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Antinociceptive interactions of mu- and kappa-opioid agonists in the colorectal distension assay in rats

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ABSTRACT

Interactions of opioid agonists, fentanyl and oxymorphone (mu-selective) and spiradoline and enadoline (kappa-selective), were examined for additive, sub-additive, or supra-additive antinociception in the colorectal distension (CRD) assay. Single-dose values (mg/kg, 0.006–0.016 for fentanyl, 0.25–1.26 for spiradoline, etc.) were summed to formulate theoretical additive-dose plots for comparison with actual combined-dose effects. Combined fentanyl and spiradoline yielded additive (low-dose levels) or supra-additive (high-dose levels) effects. Single and combined doses of fentanyl (0.012 mg/kg) and spiradoline (0.3 mg/kg) were tested after pretreatment with saline, beta-funaltrexamine (b-FNA, mu-selective antagonist), or nor-binaltorphimine (n-BNI, kappa-selective antagonist). Supra-additive effects of combined agonists were attenuated by either antagonist (greater with n-BNI). But paradoxical patterns of antagonism of single-dose effects occurred: the fentanyl antinociception was not antagonized by b-FNA, whereas the spiradoline antinociception was. The results indicate complex interactions of agonists in this visceral pain model and potential for combined agonists to improve pain relief with decreased side effects.

In the early 1980s we searched for opioid analgesics other than the morphine-type to control surgical pain in cats, this species showing mania to mu-opioid agonists. Colorectal distension (CRD) served well as a visceral pain test model in cats (Sawyer and Rech, 1987). Kappa agonists (butorphanol, nalbuphine, pentazocine) induced antinociceptive effects. Butorphanol first calmed the cats, with purring, which reverted to greater irritability than controls as the antinociception began to wane. Butorphanol, most potent (2 mg, human dose), is a mixed partial agonist at mu and kappa receptors. Nalbuphine, less potent (5–10 mg), is a mixed opioid, agonist at kappa receptors and antagonist at mu receptors. Pentazocine, least potent (15–30 mg), is also a mixed partial agonist at mu and kappa receptors, all three with limited antinociceptive efficacy (Hardman et al., 1996; Walker et al., 2001). Arylacetamide kappa-opioid agonists (U50,488H, spiradoline, and enadoline) are selective, without direct mu-opioid receptor effects and with maximal antinociceptive efficacy. Antinociception of U50,488H involves 5HT, being attenuated by 5HT antagonists and GABA disinhibitory effects, but spiradoline antinociception is less dependent upon 5HT interactions (von Voigtlander and Lewis, 1988; Nemmani and Mogil, 2003).

Using the CRD in dogs, Sawyer et al. (1991) reported enhanced antinociception by butorphanol combined with oxymorphone or ketamine. Enhanced antinociception by butorphanol plus ketamine was also observed in cats (Sawyer et al., 1990). Ketamine is an NMDA antagonist that reverses acute mu-opioid tolerance, blocking emergence of pain facilitatory systems (Fundytus, 2001). Briggs et al. (1998b) tested the combination of oxymorphone and butorphanol in cats, enhancing analgesia and reducing side effects, as was also noted in dogs by Houghton et al. (1991).

Side effects of mu agonists (euphoria, constipation, enuresis, pruritis) are often mirror images of those of kappa agonists (dysphoria, minor gastrointestinal effects, diuresis, antipruresis) (Pasternak and Wood, 1986), generally seen across mammalian species. Combining mu- and kappa-opioid agonists reduced each other's side effects, producing additive antinociception in the cold-water tail-flick (CWTF in rats, Briggs, 1996). Briggs et al. (1998a) found selective antagonism of mu- or kappa-type antinociception by b-FNA or n-BNI, respectively, using the CWTF assay. Additive or enhanced pain relief with reduced side effects by combining agonists has been observed by Verborgh et al., (1997); Sutters et al., (1990); and Ross et al., 2000, to name a few. Bie and Pan (2003), however, reported potent antagonism of mu-agonist analgesia by kappa agonists with acute doses, targeted on the brainstem nucleus raphe magnus. But they also showed that kappa agonists blocked the hyperalgesia induced by chronic treatment with mu agonists.

The seminal work of T. L. Yaksh (1997) relates to concepts of visceral pain and mechanisms of opioid antinociception. Using microinjections of drugs into supra-spinal (brain) and spinal loci, and chemical, mechanical, or thermal nociceptive stimuli, he and colleagues formulated theories of complex pain pathways and drug actions still extant today. They proposed ascending and descending

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neuronal circuits involving the brainstem hubs of periaqueductal gray (PAG, mu receptors), rostral ventral medulla (mu/delta receptors), and substantia nigra (mu receptors). From these loci, pathways ascend to higher brain centers and descend to spinal junctions, particularly the dorsal horn (mu, delta, kappa receptors), modulating and integrating nociceptive and antinociceptive impulses. Yaksh's efforts and those of later investigators using his techniques (see Discussion) have contributed much to concepts of pain pathways and mechanisms of analgesic drug actions. Combining mu- and kappa-opioid agonists was proposed to improve chronic clinical pain therapy (Smith, 2008). This hypothesis portended separate antinociceptive drug actions on junctions in parallel or serially-connected neuronal chains in pain-related central nervous regions, to effect a synergistic interaction. Chronic visceral pain, more often occurring in severe clinical cases than do cutaneous types of pain, is more difficult to manage than cutaneous pain (Joshi and Gebhart, 2000). This would seem most relevant to Smith's proposal, since visceral pain is relieved by treatment with either mu- or kappa-opioid agonists (Ness and Gebhart, 1990; von Voigtlander and Lewis, 1988).

The objective of this study was to establish synergistic antinociceptive effects of fentanyl and spiradoline interactions in a visceral antinociceptive model (CRD). Such evidence supports the potential use of combined opioids for more effective management of moderate to severe cases of clinical pain.

1. Methods

1.1. Subjects

Male Sprague–Dawley rats (227) weighing 300 to 500 g were approved for use in this study by the All-University Committee on Animal Use and Care of Michigan State University, in accord with NIH standards. All animals were trained over a two-month period to adjust to insertion of a lubricated (KY [R] jelly, Skillman, NJ, USA) colonic balloon-catheter (Pointe Medical, Crown Point, IN, USA) via the rectum. Subjects were preconditioned to lie quietly in a towel wrapped snugly around them and tolerate the catheter in place over extended periods. Cheerios [R] cereal "treats" and subsequent "play and socializing time" on a large table top with cage mates among towels, boxes and tubes ("toys") moderated stress of the testing paradigms. Play periods were interspersed between testing periods for 1 to 2 h per interval.

1.2. Drugs

Fentanyl citrate (F) was purchased from Elkins-Sims, Inc., Cherry Hill, NJ, USA. Spiradoline was generously provided by Dr. P. L. von Voigtlander, Upjohn, Kalamazoo, MI, USA. Enadoline was generously supplied by Dr. David Downs, Parke-Davis Pharmaceutical Research, Ann Arbor, MI. Oxymorphone was purchased from Mallinckrodt, Mundelein, IL. Agonists were dissolved in saline solution. The opioid antagonists beta-funaltrexamine (b-FNA) and nor-binaltorphimine (n-BNI), dissolved in sterile water, were generously supplied by the National Institute on Drug Abuse, Bethesda, MD, USA. Drugs and saline were injected in different sites and separate syringes by the subcutaneous route (SC).

1.3. Nociceptive stimulus equipment and parameters

Nociceptive thresholds were established in the colorectal distension assay (CRD) in restrained subjects by air-pressure pulse-stimuli, inflating the balloon-catheter. To insure a standard, reproducible, brief stimulus, we devised a stimulus-pulse shaper. This consisted of a 4-liter glass-jar reservoir fitted with tubing and three-way stop-cocks yoked to the jar, the catheter, a sphygmomanometer, a bicycle pump, and a port to room air. The reservoir was charged with a pressure-head between 40 and 180 mm Hg to accommodate sub-threshold, threshold, and antinociceptive responses. The nociceptive stimulus was delivered by opening the line from the reservoir to the catheter (placed within the subject's rectum), then from catheter to the open air over a maximal period of 1 s. Thus, at least 6 stimuli could be delivered over the span of 1 min. Two stimuli were delivered within 10 s, yielding essentially identical signals (or lack of), to establish a valid response.

Initial lower sub-threshold pressure-pulses, frequently and randomly presented, extinguished incidental conditioning. When a threshold pulse or greater was delivered, the rat responded with an abdominal contraction ("guarding reflex"). This nociceptive response was measured via a water-filled doughnut, Disposa-Cuff (Critikon, Tampa, FL), fitted around the subject's abdomen. Tubing from the Disposa-Cuff to a pressure transducer relayed the signal to a polygraph recorder (Grass Instruments, Quincy, MA). The maximal amplitude of pressure pulses was restricted to avoid any potential tissue damage.

1.4. Dose–response determinations of agonists

After a nociceptive threshold was determined, the balloon-catheter was removed and the rat was released from the towel to be injected SC with a coded (researcher blinded) drug or placebo, using mild hand restraint. The subject was then towel-restrained again for nociceptive testing at 15-min intervals for 30 min or as long as 3 h post-injection. Subjects that had been tested only with single drugs were used again (no more than 3 times) in later tests, but only after a minimum of a week and after three daily typical threshold (placebo) responses. The opioid agonists were first tested singly for log-dose patterns of antinociception of fentanyl, spiradoline, enadoline, and oxymorphone. Details of number of subjects, dose levels, and test parameters are presented in the Fig. 1 legend in the Results section.

Comparisons of added single doses of agonist pairs, forming theoretical additive effects, with the actual effects of combined agonist pairs were done by the following protocol. Single dose levels of agonists that produced antinociceptive effects in the range of maximal percentage effects (see below) of approximately 20 to 50% for single-dose effects were combined and tested for the actual combined-agonist scores. Statistical comparisons of the theoretical and actual scores, the method for which is described below, established the additive, sub-additive or supra-additive differences of the actual combined-agonist interactions relative to their single dose effects. See Fig. 2 legend for *n* values and more details.

Two additional tests of combined fentanyl-spiradoline and fentanyl-endoline, two dose levels (high and low) for each combination, were conducted to determine antinociceptive interactions at 15 and 30 min post-injection. See Fig. 3 legend for *n* values and more details.

1.5. Selective agonist–antagonist determinations

A set of three groups of rats was pretreated 24 or 48 h before testing with saline, another set of three 24 h before with 8 mg/kg b-FNA (mu-selective antagonist, Ward et al., 1982), and third set of three 48 h before with 10 mg/kg n-BNI (kappa-selective antagonist, Jones and Holtzman, 1998). All 3 sets then received 0.012 mg/kg fentanyl, 0.3 mg/kg spiradoline, or the combination, and were tested 15 and 30 min later. Table 1 lists the *n* values for sets and groups and depicts a grid of treatments these subjects received.

1.6. Data analysis

ED50 doses of fentanyl and spiradoline were established with the linear regression function of Sigma Plot (Jandel Corporation, San Rafael, CA, USA). A repeated measures ANOVA test of drug comparisons using Sigma Stat (Jandel) and Student–Neuman–Keul's method was used to identify significant group differences. Significance was set at $p<0.05$. Antinociceptive data were standardized as maximum percent effect

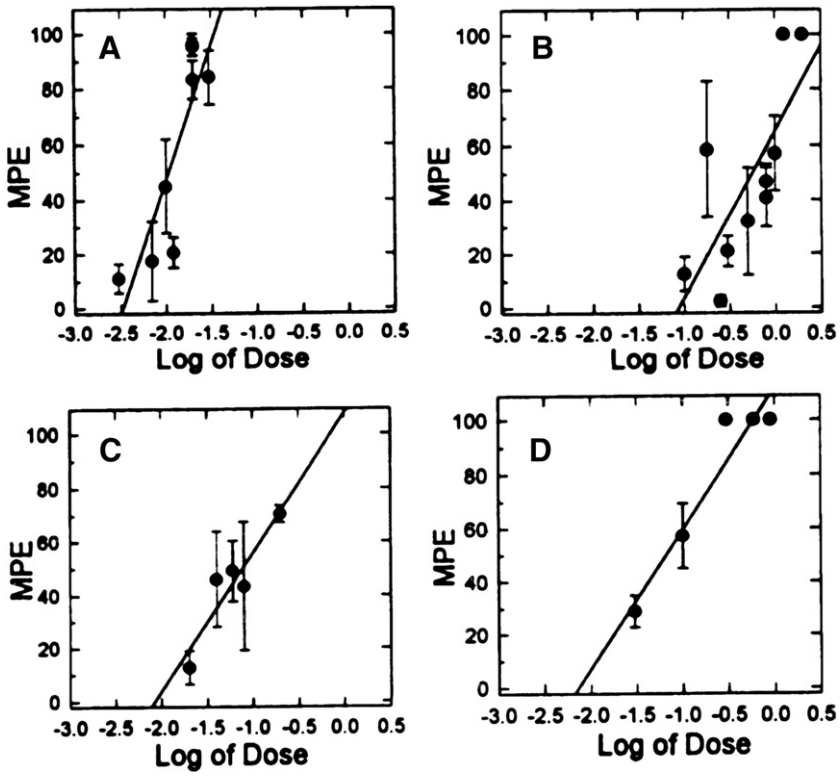

Fig. 1. Antinociceptive responses of fentanyl, spiradoline, enadoline, and oxymorphone in the CRD. A: M.P.E. means (+/−S.E.M.) are plotted for fentanyl log dose responses at 15 min post-injection. ED-50=0.01 mg/kg (range: 0.06–0.016); n=3–16 per dose. B: M.P.E. means (+/−S.E.M.) for spiradoline at 15 min post injection. ED-50=0.56 (0.25–1.26); n=3–12 per dose. C: M.P.E means (+/−S.E.M.) for enadoline at 30 min post-injection. ED-50=0.077 (0.04–0.2); n=4–7 per dose. D: M.P.E. means (+/−S.E.M.) for oxymorphone at 30 min post-injection. ED-50=0.078 (0.02–0.126); n=5–9 per dose.

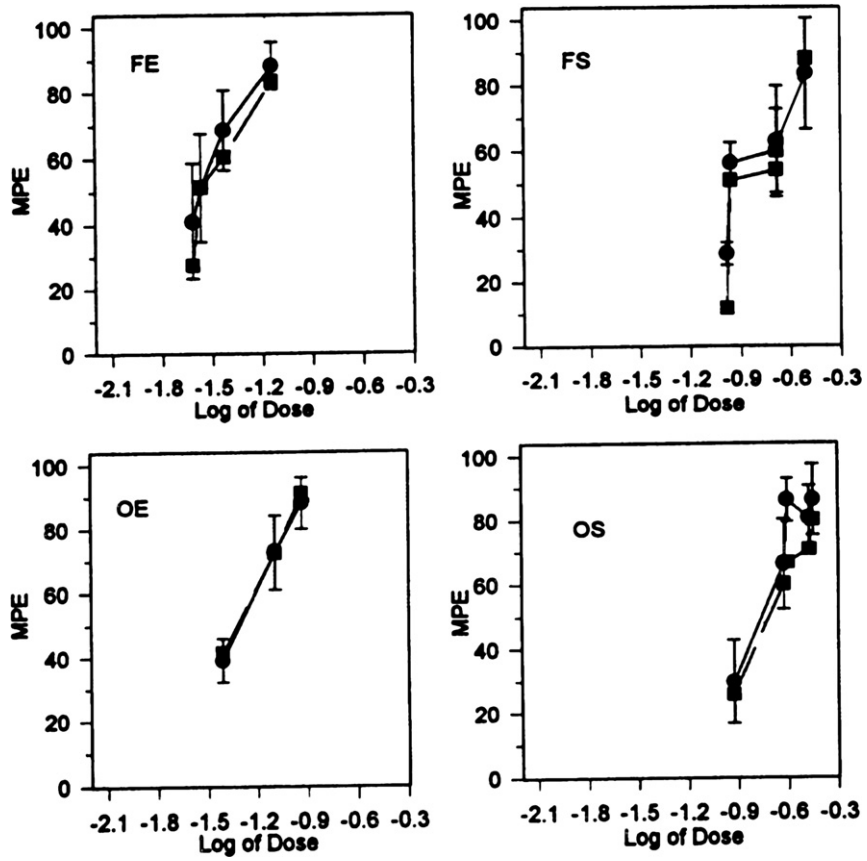

Fig. 2. Antinociceptive responses in the CRD of M.P.E. means (+/−S.E.M.) for actual combined doses of opioid agonist pairs (filled circles) vs. additive theoretical plots of combined single doses of each pair (filled squares) at 15 min post-injections. FE displays fentanyl plus enadoline plots, FS fentanyl plus spiradoline plots, OE oxymorphone plus enadoline plots, and OS oxymorphone plus spiradoline plots; n=6–9 per dose.

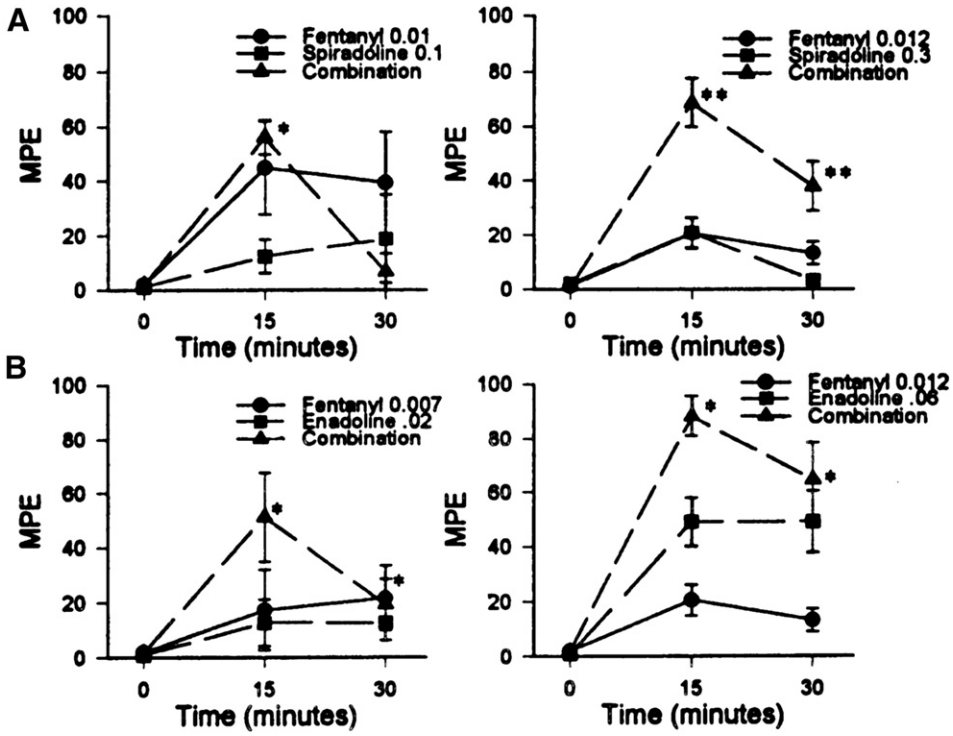

Fig. 3. Antinociceptive responses in the CRD of M.P.E. means (+/−S.E.M.) of single opioid agonists are compared with combined agonist pairs at two dose levels tested at 15 min (A) and 30 min (B) post-injection. * = Additive interactions; ** = supra-additive (synergistic) interactions; $p<0.05$; $n=8–10$ per dose.

(MPE, Harris and Pierson, 1964): MPE = PDn − *C*/Max − C × 100, where PDn = tested stimulus level at n min post-injection, *C* = the stimulus level of a naïve subject's response, and Max = the maximum stimulus level presented to any subject.

Analysis of the antinociceptive responses of actual combined doses compared to theoretical values of added sums of individual drug effects (Fig. 2) were computed using the *Z* table (Steel and Torrie, 1984). The MPE of each individual dose of fentanyl was summed with a matching MPE of a spiradoline dose. The standard error of the mean (+/−SEM) of each theoretical sum was calculated from the root mean square of individual SEM's, dividing the absolute difference between theoretical and actual values by the root mean square of theoretical and actual SEM's. Numbers in the *Z* table corresponding to $p<0.05$, *Z* greater than −1.65, and $p<0.01$, *Z* greater than −2.33, were used to establish significant differences.

2. Results

Individual mean log-dose–response patterns (+/−SEM) in the CRD for fentanyl, spiradoline, enadoline, and oxymorphone formed linear slopes from just significant to full antinociception (ANC) with little deviation (Fig. 1). See Fig. 1 legend for *n* and ED-50 values. Fentanyl duration was 50 min. Spiradoline duration was 2 h. Oxymorphone and enadoline served as class comparisons.

When we compared actual responses of the drug combinations to their theoretical sums at 15 min post-injection, results indicated mostly additive ANC interactions, with one exception (Fig. 2).

The exception was one point of actual combined-dose values of oxymorphone plus spiradoline, which yielded a supra-additive (synergistic) effect. Otherwise the actual combined effects of the 4 agonist pairs (singly scoring 20–50% MPEs) formed fairly linear slopes not significantly different from the theoretical slopes of added single doses at 15 min post-injection.

The results of low- and high-dose combinations of fentanyl plus spiradoline and fentanyl plus enadoline, tested for ANC at 15 min and 30 min post-injection, are shown in Fig. 3.

The high-dose combination of fentanyl plus spiradoline resulted in supra-additive interactions at both time periods. Tests of the other dose combinations formed additive response patterns. The single low dose of fentanyl in panel A scored a higher MPE (45) than the single high dose of fentanyl (18). This anomaly will be reviewed in the Discussion section. We never observed "fentanyl-induced freezing" behavior (catalepsy).

Single antinociceptive-dose effects of fentanyl (0.012 mg/kg), spiradoline (0.3 mg/kg), and the combined-dose effects of agonists after saline pretreatment, b-FNA pretreatment, or n-BNI pretreatment in the three sets of rats (9 groups in all) are presented in Fig. 4.

After saline pretreatment, both fentanyl and spiradoline individually produced an approximate ED-20 ANC response at the 15-min test period (mean MPE for fentanyl = 21% and for spiradoline = 22%). The drug combination after saline pretreatment induced prominent synergistic ANC (mean MPE for *C* = 68%). At the 30-min test the combined agonists continued to manifest a supra-additive effect in the saline-pretreatment group (mean MPE = 38%), compared to the mean single-dose fentanyl score of 14% and the mean single-dose spiradoline score of 3%.

Surprisingly, the fentanyl MPE score was not reduced after b-FNA pretreatment from that of the saline-pretreatment group (30% vs. 21%)

Table 1
Number of subjects, pretreatment (PreRx), and treatment grid for testing agonist–antagonist interactions in CRD

Team I: saline PreRx			Team II: b-FNA[a] PreRx			Team III: nor-BNI[b] PreRX		
F[c]	Sp[d]	C[e]	F	Sp	C	F	Sp	C
8 rats	8 rats	10 rats	4 rats	4 rats	6 rats	4 rats	4 rats	6 rats

See further details in Fig. 4 legend in the Results section.
[a] Beta-funaltrexone, 8 mg/kg SC, 24 h before test.
[b] Nor-binaltorphimine, 10 mg/kg SC, 48 h before test.
[c] F = fentanyl 0.012 mg/kg.
[d] Sp = spiradoline 0.3 mg/kg.
[e] C = combined agonists.

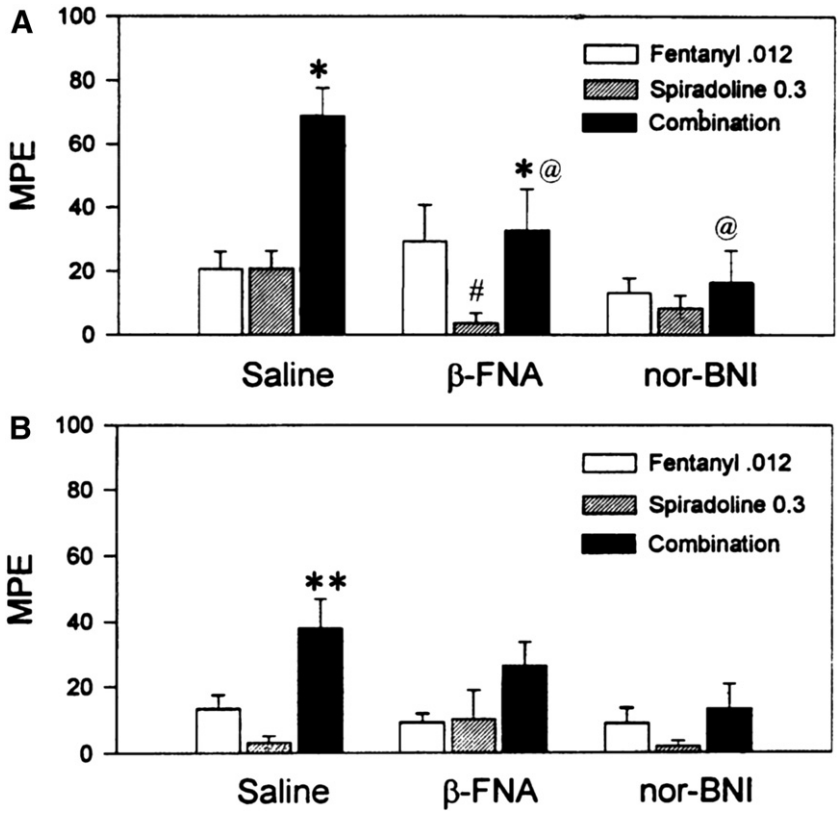

Fig. 4. Antinociceptive responses in the CRD of M.P.E. means (+/−S.E.M.) for fentanyl, spiradoline, and their combination at 15 min (A) and 30 min (B) post-injections. Three groups of rats were pretreated, the first (n=8–10) with saline 24 or 48 h before agonist testing, the second (n=4–6) with beta-funaltrexamine (b-FNA) 24 h before agonist testing, and the third (n=4–6) with nor-binaltorphimine (n-BNI) 48 h before agonist testing. * = supra-additive (synergistic) interaction vs. saline control single-agonist responses. # = antagonism by b-FNA vs. saline control spiradoline response. @ = antagonism vs. the saline control combined-agonist response for both b-FNA and n-BNI pretreatments, as well as a significant decrease of the n-BNI combined-agonist response vs. the b-FNA combined-agonist response. ** = supra-additive interaction of combined agonists vs. all other responses at the 30 min test period. For all comparisons, significance was set at $p<0.05$.

at the 15-minute test period. Furthermore, the spiradoline MPE was significantly decreased (4% vs. 22%) after b-FNA in this period. The combined agonists after b-FNA resulted in a score significantly reduced (33%) from the combined agonists' score in the saline-pretreatment group (68%).

The n-BNI pretreatment failed to alter significantly the individual agonist scores at either the 15- or 30-min test periods compared to those of saline controls. However, the score of the combined drugs after n-BNI was much reduced from those of saline-pretreatment rats (18% vs. 68% at the 15-min test, and 13% vs. 38% at the 30-min test).

To emphasize the difference of the paradoxical effects in Fig. 4 compared to our results of agonist/antagonist interactions in the CWTF (Briggs et al., 1998a), the results from the CWTF study are repeated here. Using the CWTF, the mean MPE of fentanyl was 86% and that of spiradoline was 77% after saline pretreatment. After b-FNA pretreatment the fentanyl score was significantly reduced to 21%. The spiradoline score was a non-significant decrease of 67%. After n-BNI pretreatment, the fentanyl score was a non-significant decrease of 73% and the spiradoline score was significantly reduced to 13%. In addition, combined agonists in CWTF had an antinociceptive MPE that was additive relative to the single dose effects, rather than the supra-additive effect observed in the CRD assay.

3. Discussion

Dose–response patterns of individual doses of the agonists, fentanyl and spiradoline, included maximal antinociceptive effects (ANC) in the CRD visceral pain assay. Comparison of the theoretical combination effects of agonists, added sums of individual agonist responses, with actual combined effects of fentanyl plus spiradoline, indicated primarily additive-response patterns of ANC for these combinations. Higher-dose combinations of fentanyl plus spiradoline produced supra-additive ANC at both 15 and 30 min post-injection (Figs. 3 and 4). Thus, combination of this mu-opioid agonist and this kappa-opioid agonist at some dose levels enhanced the increase in the threshold for visceral pain in the CRD test model beyond an additive level.

In our previous investigation of the ANC of fentanyl and spiradoline in the cold-water tail-flick assay (CWTF, Briggs et al., 1998a) we also observed maximal ANC for either class of opioid agonist. However, combined-agonists dose–effect patterns of ANC in CWTF differed from those in the CRD. In CWTF low-dose combinations led to additive effects, while high-dose combinations led to sub-additive or antagonistic interactions. In CRD, low doses in combination induced additive effects while combinations with high doses resulted in supra-additive ANC patterns. The maximal antinociceptive effects of spiradoline and enadoline distinguish them from antinociceptive effects of butorphanol, nalbuphine and pentazocine (mixed partial agonists or agonist–antagonists, Hardman et al., 1996; Walker et al., 2001). The latter kappa opioids show limited efficacy and sub-additive effects when combined at higher doses with mu-opioid agonists such as oxymorphone (Sawyer and Rech, 1987; Briggs et al., 1998b).

Regarding agonist–antagonist interactions (fentanyl and spiradoline, b-FNA and n-BNI), prior results in CWTF were straightforward. b-FNA (mu-selective antagonist) markedly decreased the ANC of fentanyl without a significant change in spiradoline ANC. After n-BNI (kappa-specific antagonist), a reduced ANC of spiradoline (selective kappa agonist, von Voigtlander and Lewis, 1988) occurred, while no significant change in the ANC of fentanyl was observed. Agonist–antagonist interactions in CRD (Fig. 4) resulted in paradoxical

reactions. After b-FNA, fentanyl ANC tended to increase (non-significantly) while spiradoline ANC was attenuated, relating to individual agonist effects in saline-pretreatment subjects. After n-BNI, neither fentanyl nor spiradoline single-dose ANC was significantly altered from those of the saline-pretreated subjects. The use of low ANC doses of agonists in the CRD tests (MPE of approximately 20%), intended to optimize synergistic ANC interactions of the two agonists, perhaps compromised the extent of the antagonisms. Other possible explanations for such complex opioid interactions are discussed below.

In Fig. 3, left-hand panel A, ANC of the low-dose fentanyl was greater than that of the higher-dose fentanyl response, right-hand panel A. This anomaly may relate to the repeated testing of the subjects (maximum of 3 treatments) with single doses of opioid agonists, even though we spaced a week and at least 3 days of placebo test results between treatments. Pearl and Glick (1996) reported interactions of U50,488H or spiradoline with morphine, reducing morphine enhancement of locomotor activity when morphine was injected 19 h after either kappa-opioid agonist. The kappa-opioid antagonism was further strengthened by 2 days of morphine pretreatment. Thus, mu- plus kappa-opioid agonistic influences on neuroplasticity appear to far outlast (45 h or more) the usual ANC duration of single-dose effects.

Generally, mu- and kappa-opioid receptor activities modulate preferentially the somatic (cutaneous) and visceral types of pain impulses, respectively (Ness and Gebhart, 1990). Mu receptors predominate on C fibers, while kappa receptors predominate on A-delta fibers (Werz et al., 1987). Nociceptive stimuli from peripheral organs enter the spinal cord mostly via dorsal roots. Those carried by A-delta fibers are characterized as immediate, “sharp,” and time-limited (“first pain,” cutaneous, somatic, as in the CWTF). They distribute to dorsal-root laminae I, V, and perhaps X. They transmit to the neospinothalamic tract, rapidly relaying the discriminative signals to the ventrolateral thalamus, and thence to S1 of sensory cortex. These components contain a paucity of opioid receptors.

C fibers entering dorsal roots carry impulses with slow onset, more powerful affectively, characterized by “burning” and aching, and being more protracted (“second pain,” more typical of visceral pain and CRD). Convention states that they end in lamina II, but recent information suggests projections to deeper laminae. These signals are relayed via ascending spinoreticulodiencephalic pathways, with branching and collaterals at spinal nodes, to distribute primarily to brainstem nuclei. These slower, diffuse relays are generously endowed with mu- and kappa-opioid modulating receptors, the mu type predominating in brainstem nuclei and the kappa type predominant in spinal column nodes (Willis and Westlund, 1997). Kappa receptors show a greater association with visceral pain systems (Black and Trevethick, 1998). Dorsal horn visceral afferents have less dense A-delta than C fibers (1/8–1/10), whereas somatic afferents have the opposite (2/1) (Bonica, 1990; Janig and Morrison, 1986), for diffuse vs. focal emphasis.

In a series of studies following in the footsteps of Yaksh, Miaskowski and colleagues (see Miaskowski et al., 1993) described mu- and kappa-opioid agonist interactions as producing antagonistic or synergistic ANC, the latter accompanied by reduced side effects. They used mechanical nociceptive stimuli, implying visceral-type pain mechanisms. Intracerebroventricular (i.c.v.) injections delivered agonists to brain sites while intrathecal (i.t.) injections delivered them to spinal sites. DPDPE (delta agonist) i.c.v. combined with i.t. DAMGO (mu agonist) induced antagonistic effects. However, most combinations produced enhanced ANC. The greatest synergy was seen after combined i.c.v. DAMGO and i.t. U50, 488H. They proposed a mechanism of multiple brain-spinal ascending–descending neuronal loops, with mu and kappa receptors residing at junctions of shared components. Multiple agonistic actions at receptors in serial or parallel arrangements were proposed to amplify the total ANC effect beyond the sum of the parts.

Consistent with the above theories, supra-spinal dynorphin (endogenous kappa agonist) antagonized the ANC of morphine also injected supra-spinally, but supra-spinal dynorphin potentiated spinally-induced morphine ANC (Ren et al., 1985). Also, Stachura and Herman (1994) reported potentiated ANC of SC morphine by spiradoline injected intrathecally. ANC from morphine or U50, 488H in mice was attenuated by increasing brain GABA activity or reducing brain 5HT activity (Nemmani and Mogil, 2003), indicating that complex multiple interactions between opioid agonists and other neurotransmitter systems also occur.

Neurochemical studies support Yaksh's and Miaskowski's hypotheses. Both mu- and kappa-opioid receptors were found on most nociceptive neurons throughout central and peripheral mammalian nervous systems (Atweh and Kuhar, 1997; Allerton et al., 1989). Interactions may occur on peripheral A-delta fibers and C fibers, on dorsal root ganglion cells and synaptic endings, and on interneurons in dorsal horn or spinal projection cells. Also, interactions do occur in supra-spinal nuclei (especially PAG, PVG, RVM, and raphe nuclei), as well as in forebrain loci (see Bie and Pan, 2003; He and Lee, 1997).

Similar constructs were put forth by Narita et al. (2005) and Khotib et al. (2004) in a number of articles. Kappa-opioid receptors were found to be involved in the same neuronal network in rat PAG that controls morphine tolerance and dependence (Herra'ez-Baranda et al., 2005). This discovery relates to studies by He and Lee (1997), Jang et al. (2006), Song and Takemori (1992), Tao et al. (1994), and Yamamoto et al. (1988), in which kappa-opioid agonists enhanced morphine ANC, reversing tolerance and/or dependence. Acute mu- and kappa-opioid agonists both inhibited glutamate input to brain-stem ventral tegmental area neurons, but from different sources (Margolis et al., 2005). But chronic opioid agonists activate glutamate mechanisms, promoting opioid-agonist tolerance and dependence (Fundytus, 2001). These types of neuronal dichotomy would allow for potential synergistic or occlusive effects of combined agonists. Complex mu-/kappa-opioid interactions, with differential relationships of opioid receptors in visceral and cutaneous types of pain, were also elaborated by Schmauss and Yaksh (1984) and Gebhart (1992).

Based upon the above-reviewed research, failure of b-FNA pretreatment to alter ANC of fentanyl in CRD (Fig. 4) could occur by several mechanisms. A supra-spinally or spinally innervated mu-opioid receptor link: (1) may exert tonic inhibition of spinal kappa-opioid-agonist mechanisms; then: (2), by blockade of the mu receptors by b-FNA, a disinhibition of the spinal kappa mechanism could cause ANC to be induced by release of an endogenous kappa agonist. Likewise, the decreased ANC of spiradoline after b-FNA could relate to: (1) chronic supra-spinal or spinal kappa-opioid mechanisms activating release of an endogenous mu-opioid agonist. In turn: (2) the mu-agonist would inhibit spinal pain-projection neurons reacting to incoming distal nociceptive stimuli, causing ANC. Though spiradoline would still release endogenous mu agonist, b-FNA blockade of post-junctional mu receptors would attenuate the ANC.

Such interactions would be consistent with the synergism of ANC by combined agonists in the saline-pretreatment group (Fig. 4) being decreased after either antagonist pretreatment, b-FNA or n-BNI. The greater antagonism by n-BNI of the combined agonist ANC synergy may indicate (as suggested by Schmauss and Herz, 1987) a dominant role of kappa-opioid receptor mechanisms in the suppression of visceral pain. Staahl et al. (2006) showed oxycodone to induce superior ANC vs. morphine in human subjects exposed to experimental visceral nociception. Since oxycodone is a kappa agonist metabolized to a mu agonist (Ross et al., 2000), these results imply a mu- and kappa-opioid interaction.

As indicated above, interactions between exogenous mu- and kappa-opioids, as well as those between endogenous opioids, seem to be most implicated in conditions involving chronic visceral pain. Several clinically-oriented reviews have promoted the concept of employing opioid drug combinations for improved therapeutic management of pain while reducing adverse drug side effects (Coop and MacKerell, 2002; Smith, 2008). Joshi and Gebhart (2000) reviewed

the need for greater knowledge and research in areas of visceral pain for candidate opioid and non-opioid therapies. They emphasized the recent discovery of the dorsal column pain pathway, further integrating spinal and supraspinal nociceptive and ANC mechanisms. Thus, sites were identified at which drugs may act to modulate visceral pain mechanisms. More extensive research on this topic would likely aid in the development of more effective therapies.

Larsson et al. (2003) used the CRD assay in mice, testing ANC responses to U69593 (a kappa agonist) and fentanyl, 0.5 and 0.05 mg/kg, respectively. These data are in close agreement with our results testing fentanyl and spiradoline in CRD using rats. Christianson and Gerhart (2007) reviewed the use of CRD in a number of non-anesthetized species, including humans. Drug efficacy, strain, species, gender, and genetic differences were shown to be important variables. Rodent strain differences in gene mechanisms that control synthesis of opioid receptors and endorphins can markedly alter ANC responses to exogenous opioids.

Fentanyl and the kappa-opioid agonist U69593 were evaluated for effects on FR-30 food-reinforced responding, ANC, and self-administration in rhesus monkeys (Negus et al., 2008). The drugs singly decreased response rates for food, and, when combined, resulted in sub-additive effects. Using tail emersion in 50-degree-C water, a cutaneous type of nociception, each agonist induced dose-dependent ANC. Combined agonists produced additive ANC responses. Fentanyl access supported self-administration of the mu-opioid agonist, but U69593 access did not induce U69593 self-administration. Combined agents increased the sensitivity of fentanyl self-administration to increases in FR demand values. The authors suggest that mu/kappa opioid combinations, or single agents with mixed mu/kappa agonist effects, may reduce abuse liability without compromising analgesic efficacy as related to single selective opioid agonist effects. We are in total agreement with their projections, having advanced similar predictions (Briggs et al., 1998a,b; Sawyer and Rech, 1987; Sawyer et al., 1991). The ANC results of Negus et al. (2008) resemble ours with fentanyl-spiradoline single and combined drug effects in the CWTF in rats, but are different from our results using rats in the CRD.

Regarding speculations on the clinical utility of mu-opioid and kappa-opioid combinations, a major drawback to the use of selective kappa-opioid agonists of the spiradoline type is the prominent dysphoria they induce. We have submitted a manuscript for publication, entitled “Fentanyl and Spiradoline Interactions for Place Conditioning Responses in a Black–White Shuttle-Box.” In this study we describe the dose-related preference of fentanyl to be suppressed by combination with spiradoline, supporting the thesis that the combined agonists would reduce the addiction liability of fentanyl. But we also found that aversion in spiradoline-trained rats was attenuated by combining the kappa-opioid agonist with fentanyl. The potential for decreased aversion with combined agonists may make them more acceptable to patients, as was intimated by Preston and Bigelow (1993) in a study of mu- and kappa-opioid interactions in humans.

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