Interaction Between Medullary and Spinal δ₁ and δ₂ Opioid Receptors in the Production of Antinociception in the Rat

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ABSTRACT

Previous work supports the existence of two types of δ opioid receptor (δ₁ and δ₂) and a role of both subtypes in the spinal cord and the ventromedial medulla (VMM) in the production of antinociception. Although it is well established that spinal and supraspinal μ opioid receptors interact in a synergistic manner to produce antinociception, little is known about the interaction of δ opioid receptors. This study used isobolographic analysis to determine how δ₁ and δ₂ opioid receptors in the VMM interact with their respective receptors in the spinal cord to produce antinociception. Concurrent administration of the δ₁ opioid receptor agonist [d-Ala²,Glup⁴]deltorphin at spinal and supraspinal sites produced antinociception in an additive manner. However, as the total dose of [d-Ala²,Glup⁴]deltorphin increased, this interaction converted to additivity. These observations suggest that different mechanisms mediate the antinociceptive effects of different doses of δ₂ opioid receptor agonists. The difference in the nature of the interaction produced by δ₁ and δ₂ opioid receptor agonists provides additional evidence for the existence of different subtypes of the δ opioid receptor. These results also suggest that δ₂ opioid receptor agonists capable of crossing the blood-brain barrier will be more potent or efficacious analogues than δ₁ opioid receptor agonists after systemic administration.

Studies in both the mouse (Roerig and Fujimoto, 1989) and the rat (Yeung and Rudy, 1980; Siuciak and Advokat, 1989; Miyamoto et al., 1991) indicate that the concurrent administration of μ opioid receptor agonists at supraspinal and spinal sites produces antinociception in a synergistic manner. This synergistic interaction is thought to be responsible for the potent antinociception produced by systemically administered μ opioid receptor agonists, such as morphine. Although there is good agreement that supraspinal and spinal μ opioid receptor agonists interact synergistically, there is less consensus about the manner in which supraspinal and spinal δ opioid receptor agonists interact. Concurrent i.c.v. and intrathecal (i.t.) administrations of δ opioid receptor agonists such as [d-Ala²,d-Leu⁵]enkephalin (Roerig et al., 1991) or [d-Pen²,d-Pen⁵]enkephalin (DPDPE) (Roerig and Fujimoto, 1989) produce antinociception in an additive manner in the radiant-heat tail-flick test in the mouse. In contrast, i.c.v. and i.t. administration of DPDPE produces antinociception in a synergistic manner in a test of mechanical nociception in the rat (Miaskowski and Levine, 1992; Miaskowski et al., 1993).

Since these studies were conducted, two subtypes of the δ opioid receptor, δ₁ and δ₂, have been described pharmacologically (Hammond, 1993; Porreca and Burks, 1993; Zaki et al., 1996). Both subtypes are implicated in the modulation of nociception in the spinal cord (Stewart and Hammond, 1993; Hammond et al., 1995) and the brain stem (Ossipov et al., 1995; Thorat and Hammond, 1997) of the rat. Although the earlier studies with DPDPE suggest that supraspinal and spinal δ₁ receptor agonists interact in an additive or a synergistic manner to produce antinociception, nothing is known about the manner in which δ₂ opioid receptor agonists interact. A better understanding of how δ opioid receptor subtype-selective agonists interact at supraspinal and spinal sites could facilitate the development of systemically bioavailable δ opioid receptor agonists as analogues. The present study was therefore undertaken to determine how δ₁ and δ₂ opioid receptors in the ventromedial medulla (VMM) interact with their respective subtype in the spinal cord to produce antinociception. An isobolographic analysis was con-

ABBREVIATIONS: DELT, [d-Ala²,Glup⁴]deltorphin; DPDPE, [d-Pen²,d-Pen⁵]enkephalin; NRM, nucleus raphe magnus; NGCPm, nucleus reticularis gigantocellularis pars α; VMM, ventromedial medulla; i.t., intrathecal; SNC80, (+)-4-[(αR)-α-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N,N-diethylbenzamide; TAN67, 2-methyl-4-α(3-hydroxyphenyl)-1,2,3,4,4a,5,12,12a-octahydro-quinoline[2,3,3-g]isoquinoline.
ducted in which either the δ opioid receptor agonist DPDPE or the δ opioid receptor agonist DELT was administered concurrently to the VMM and the spinal cord in a fixed dose-ratio that approximated their respective ED_{50} values at each site. Alterations in nociceptive threshold were determined by the tail-flick and hot-plate tests.

Materials and Methods

These experiments were approved by the Institutional Animal Care and Use Committee of the University of Chicago. All procedures were conducted in accordance with the “Guide for Care and Use of Laboratory Animals” published by the National Institutes of Health and the ethical guidelines of the International Association for the Study of Pain.

Animals. Male Sprague-Dawley rats (Sasco, Kingston, NY) weighing 300 to 350 g were anesthetized with halothane and prepared with an i.t. catheter that terminated at the L4 or L5 segment of the spinal cord (Yaksh and Rudy, 1976). Rats that exhibited motor impairments such as hindlimb or forepaw paresis were euthanized. Five to 6 days later, the rats were reanesthetized with a mixture of ketamine hydrochloride (85 mg/kg i.p.) and xylazine (9 mg/kg i.p.) and implanted with an intracerebral guide cannula (26 gauge; Plastic One, Inc., Roanoke, VA) that terminated 3 mm dorsal to either the nucleus raphe magnus (NRM) or the nucleus reticularis gigantocellularis pars α (NGCparα) in the VMM. The cannula was secured to the skull with stainless steel screws and dental acrylic. A 30-gauge stainless steel stylet was placed in the guide cannula to maintain its patency. Rats were housed individually after surgery under a 12-h light/dark cycle with food and water available ad libitum. Seven days elapsed before behavioral testing began. Rats received only one dose combination and were used only once in this study.

Behavioral Tests. Nociceptive threshold was assessed by the radiant heat tail-flick and 55°C hot-plate tests. In the tail-flick test (D’Amour and Smith, 1941), the rat’s blackened tail was positioned under an intense light beam, and the time for the rat to remove its tail from the thermal stimulus was recorded. This test was performed twice at each time point on two different regions of the distal tail. The results of the two trials were averaged and recorded as the tail-flick latency. In the event that the rat did not withdraw its tail from the stimulus by 14 s, the test was terminated to prevent tissue damage, and the rat was assigned this cutoff latency. In the hot-plate test (Woolfe and MacDonald, 1944), the rat was placed on an enclosed copper plate heated to 55°C. The time between placement of the rat on the hot-plate and the occurrence of either a hindpaw lick or a jump off the surface was recorded as the hot-plate latency. Hot-plate latency was measured once per time period. In the absence of a hindpaw lick or a jump by 40 s, the test was terminated to prevent tissue damage, and this cutoff latency was assigned. Motor function was evaluated using the inclined-plane test (Rivlin and Tator, 1977). The tail-flick, inclined-plane, and hot-plate tests were performed in succession.

Experimental Design. Measurements of nociceptive threshold and motor competency were made before the injection of drug. Those rats that responded in ≤5.0 s on the tail-flick test and ≤15.0 s on the hot-plate test and had inclined-plane angles of ≈40 degrees were used in this study. Mean baseline tail-flick and hot-plate latencies among the different dose treatment groups ranged from 3.5 to 4.0 s and from 7.9 to 12.5 s, respectively. After baseline nociceptive threshold was determined, DPDPE (0.49 ng to 4.9 μg) was microinjected into the VMM followed 25 min later by an i.t. injection of DPDPE (2.3 ng to 25 μg). The intracerebral and i.t. doses of DPDPE were administered in a fixed ratio of 1:4.7 that approximated the ratio of the ED_{50} values in mass units (i.e., nanograms or micrograms) of DPDPE at medullary and spinal sites, respectively. Tail-flick latency, hot-plate latency, and inclined-plane angle were then redetermined 45 and 60 min after the intracerebral injection. A similar paradigm was used to characterize the interaction of DELT. After determination of baseline nociceptive threshold, DELT (0.023 ng to 0.94 μg) was microinjected into the VMM, followed 10 min later by an i.t. injection of DELT (0.063 ng to 2.51 μg). The intracerebral and i.t. doses of DELT were administered in a fixed ratio of 3:8 based on the ratio of the ED_{50} values of DELT at medullary and spinal sites, respectively. Tail-flick latency, hot-plate latency, and inclined-plane angle were then redetermined 30 and 40 min after the intracerebral injection. This order of drug administration ensured that the peak effects of DPDPE (Hammond et al., 1995; Thorat and Hammond, 1997) or DELT (Stewart and Hammond, 1993; Thorat and Hammond, 1997) at medullary and spinal sites would coincide and encompass both testing times. The agonists were administered in a fixed-dose ratio to allow characterization of the interaction between medullary and spinal sites by the isobolographic method (Boerig and Fujimoto, 1988, 1989; Tallarida et al., 1989; Tallarida, 1992b). Data on the hot-plate test were, by default, obtained at the dose-ratio determined for the tail-flick test because neither DPDPE nor DELT increased hot-plate latency in a dose-dependent manner after microinjection in the medulla. It was therefore not possible to calculate a ratio of ED_{50} values to be administered in the hot-plate test.

Statistical Analysis. A two-way ANOVA for repeated measures was used to compare the effects of DPDPE or DELT with those of the vehicle control. The Newman-Keuls test was used for post-hoc comparisons among the individual group mean values. Dose-response relationships for DPDPE or DELT at each site alone or in combination were determined using the individual tail-flick and hot-plate latencies obtained at the time of peak effect. For concurrent injection, the dose was expressed as the total dose (VMM + i.t.) of drug administered. The ED_{50} value was defined as the dose that produced the half-maximal possible increase in response latency. This value corresponded to 9.0 s in the tail-flick test and 25.0 s in the hot-plate test. Fieller’s theorem as applied by Finney (1964) was used to determine the 95% confidence limits. The experimentally derived dose-response relationship for the total dose of drug was then compared with its theoretical dose-additive relationship by standard parallel line assay methods (Finney, 1964; Tallarida et al., 1989; Tallarida, 1992b). In addition, an isobologram was constructed using the ED_{50} values for each agonist for the individual VMM and i.t. administrations and their 95% confidence limits (Tallarida et al., 1989). The experimentally derived ED_{50} value of the agonist combination was then plotted on the isobologram and statistically compared with the theoretical dose-additive point (Tallarida, 1992b).

Data on the effects of DPDPE (Hammond et al., 1995) and DELT (Stewart and Hammond, 1993) at i.t. or medullary (Thorat and Hammond, 1997) sites of injection were taken from previous studies published by personnel from this laboratory. Because the data on the antinociceptive potency of DPDPE or DELT in the VMM were obtained at about the same time as this study was conducted, replication of these dose-effect curves was not necessary. The data on the effects of i.t. administered DPDPE and DELT were obtained several years earlier. However, two independent estimates of the ED_{50} values of both DPDPE and DELT conducted several years apart yielded ED_{50} values for each drug that were not significantly different (Stewart and Hammond, 1993; Hammond et al., 1995). Given that the same sources of drug and animals were used in all studies, a third replication of the dose-effect curves of i.t. administered DPDPE or DELT was not justified.

Histology. At the conclusion of testing, the rats were euthanized by CO_{2} inhalation. The location and patency of the i.t. catheter were determined by direct visual inspection after a laminctomy and an i.t. injection of India ink. The brains were removed and fixed by immersion in a 4% formaldehyde and 30% sucrose solution. Transverse sections of the brain stem (35 μm) were cut on a cryostat microtome and stained with cresyl violet. The location of each microinjection site was plotted on transverse sections of the rat brain.
stem modified from those provided by Neurographics (Kanata, Ontario) and was verified by a person unaware of the treatment.

**Drugs.** DPDPE (lot no. 116H58302) and DELT (lot no. 44H08641) were purchased from Sigma Chemical Co. (St. Louis, MO) and dissolved in saline. Intracerebral microinjections were made over a 60- to 120-s period in a volume of 0.4 μl via a 33-gauge stainless steel injector that extended 3 mm beyond the tip of the guide cannula. After injection, the cannula was left in place for an additional 60 s to allow the drug to diffuse locally and to limit its diffusion up the injection track. Intrathecal injections were made over a 60-s period in a volume of 10 μl and were followed by 10 μl of saline to flush the catheter. The progress of drug delivery to supraspinal and spinal sites was monitored by the movement of an air bubble in the polyethylene tubing that connected the injector to the syringe pump.

**Results**

**Distribution of Microinjection Sites in VMM.** Histological analysis revealed that the microinjection sites were distributed throughout the rostrocaudal extent of the NRM and NGCpα. The large number of rats and different treatment groups in this study precluded presentation of all the injection sites for each treatment group; therefore, because there were no major differences in the distribution of microinjection sites among the various treatment groups, only the distribution of microinjection sites for the total dose of 0.86 ng of DELT is presented (Fig. 1). Microinjection sites located outside the NRM and NGCpα included sites in the dorsal, lateral, or caudal aspects of the nucleus reticularis giganto-cellularis, as well as the pyramids, inferior olive, trapezoid body, and nucleus raphe obscurus. These sites were excluded from the analysis because previous data indicated that microinjection of DPDPE or DELT at these sites did not increase tail-flick or hot-plate latency (Thorat and Hammond, 1997).

**Effect of Concurrent Administration of DPDPE in VMM and Spinal Cord.** Total doses of ≥0.92 μg of DPDPE administered concurrently in the VMM and spinal cord significantly increased tail-flick latency. This effect was maximal at 45 min and persisted through 60 min after the intracerebral injection (data not shown). Total dose combinations of >27.9 μg could not be tested because it was not possible to administer >4.9 μg of DPDPE in the VMM due to its limited solubility. Figure 2 illustrates the experimentally derived dose-response relationship for the concurrent administration of the δ opioid receptor agonist DPDPE, as well as the theoretical additive dose-response relationship constructed for the 1:4.7 ratio of DPDPE at these sites. Although the experimentally derived dose-response relationship was situated to the right of the theoretical additive dose-response relationship (Fig. 2A), suggestive of a subadditive interaction, statistical comparison of the regression lines and their variances revealed that they did not differ significantly (P = .06), indicating that the interaction of DPDPE at medullary...
and spinal sites was additive. Because dose combinations of DPDPE that increased tail-flick latency beyond the 9.0-s criterion value could not be administered due to solubility limitations, an isobologram for this level of effect could not be constructed.

Concurrent administration of total doses ranging from 0.28 to 27.9 μg of DPDPE in the VMM and spinal cord did not significantly increase hot-plate latency (Fig. 2B). Because it was not possible to test higher total dose combinations of DPDPE, it cannot be definitively stated that DPDPE interacts in an additive or infra-additive manner in the hot-plate test. It is clear, however, that the interaction of DPDPE at medullary and spinal sites is not synergistic. No dose combination of DPDPE produced motor deficits, as determined by the inclined-plane test (data not shown), although the highest dose of DPDPE did induce transient circling and cervical flexion in 5 of 10 rats.

To ensure that synergism had not been overlooked in either the tail-flick or hot-plate tests, 10- and 100-fold lower total doses of DPDPE were also tested. In rats that received a combined total dose of 28 ng of DPDPE, tail-flick and hot-plate latencies were 3.3 ± 0.1 and 10.5 ± 1.4 s, respectively. In rats that received a combined total dose of 2.8 ng of DPDPE, tail-flick and hot-plate latencies were 3.6 ± 0.3 and 11.0 ± 1.8 s, respectively. These latencies were not significantly different from baseline values.

**Effect of Concurrent Administration of DELT in VMM and Spinal Cord.** Concurrent administration of DELT to the spinal cord and VMM produced an uncharacteristic dose-response relationship (Fig. 3A). Extremely low total dose combinations (86 pg to 43 ng) of DELT produced a dose-dependent increase in tail-flick latency, with a peak effect apparent by 30 min and persisting through 40 min after the intracerebral injection. Unexpectedly, the near-maximal increase in tail-flick latency produced by 43 ng was not sustained at higher doses; rather, a decrement in effect occurred. However, at total dose combinations of 0.22 to 3.46 μg, DELT again produced a dose-dependent increase in tail-flick latency. In view of the dual effect of DELT, the data for doses ranging from 86 pg to 0.043 μg of DELT and from 0.22 to 3.46 μg of DELT were separately fit by linear regression analysis and compared with the theoretical dose-additive line for the 3:8 ratio of DELT. The dose-response relationship for the very low doses of DELT was situated about 400-fold to the left of the theoretical dose-additive line and was significantly different from the dose-additive line, consistent with a synergistic interaction (*P* < .001). This conclusion is supported by examination of the isobologram (Fig. 4, filled circles) in which the 95% confidence limits for the ED$_{50}$ value of the experimental mixture do not overlap those determined for the theoretical dose-additive point. In contrast, the dose-response relationship for the total doses of DELT between 0.22 and 3.46 μg did not differ from the dose-additive line (Fig. 3A). Moreover, the experimentally derived ED$_{50}$ for the dose combination (Fig. 4, open circles) was superimposed on the theoretical dose-additive point in the isobologram. These data indicate an additive interaction for the higher total doses of DELT.

Concurrent administration of total doses of DELT ranging from 86 pg to 0.22 μg did not increase hot-plate latency compared with latencies in saline-treated rats. A small increase in hot-plate latency occurred after the administration

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**Fig. 3.** Dose-response relationships for DELT administered to the VMM (○; dashed line) the i.t. space (□; dashed line), and both the VMM and i.t. space in a fixed dose-ratio of 3:8 (●; solid line). The theoretical dose-additive line for this combination is represented by the solid line (Δ). A, tail-flick test. The slopes of the dose-response curves for DELT microinjected in the VMM and for i.t. administered DELT did not differ significantly and therefore are drawn using the common slope as described by Tallarida (1992b). When fitted to this common slope, the ED$_{50}$ values and 95% confidence limits of DELT in the VMM and spinal cord were 0.63 μg (0.3–1.3 μg) and 2.0 μg (0.9–4.3 μg), respectively. The common slope was then used to construct the theoretical dose-additive line for the total dose of DELT administered at both medullary and spinal sites; the ED$_{50}$ value was 1.26 μg (0.6–1.9 μg). The ED$_{50}$ values for the experimentally derived data for the low and high total dose combinations of DELT administered at both medullary and spinal sites, which were also constructed using the common slope, were 0.003 μg (0.002–0.006 μg) and 1.5 μg (0.8–2.9 μg), respectively. B, hot-plate test. Microinjection of DELT in the VMM or the i.t. space did not increase hot-plate latency. These data therefore were not fit by linear regression. Symbols represent the mean ± S.E.M. of response latencies from 7 to 21 rats.
used in this study produced motor deficits on the inclined-plane test (data not shown).

Discussion

In the present study, we used an approach in which the δ opioid receptor agonists were administered to medullary and spinal sites in a fixed dose-ratio. Two factors can guide the selection of the dose-ratio. The first factor is the empirical value of examining a combination that corresponds to the ratio of the equieffective (e.g., ED_{50}) doses of a drug at each site. The second factor is the variance of the theoretical dose-additive line. The selection of a dose-ratio with a small variance will facilitate detection of synergy or subadditivity. This analysis was limited to a single-dose combination that approximated the ratio of the ED_{50} values of these drugs at each site. In the case of DELT, the variance of the dose-additive line for this ratio was near optimal. In the case of DPDPPE, the variance of this ratio was not optimal. However, dose combinations with lower variance required administering a higher proportion of the total dose in the medulla; solubility limitations precluded this approach. Thus, technical limitations and the empirical value of testing equieffective doses of DPDPPE at each site prevailed in the study design.

The present results indicate that concurrent administration of the δ opioid receptor agonist DPDPPE to the VMM and spinal cord produced antinociception in an additive manner in the tail-flick test. This finding is consistent with a previous study in the mouse in which DPDPPE was administered i.c.v. and i.t. in a fixed-dose ratio and antinociception was assessed by the tail-flick test (Roerig and Fujimoto, 1989). However, these results are not consistent with prior studies in the rat that concluded that i.c.v. and i.t. administered DPDPPE interact synergistically to produce antinociception (Miauskowski and Levine, 1992; Miauskowski et al., 1993). One factor that may contribute to the discrepancy in findings is the use of a different measure of nociception. Miauskowski and colleagues used a modification of the Randall-Selitto paw pressure test to assess antinociception. Another factor may relate to experimental design. In these earlier studies, the researchers did not use a fixed dose-ratio analysis but rather administered increasing doses of DPDPPE at one site in the presence of a fixed dose of DPDPPE at the other site. This study design, in which the ratio of drug doses continuously varies, has been the subject of some debate (Tallarida et al., 1989; Tallarida, 1992a; Caudle and Williams, 1993).

The interaction of δ opioid receptor agonists at supraspinal and spinal sites has not been previously examined. The present findings indicate that the interaction is complex and dose dependent. Very low total dose combinations produced antinociception in the tail-flick test in a synergistic manner, whereas higher total doses produced antinociception in an additive manner. The conversion from synergy to additivity as the total dose increased was unexpected but is not without precedent. In a study of the immunosuppressive effects of µ and δ opioid receptor agonists using the spleen cell plaque-forming assay, the interaction of morphine and DELT converted from synergy to subadditivity as the total dose increased from femtomolar to nanomolar concentrations (Meissler et al., 1998).

At present, one can only speculate as to the mechanisms that subserve the complex interaction of medullary and spinal δ opioid receptors. The conversion from synergy to additivity suggests that the effects of different dose ranges of DELT are mediated by different mechanisms. One mechanism by which synergism could occur would involve the spinal release of norepinephrine. Preliminary studies from this laboratory indicate that the antinociception produced by microinjection of DELT in the VMM can be partially attenuated by i.t. administration of the α_{2}-adrenoceptor antagonist yohimbine (P. Banfor and D. L. Hammond, unpublished observations). Furthermore, i.t. administration of DELT with the α_{2}-adrenoceptor agonist dexmedetomidine produces antinociception in a synergistic manner (Grabow and Hammond, 1998). Finally, i.t. administration of yohimbine prevents the synergistic antinociception produced by coincident medullary and i.t. administration of DELT (Grabow and Hammond, 1998). Taken together, these data suggest that microinjection of low doses of DELT in the VMM evokes a release of endogenous norepinephrine in the spinal cord that interacts in a synergistic manner with exogenously administered DELT. However, as the dose of DELT increases, additional mechanisms appear to be recruited. One possible mechanism would involve an additional release of enkephalins in the spinal cord by higher doses of DELT in the VMM. Endogenously released enkephalins interact preferentially with δ opioid receptors in the spinal cord (Takekomi and Portoghese, 1993; Tseng et al., 1995; Hammond et al., 1997) and therefore would be expected to interact in an additive manner with i.t. administered DELT. Additionally, there is new evidence that supraspinally administered morphine activates a bulbo spinal pain facilitatory pathway mediated by α_{1}-adrenoceptors.
agonists, provided an opportunity to test the hypothesis that yet to be developed for clinical use. The recent introduction of reached in the case of DELT. This though comparison of the experimentally derived dose-re-“diluted” the i.t. administered drug (Tallarida, 1992b). Al-
ing the VMM site as an ineffective agent that in effect nevertheless, a theoretical dose-additive relationship for concur-
rently administered PDPDE could be constructed by model-
ling the VMM site as an ineffective agent that in effect “diluted” the i.t. administered drug (Tallarida, 1992b). Al-
though comparison of the experimentally derived dose-re-
sp忽nce relationship to the theoretical dose-additive line did not definitively demonstrate an additive interaction, it did indicate that the interaction was not synergistic in accor-
dance to the tail-flick test. No definitive conclusions could be reached in the case of DELT. This δ opioid receptor agonist did not produce a significant and sustained increase in hot-
plate latency when administered at either VMM (Thorat and Hammond, 1997; but see Ossipov et al., 1995) or spinal sites (Stewart and Hammond, 1993) or when administered concur-
rently (this study). Although the three highest total doses produced a modest but significant increase in hot-plate latency, higher total doses could not be tested due to solubility limitations. Therefore, the existence of synergism could not be con-

Support for the existence of at least two subtypes of δ opioid receptor has been provided by studies that used com-
peted and noncompetitive antagonists of the δ1 and δ2 opioid receptor (Jiang et al., 1991; Mattia et al., 1992; Stew-
art and Hammond, 1993; Hammond et al., 1995) or antisense probes for the δ opioid receptor (Bilsky et al., 1996) or that assessed the development of tolerance and cross-tolerance to the different prototypic agonists (Mattia et al., 1991; Sofuo-
glu et al., 1991). The finding that spinally and supraspinally administered PDPDE interacts in an additive manner whereas very low doses of DELT interact in a synergistic manner is additional evidence in support of the existence of different subtypes of δ opioid receptor. It is unlikely that the additive interaction observed at higher total doses of DELT reflects a nonspecific action of DELT at δ2 receptors because previous studies have established that the effects of these doses of DELT in the VMM and the spinal cord are mediated by δ1 opioid receptors (Stewart and Hammond, 1993; Thorat and Hammond, 1997).

Although the existence of δ opioid receptors has been rec-
ognized since the late 1970s, it is perplexing that systemi-
cally bioavailable δ opioid receptor agonist analgesics have yet to be developed for clinical use. The recent introduction of SNC80 and TAN67, two structurally dissimilar nonpeptidic agonists, provided an opportunity to test the hypothesis that δ opioid receptor agonists were a feasible approach to the development of potent, efficacious opioid analgesics that lacked the adverse effects associated with μ opioid receptor agonists. SNC80, which has affinity for both the δ1 and δ2 opioid receptors, was of limited potency in the tail-flick and hot-plate tests when administered systemically to the mouse (Bilsky et al., 1995). TAN67, which has preferential affinity for the δ1 opioid receptor, also exhibited limited efficacy and potency in tail-flick and hot-plate tests when administered systemically to the mouse (Kamei et al., 1995; Suzuki et al., 1995). The present finding that medullary and spinal δ1 opioid receptors interact in an additive, and not a synergistic, manner may be one basis for the relatively poor efficacy and potency of systemically administered SNC80 and TAN67. In contrast, the finding that medullary and spinal δ1 opioid receptors can interact in a synergistic manner within a limited dose range suggests that the development of systemi-
cally bioavailable δ1 opioid receptor agonist analgesics remains a fea-
sible approach to the development of potent non-μ, non-κ opioid analogues. However, in future studies of this pharma-
cological class of analgesic, close attention to the selection of dose will be necessary.

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