 2).

The interaction among the various classes of agonists operative in the spinal cord is an important field of study for the advancement of spinal analgesia. The synergistic antinociceptive effects between opiates interacting at delta-opiate receptors and the alpha-2 adrenergic agonist clonidine, as shown in the current study, may be extremely important as tools with which to produce adequate spinal analgesia while minimizing doses of agents and thus avoiding the potential side effects associated with any one individual agent.

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