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Role of Central Mu, Delta-1, and Kappa-1 Opioid Receptors in Opioid-induced Muscle Rigidity in the Rat

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Background: Opioids appear to produce their physiologic effects by binding to at least three types of opioid receptors, the mu (μ), delta (δ), and kappa (κ) receptors. Muscle rigidity occurs after administration of supra-analgesic doses of potent μ -preferring agonists like alfentanil. The role of different supraspinal opioid receptors in this rigidity has been addressed only recently. To elucidate the contribution of central μ , δ , and κ receptors to muscle rigidity, the effects of intracerebroventricularly administered opioid receptor-selective agonists and antagonists on alfentanil-induced muscle rigidity were examined in rats.

Methods: Rats in which chronic intracerebroventricular cannulae had been implanted received an intracerebroventricular infusion of either saline or a μ (D-Ala²,N-Me-Phe⁴-Gly⁵-ol-enkephalin; DAMGO), δ_1 (D-Pen²,D-Pen⁵-enkephalin; DPDPE), or κ_1 (trans-(\pm)-3,4-dichloro-N-methyl-N-(2-(1-pyrrolidinyl)cyclohexyl)-benzene-acetamide methane sulfonate; U50,488H)

opioid agonist. Ten minutes later, they received either saline or the μ -agonist alfentanil subcutaneously. Muscle rigidity was assessed using hindlimb electromyographic activity. Different groups of animals were pretreated with an intracerebroventricular infusion of either saline or a μ (D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH₂; CTAP), δ (naltrindole), or κ_1 (norbinaltorphimine) opioid antagonist before administration of either saline or a selective intracerebroventricular agonist.

Results: The μ agonist DAMGO alone dose-dependently induced muscle rigidity. This effect was antagonized by pretreatment with the μ -selective antagonist CTAP. Neither DPDPE nor U50,488H, when administered alone, affected muscle tone. However, both the δ_1 and κ_1 agonists dose-dependently attenuated alfentanil-induced rigidity. This antagonism of alfentanil rigidity was abolished after pretreatment with the δ (naltrindole) and κ_1 (norbinaltorphimine) antagonists, respectively.

Conclusions: The present data demonstrate that whereas systemic opiate-induced muscle rigidity is primarily due to the activation of central μ receptors, supraspinal δ_1 and κ_1 receptors may attenuate this effect. This finding is consistent with previous demonstrations of functional interactions between different central opioid receptor populations in other opiate effects, and could have important pharmacotherapeutic implications. (Key words: Anesthetics, intravenous: alfentanil. Complications: muscle rigidity. Analgesics, opioid: receptors. Antagonists: narcotic. Brain: drug injections. Measurement techniques: electromyography. Animals: rat.)

HIGH-DOSE opiate administration produces intense analgesia and decreased sympathetic response to painful stimulation. Because of these advantageous clinical properties, opiates have increasingly enjoyed widespread use in anesthesia. Unfortunately, the profound analgesia of acute high-dose opiate# administration may be accompanied by prolonged respiratory depression and intense generalized muscle rigidity. Laboratory efforts have been undertaken to understand the mechanism of opiate-induced muscle rigidity.** The development of opiate agonists that do not produce muscle rigidity or other undesirable side effects would be a major advance in anesthesia and clinical pain management.

The cloning, localization, and functional validation of

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three similar but distinct gene products¹ now provide convincing evidence for the existence of at least three opioid receptor types (*i.e.*, mu [μ], delta [δ], and kappa [κ]) with different distributions throughout the central nervous system (CNS).²⁻⁴ These three receptors are also differentially implicated in the mediation of various physiologic and behavioral effects of opiates.^{1,4-7} $\dagger\ddagger$ *In vitro* and *in vivo* pharmacology strongly substantiates a further subdivision of each of the receptor types into subtypes (or isoreceptors).^{4,6,7,8}

With respect to the role of different receptors in opiate-induced muscle rigidity, initial studies focused on the μ receptor and used relatively poorly selective opioid receptor agonists.⁹ The elucidation of the *in vivo* receptor selectivity of opioid agonists, such as D-Ala²,N-Me-Phe⁴-Gly⁵-ol-enkephalin (DAMGO; μ selective),^{10,11} D-Pen²,D-Pen⁵-enkephalin (DPDPE; delta-1-selective),¹²⁻¹⁴ as well as the non-peptide agonist trans-(\pm)-3,4-dichloro-N-methyl-N-(2-(1-pyrrolidinyl)cyclohexyl)-benzene-acetamide methane sulfonate (U50,488H; kappa-1),^{15,16} now allows a more detailed study of the central opioid receptors mediating systemic opiate-induced muscle rigidity.

The purpose of the current study was to determine whether opiate-induced muscle rigidity, as measured by extremity electromyographic activity, is the result solely of activation of central μ opioid receptors or whether central delta and/or kappa receptors might also play a role. To pursue the hypothesis that central delta or kappa opioid receptors do mediate systemic opiate-induced muscle rigidity, the effect of intracerebroventricular administration of opioid receptor-selective agonists was investigated in intact, healthy spontaneously ventilating rats. In addition, the interaction between central opioid receptor activation and the rigidity induced by systemic administration of the potent μ -preferring agonist alfentanil^{17,18} was examined. The receptor specificity of the effects observed was

confirmed with the use of opioid receptor-selective antagonists.

Methods

Animals

All procedures were approved by our institution's Animal Care Committee. The subjects were 114 male albino Wistar rats (Harlan Laboratories, Indianapolis, IN) that weighed approximately 250–320 g at the time of the experiments. Animals were housed 2–3 per cage in a temperature-controlled room with a 12-h light/dark cycle.

Drugs

The drugs administered intracerebroventricularly were DAMGO (courtesy of NIDA, Bethesda, MD), DPDPE (courtesy of NIDA), U50,488H (gift of Upjohn Laboratories, Kalamazoo, MI), D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH₂ (CTAP; Peninsula Laboratories, Belmont, CA), naltrindole hydrochloride (NTI, Searle Research and Development, Skokie, IL), and nor-binaltorphimine (Research Biochemicals International, Natick, MA). All drugs were freshly dissolved in sterile physiologic saline (0.9%) and administered in a volume of 5 μ l. The doses are expressed as free base.

In some of the experiments, systemic opiate-induced muscle rigidity was induced with alfentanil (Alfenta; Janssen Pharmaceutica, Piscataway, NJ) dissolved in saline and administered subcutaneously at a dose of 500 μ g/kg in a volume of 1 ml/kg. This dose of alfentanil was chosen because it was shown, in previous studies, to consistently produce profound muscle rigidity.¹⁷⁻¹⁹

Surgical Procedure and Habituation

All rats were anesthetized with halothane and secured in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). Under aseptic conditions, a 7-mm, 23-gauge, stainless-steel guide cannula was implanted in the lateral ventricle. The coordinates used were: –0.6 mm to bregma, 2.0 mm lateral to the midline, and –3.2 mm to the skull surface at the point of entry.²⁰ The cannula was anchored to the skull with stainless-steel screws and dental cement and kept patent with a 30-gauge stainless-steel dummy stylet. The rats were allowed to recuperate for at least 5 days and to acclimate to the experimental cylindrical restraining apparatus during three separate daily 2-h sessions during this postsurgical period.

* In this paper, the term "opiate" is used to refer to exogenously administered drugs with morphine-like properties (*e.g.*, alfentanil is an opiate, and alfentanil produces opiate-induced muscle rigidity). In contrast, the term "opioid" is used to refer to endogenous peptides, their derivatives, and their receptors (*e.g.*, D-Ala²,N-Me-Phe⁴-Gly⁵-ol-enkephalin [DAMGO] is an opioid agonist that primarily acts at mu opioid receptors).

** Weinger MB: Opiate-induced muscle rigidity: Clinical implications and pathophysiology. *Progress in Anesthesiology* 1993; 7:198–212.

†† Weinger MB: Opiates: Basic pharmacology. *Progress in Anesthesiology* 1995; 9:151–65.

Experimental Design

The first series of experiments was undertaken to define agonist dose-response relations. Rats were pretreated with a single intracerebroventricular dose of either the δ_1 receptor agonist DPDPE, the κ_1 receptor agonist U50,488H, or the μ -receptor agonist DAMGO, and then randomly assigned to one of two groups. In one group, alfentanil was injected subcutaneously 10 min after agonist administration, whereas the other received the same volume of physiologic saline. The animals pretreated with DAMGO intracerebroventricularly received only saline subcutaneous injection. The doses tested were: DPDPE at 0, 50, or 150 nmol; U50,488H at 0, 22, or 107 nmol; and DAMGO at 0, 4, or 8 nmol.

In the second series of experiments, the same agonists were used at doses that were demonstrated in the first experiment to have an effect alone or in combination with alfentanil on muscle rigidity. In these experiments, the rats first received an intracerebroventricular injection of either an opioid receptor-selective antagonist or saline. Then, the rats received a second intracerebroventricular injection of either the corresponding agonist or saline. The receptor-selective antagonists used in these experiments were as follows: (1) for δ opioid receptors, NTI (10 nmol); (2) for κ_1 receptors, nor-binaltorphimine (10 nmol); and (3) for μ receptors, CTAP (3 nmol). Ten minutes later, alfentanil was injected (except for the DAMGO group, which received only the intracerebroventricular treatments).

In all tests, animals were assigned randomly to treatment groups, and the observer was blinded to the experimental conditions. Each rat was studied only once. The drug doses and the time interval between treatments were chosen based on preliminary experiments^{‡‡} and were consistent with those found, in this laboratory, to produce other relevant physiologic effects, including antinociception,^{§§} respiratory effects,^{21,22} and reinforcement.^{23,24}

Experimental Protocol

One to two hours before experiments, animals were transported from the animal care facility; food and wa-

ter was withheld. Rats were placed individually into the cylindrical holding apparatus inside a sound-attenuating box (Coulbourn Instruments, Lehigh Valley, PA). Muscle rigidity was assessed by measuring hindlimb electromyographic activity, as described previously.^{17-19,25,26} Briefly, two monopolar electrodes were inserted percutaneously into the left gastrocnemius muscle of each rat, and a third (ground) electrode was inserted into the right hind limb. Leads were held in place with cellophane tape. Limbs were secured with surgical tape in a manner that allowed unimpeded joint movement. After lead placement, the door of the box was closed, and baseline electromyographic activity was recorded for 15 min. Then, one or two (according to the experimental design) intracerebroventricular injections were performed, using an 8-mm, 30-gauge, stainless-steel injector cannula attached to polyethylene tubing. Five microliters of drug were injected by pressure using a Hamilton pump for 2 min. Electromyographic activity was then recorded at 5-min intervals for 60 min after the alfentanil (or saline) injection.

Raw muscle potentials were differentially amplified 200 times and band-pass filtered (10 Hz-3 KHz). The resulting signals, viewed on an oscilloscope, were converted with a root-mean-squared voltage rectifier ($t^{1/2} = 3$ s) to produce time-varying analog deflections on Triplet 200 mV meters.^{17-19,26} Full-scale deflection of the meter corresponded to 100 μ V of electromyographic activity. During data collection, care was taken to exclude the effects of transient movement artifacts, thereby permitting assessment of tonic rather than phasic muscle activity. At the conclusion of the experiment, the position of each cannula was verified with an intracerebroventricular infusion of India ink followed by necropsy.

Data Analysis

Data from the two experimental series were analyzed separately. In the first series, the mean area under the electromyographic curve was calculated and, for each agonist, a two-way analysis of variance was performed to evaluate drug effect (alfentanil or saline) and the effect of drug dose. In the second experimental series, statistical differences between agonist, antagonist, antagonist-agonist, and saline (control) groups were determined using two-way analysis of variance with repeated measures.²⁷ Then, Student-Newman-Keuls *a posteriori* tests were used to assess differences between treatment groups at individual time points, as well as differences over time within each treatment

^{‡‡} Bronson JB, Weinger MB: Opiate-induced muscle rigidity is mediated by mu, and not delta or kappa receptors, in the rat (abstract). ANESTHESIOLOGY 1989; 71:A600.

^{§§} Weinger MB, Tang R, Wood DL: The effects of opioid receptor selective agonists on three anesthetic endpoints in the rat (abstract). ANESTHESIOLOGY 1993; 79:A777.

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group. Mean animal weights between different treatment groups were analyzed in the same manner. Data were expressed as mean \pm SEM ($P < 0.05$ considered statistically significant).

Results

In all experimental groups, the baseline electromyographic activity, recorded during the 15-min pretreatment period, ranged from 2 to 5 μ V root-mean-squared. No significant differences in body weight were found among the different treatment groups.

When intracerebroventricular administration of either DPDPE (50 or 150 nmol) or U50,488H (43 or 107 nmol) was followed by a saline subcutaneous injection, the electromyographic activity was not significantly different from its own preinjection values or from the values in the saline-saline control groups (figs. 1a and 2a). In contrast, the intracerebroventricular administration of DAMGO resulted in a dose-dependent increase in muscle tone (fig. 3).

The pretreatment with 50 nmol DPDPE failed to alter the alfentanil-induced muscle rigidity. However, 150 nmol DPDPE significantly attenuated the alfentanil-induced muscle rigidity (fig. 1b). Similarly, pretreatment with U50,488H dose-dependently decreased electromyographic activity after alfentanil injection, although this effect only attained statistical significance with the 107-nmol dose (fig. 2b).

In the second experimental series, DPDPE 150 nmol or U50,488H 107 nmol was administered 10 min before alfentanil. DPDPE preceded by saline intracerebroventricularly decreased the alfentanil-induced muscle rigidity at all time points when compared with the control group (two saline intracerebroventricular treatments followed by alfentanil). In addition, when the δ antagonist NTI was injected before DPDPE, the electromyographic activity after alfentanil administration was not significantly different from control (alfentanil produced its usual full effect; fig. 4). Intracerebroventricular administration of NTI followed by saline failed to alter the alfentanil-induced muscle rigidity.

The administration of U50,488H preceded by saline decreased alfentanil-induced rigidity at most time points compared with the control group (fig. 5). This effect of U50,488H was abolished by pretreatment with nor-binaltorphimine. The intracerebroventricular injection of nor-binaltorphimine followed by saline intracerebroventricularly appeared to increase muscle tone before and after alfentanil compared with the control

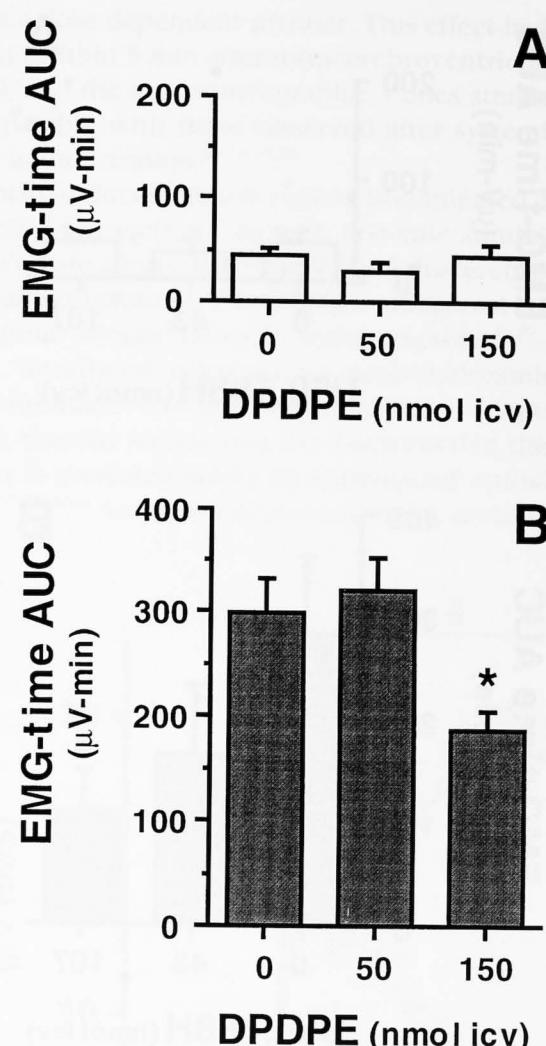


Fig. 1. Effect of different doses of DPDPE on electromyographic activity. Y axis gives the area under the electromyographic data versus time curve (AUC): (a) saline-treated groups; and (b) alfentanil-treated groups (500 μ g/kg subcutaneously). N = 6 for each dose examined. Significant decrease in alfentanil-induced muscle rigidity (* $P < 0.05$) after intracerebroventricular pretreatment with DPDPE (150 nmol), compared with saline intracerebroventricularly (zero dose group).

group; however, this effect just failed to attain statistical significance ($P < 0.10$) because of appreciable variability between animals.

Finally, the effect of the μ agonist DAMGO was studied. When preceded by saline intracerebroventricularly, DAMGO increased muscle tone within 5 min of intracerebroventricular administration, and this effect lasted for at least 80 min (fig. 6). The DAMGO time-effect curve appeared biphasic in some rats, although the group mean differences in electromyographic values over time were not statistically significant. Pretreatment with the μ antagonist CTAP significantly attenu-

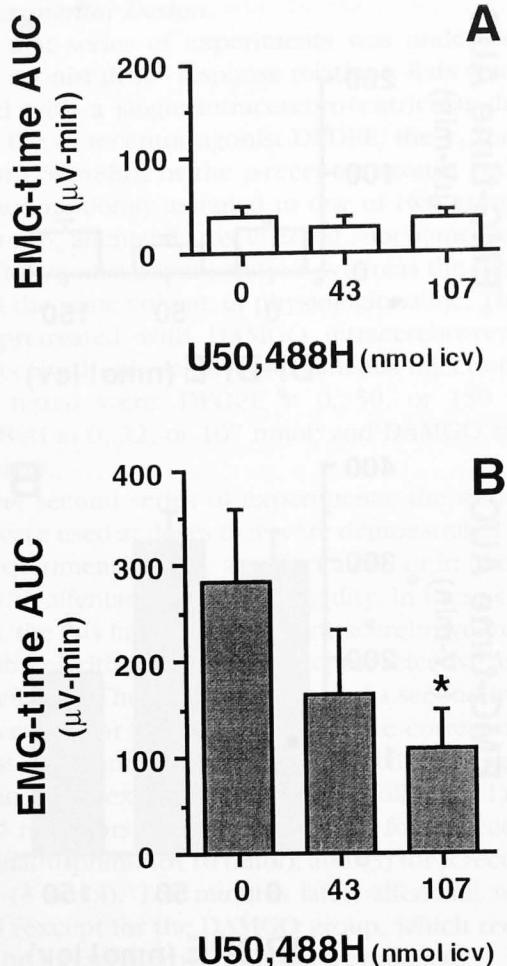


Fig. 2. Effect of different doses of U50,488H on the electromyographic activity (electromyography-time area under the curve): (a) saline-treated groups; and (b) alfentanil-treated groups. N = 6 for each dose. U50,488H (107 nmol) pretreatment significantly decreased alfentanil-induced muscle rigidity (* $P < 0.05$) when compared with saline intracerebroventricularly (zero dose group).

ated the muscle rigidity caused by DAMGO, although the CTAP/DAMGO group still had a significant increase in electromyographic activity for the first 30 min after DAMGO, compared with the pretreatment baseline. Thereafter, electromyographic activity was not different from baseline values.

Discussion

The current study examined the role of three major opioid receptor types in muscle rigidity by means of *in vivo* pharmacologic manipulation. The dose of alfen-

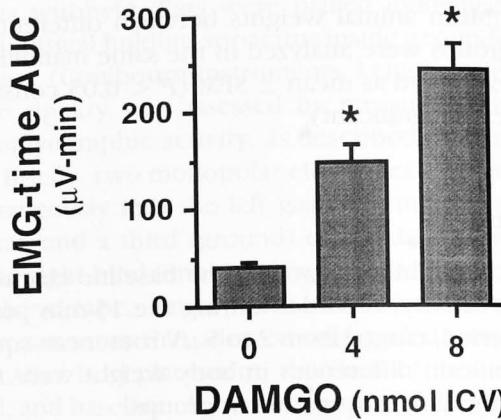


Fig. 3. Effect of different doses of the μ receptor agonist DAMGO on electromyographic activity (electromyography-time area under the curve). N = 6 for the saline group, n = 7 for the 4 nmol, and n = 8 for the 8 nmol group. * $P < 0.005$ compared with saline group.

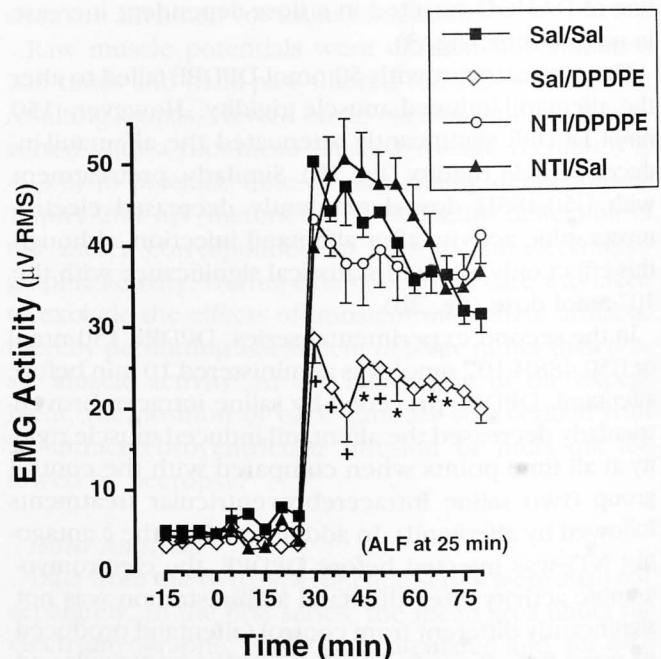


Fig. 4. Effect of pretreatment with 150 nmol DPDPE (Sal/DPDPE group); 10 nmol NTI (NTI/Sal group); 10 nmol NTI and 150 nmol DPDPE (NTI/DPDPE group); or saline (Sal/Sal group) on the alfentanil-induced muscle rigidity. The first intracerebroventricular injection (saline or NTI) was performed at 0 min, the second intracerebroventricular injection (DPDPE or saline) was at 15 min. All animals received 500 μ g/kg alfentanil subcutaneously at 25 min. N = 7 for all experimental groups. The administration of DPDPE (Sal/DPDPE group) significantly decreased alfentanil-induced muscle rigidity compared with Sal/Sal group (* $P < 0.05$; + $P < 0.001$). There was no significant difference in electromyographic activity between Sal/NTI; NTI/DPDPE, and Sal/Sal groups.

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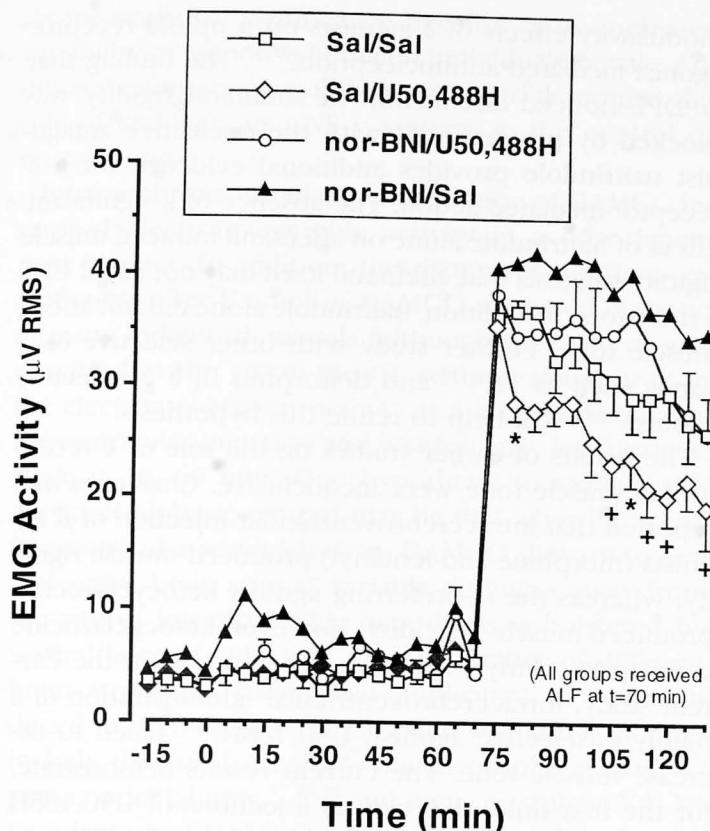


Fig. 5. Effect of pretreatment with 107 nmol U50,488H (Sal/U50,488H group, n = 7); 10 nmol nor-binaltorphimine (nor-binaltorphimine/Sal group, n = 6); 10 nmol nor-binaltorphimine and 107 nmol U50,488H (nor-binaltorphimine/U50,488H group, n = 6); or saline (Sal/Sal group, n = 7) on alfentanil-induced muscle rigidity. The intracerebroventricular injections were performed at time points 0 (saline or nor-binaltorphimine) and 60 (saline or U50,488H); alfentanil was administered subcutaneously at 70 min. The pretreatment with U50,488H (Sal/U50,488H group) significantly decreased the alfentanil-induced muscle rigidity compared with the control group (Sal/Sal) (*P < 0.05; +P < 0.005). No significant differences between Sal/Sal, nor-binaltorphimine/Sal, and nor-binaltorphimine/U50,488H groups were observed.

tanil chosen for this study (500 μ g/kg) produced a sustained and near maximal level of muscle rigidity after systemic administration.¹⁸ The selective delta-1 agonist DPDPE alone had no effect on hindlimb muscle tone; however, at an antinociceptive dose (150 nmol), DPDPE significantly decreased the alfentanil-induced muscle rigidity. Similarly, the kappa-1 agonist U50,488H, although having no effect on electromyographic activity when given alone, produced a dose-related decrease in alfentanil-induced rigidity. Both of the non- μ opioid agonists failed to alter baseline muscle tone during the first 10 min after intracerebroventricular administration. In contrast, intracerebroventricular injection of the μ agonist DAMGO alone produced muscle rigidity in a dose-dependent manner. This effect had a rapid onset (within 5 min after intracerebroventricular injection), and the electromyographic values attained were comparable with those observed after systemic alfentanil administration.^{18,19,25,26}

Opiate-induced muscle rigidity is eliminated after spinal cord transection²⁸ or with systemic administration of an opiate antagonist.²⁵ Similarly, intracerebroventricular administration of the opiate antagonist methylnaloxonium blocks systemic opiate rigidity.^{26,29} In contrast, intrathecal naloxone or methylnaloxonium will not attenuate systemic opiate rigidity (unpublished data), thereby supporting the assertion that this opiate effect is mediated solely by supraspinal opioid receptors.^{17,26,29**} An intracerebroventricular route for study

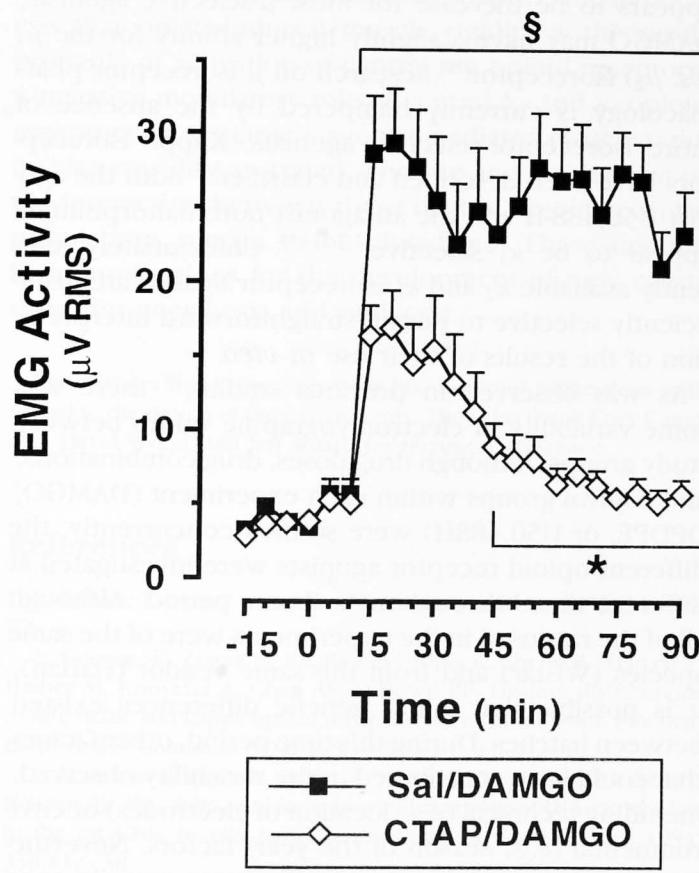


Fig. 6. Effect of 8 nmol DAMGO on muscle tone. Two intracerebroventricular injections were performed: one at time 0 and one at 10 min. Experimental group Sal/DAMGO (n = 9) received first saline and then DAMGO; the CTAP/DAMGO group first received 3 nmol CTAP and then DAMGO (n = 10). The administration of DAMGO significantly increased the muscle tone (§P < 0.001) compared with the baseline values. This effect was attenuated by pretreatment with CTAP (P < 0.05 for all time points). The electromyographic activity at some time points (indicated by *) within CTAP/DAMGO group was no different from the baseline values.

drug administration was chosen in the current experiments to assure a CNS site of action of the effects under study, and also because DPDPE, DAMGO, and CTAP can be metabolized in plasma and, therefore, are not very active systemically.

There is substantial evidence for the existence of different opioid isoreceptors.⁶⁻⁸ The δ isoreceptors (δ_1 and δ_2) have been the most fully described.⁶ DPDPE is a relatively δ_1 -selective agonist,^{6,30} whereas the δ antagonist naltrindole is either not particularly isoreceptor selective or is, at best, weakly δ_1 -selective.^{6,30,31} The role of δ_2 receptors in opiate rigidity remains to be elucidated.

The μ_1 receptor is postulated to mediate analgesia⁵ and muscle rigidity,²⁵ whereas the μ_2 receptor is hypothesized to play a role in respiratory depression.⁵ As appears to be the case for most μ -selective agonists, DAMGO may have a slightly higher affinity for the μ_1 (*vs.* μ_2) isoreceptor.³² Research on μ isoreceptor pharmacology is currently hampered by the absence of more isoreceptor-selective agonists. Kappa isoreceptors have been described and classified.⁷ Both the agonist U50,488-H and the antagonist norbinaltorphimine appear to be κ_1 selective.^{1,7,33-35} Unfortunately, currently available κ_2 and κ_3 isoreceptor ligands are insufficiently selective to permit straightforward interpretation of the results of their use *in vivo*.

As was observed in previous studies,¹⁹ there was some variability in electromyographic values between study groups. Although drug doses, drug combinations, and control groups within each experiment (DAMGO, DPDPE, or U50,488H) were studied concurrently, the different opioid receptor agonists were investigated at different times throughout a 2-year period. Although all of the rats used in the experiments were of the same species (Wistar) and from the same vendor (Harlan), it is possible that minor genetic differences existed between batches. During this time period, other factors that could have contributed to the variability observed, including technical (*e.g.*, location of electrodes) or environmental (*e.g.*, season of the year) factors. Nevertheless, significant dose-related effects on muscle tone caused by intracerebroventricular injection of different opiate agonists (compared with parallel controls) were easily demonstrated.

The δ_1 agonist DPDPE, at a dose of 150 nmol, significantly attenuated systemic alfentanil-induced muscle rigidity, suggesting a modulatory role for CNS δ_1 receptors in opiate rigidity. This finding appears consistent with other studies, demonstrating negative or positive

modulatory effects of δ agonists on μ opioid receptor agonist-mediated antinociception.^{36,37} The finding that DPDPE-induced attenuation of alfentanil rigidity was blocked by pretreatment with the δ -selective antagonist naltrindole provides additional evidence for a δ receptor-mediated action. The absence of a significant effect of naltrindole alone on alfentanil-induced muscle rigidity suggests that alfentanil itself may not act at CNS δ receptors. In addition, naltrindole alone did not affect muscle tone. Further study with other selective δ ligands such as TIPP³⁸ and deltorphin II, a δ_2 -selective agonist,^{13,14} will help to refine this hypothesis.

The results of earlier studies on the role of κ receptors in muscle tone were inconclusive. Chaillet *et al.*⁹ reported that intracerebroventricular injection of μ agonists (morphine and fentanyl) produced muscle rigidity, whereas the κ_1 -preferring agonist ketocyclazocine produced muscle flaccidity. However, ketocyclazocine is not particularly κ -selective.⁸ In contrast, in the current study, intracerebroventricular administration of a highly κ_1 -selective agonist, U50,488H,^{7,15} failed to decrease muscle tone. The current results demonstrate, for the first time, that central injections of U50,488H antagonize alfentanil-induced muscle rigidity. The antagonism of μ receptor-mediated muscle rigidity by U50,488H is consistent with previous data on negative modulatory effects of the kappa system in other μ opioid effects. Using the κ antagonist nor-binaltorphimine, Spanagel and Shippenberg³⁹ demonstrated that the endogenous κ opioids play a role in morphine-induced behavioral sensitization. Kappa agonists can antagonize μ receptor-mediated decreased bladder motility,⁴⁰ dopamine release in locomotor systems,⁴¹ respiratory depression,⁴² alteration of seizure threshold,⁴³ and antinociception.⁴⁴ However, it is still unclear whether these κ -mediated effects are due to a direct effect of κ agonists on μ receptors or a functional interaction between the two receptor populations.

In the current study, the role of κ_1 receptors in U50,488H's effects was confirmed by pretreatment with nor-binaltorphimine, a specific κ_1 -opioid receptor antagonist.^{45,46} Because nor-binaltorphimine is an irreversible antagonist with a slow onset, rats were pretreated intracerebroventricularly with nor-binaltorphimine 60 min before U50,488H.³⁴ Nor-binaltorphimine pretreatment effectively blocked U50,488H's effect on alfentanil-induced rigidity. The group that received nor-binaltorphimine alone appeared to have slightly higher electromyographic values after alfentanil than the saline group, although this effect failed to meet the cri-

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ria for statistical significance because of an increased variability of response between individual animals. Additional experiments will be required to determine the role of endogenous CNS κ systems in the control of muscle tone.

Intracerebroventricular administration of DAMGO increased electromyographic activity in a dose-dependent manner. In addition, the pattern of the time-effect curve after 8 nmol of DAMGO appeared biphasic in many individual animals (although this pattern was obscured in the group mean), with an initial peak in the electromyographic activity at 5 min postintracerebroventricular injection and a secondary, less distinct, peak at 40–60 min. One hypothesis to explain this apparent biphasic pattern may be that, after intracerebroventricular administration, DAMGO diffuses to several active brain sites at variable distances away from the site of injection. This hypothesis is bolstered by several lines of evidence. First, a number of different brain structures have been implicated in opioid-induced muscle rigidity. These putative brain structures include the basal ganglia,⁴⁷ nucleus raphe pontis,^{17,29} periaqueductal gray,^{17,29,48} and locus coeruleus.^{49,50} Microinjection of DAMGO into the periaqueductal gray⁴⁸ or of fentanyl into the locus coeruleus⁵¹ both produce significant muscle rigidity. Similarly, microinjection of the antagonist methylnaloxonium into periventricular or pontine sites blocked systemic alfentanil-induced rigidity.¹⁷ A significant decrease in alfentanil rigidity also was observed after microinjections of very small doses (0.125 μ g in 0.1 μ l) of methylnaloxonium into the periaqueductal gray (unpublished data). Second, the distribution after intracerebroventricular administration of a hydrophilic peptide like DAMGO has been shown to initially be to periventricular structures (e.g., periaqueductal gray) and then, after a time delay (to allow for tissue diffusion), to sites more distant from the ventricular system (e.g., substantia nigra, raphe nuclei, and globus pallidus).⁵¹ This hypothesis must be tested with experiments specifically designed to examine the intracranial pharmacokinetics of intracerebroventricular drug administration.

Pasternak postulated the existence of two subtypes of the μ -opioid receptor: μ_1 and μ_2 .^{5,8} Using naloxonazine, a putative μ_1 -selective irreversible antagonist, they demonstrated that catalepsy and analgesia⁵² were mediated by the putative μ_1 receptor, whereas respiratory depression⁵³ was mediated by the μ_2 receptor. Subsequently, Negus *et al.*²⁵ showed that both naloxonazine and β -funtrexamine, a nonsubtype selective irrevers-

ible μ antagonist, produced similar rightward shifts in the alfentanil antinociception and muscle rigidity dose-effect curves. These data suggested that opiate-induced muscle rigidity may not be pharmacologically separable from opiate-induced antinociception on the basis of μ isoreceptor specificity. Consequently, development of even more potent μ -selective agonists may not represent an advance in pharmacotherapeutics, because opiates with greater efficacy at the μ receptor may be more likely to induce muscle rigidity. Therefore, the finding of antagonist effects of central delta and kappa agonists on mu agonist-induced rigidity potentially take on increased practical significance. In fact, significant pharmacotherapeutic benefit may accrue from the development of δ - and κ -selective analgesics.

In conclusion, the current study supports the hypothesis that opiate-induced muscle rigidity is the result primarily of activation of central mu opioid receptors. A negative modulatory role of central δ_1 - and κ_1 -opioid receptors on systemic μ -agonist-mediated muscle rigidity also was demonstrated. The site and mechanism of the interaction between these central opioid receptor populations remain to be elucidated. These findings have implications for the development of new opiate drugs for anesthesia and analgesia.

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