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Pharmacological Profile of the Potentiation of Opioid Analgesia by Restraint Stress

DANIEL J. CALCAGNETTI,[1] SHARON W. FLEETWOOD[2] AND STEPHEN G. HOLTZMAN

Department of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322

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CALCAGNETTI, D. J., S. W. FLEETWOOD AND S. G. HOLTZMAN. *Pharmacological profile of the potentiation of opioid analgesia by restraint stress.* PHARMACOL BIOCHEM BEHAV 37(1) 193–199, 1990.—Morphine-treated rats exposed to restraint stress show potentiated magnitude and duration of analgesia compared to unstressed rats. The present study was performed to assess the pharmacological characteristics of stress-induced potentiation of opioid analgesia. We tested 10 opioids to determine whether restraint stress treatment would potentiate their ability to produce antinociception indexed by the tail-flick assay. We tested full mu, delta and kappa opioid receptor agonists (fentanyl, meperidine, DPDPE, U50488H, ethylketocyclazocine), and mixed agonist/antagonists representing a range of receptor selectivities and intrinsic activities (profadol, buprenorphine, pentazocine, butorphanol and nalbuphine). Dose-effect and time-response curves were generated for unrestrained and restrained rats after either subcutaneous (SC) and/or intracerebroventricular (ICV) injections. In restrained rats, all drugs except for SC-administered nalbuphine produced dose- and time-dependent analgesic effects of greater magnitude (1.5–3 times) than they produced in unrestrained rats. However, restrained rats given agonists with high intrinsic activity at the μ receptor displayed the most potent and consistent potentiation of analgesia compared to unrestrained controls. Our results suggest that activation of the μ receptor is of primary importance for restraint to potentiate analgesia, because restrained rats injected with δ and κ agonists displayed potentiation of analgesia only at doses high enough to possibly exceed the selective activation of their respective receptor types.

Endogenous opioids Restraint stress Analgesia Agonist/antagonists Intracerebroventricular
Mu, kappa and delta opioid receptors Fentanyl Profadol Buprenorphine Butorphanol DPDPE
Meperidine Ethylketocyclazocine Nalbuphine U50488H Pentazocine Tail-flick assay

THE analgesic effect of opioids is potentiated in rats subjected to restraint stress. For example, both the magnitude and the duration of morphine-induced analgesia, as measured by the tail-flick test, are increased in restrained rats (1,2). Systematic examination of the phenomenon revealed that it is not dependent upon the pituitary-adrenocortical axis and that brain and plasma levels of morphine are not significantly different for restrained and unrestrained treated rats (1). Moreover, restraint does not appear to alter the opioid receptor affinity for naloxone compared to unrestrained subjects (2).

Potentiation of opioid analgesia by restraint stress is believed to be a centrally mediated phenomenon because the analgesic effects of intracerebroventricularly (ICV) injected morphine (3), D-Ala^2-N-MetPhe^4-Gly^5(ol) enkephalin (DAGO) and D-Ala^2-D-Leu^5-enkephalin (DADLE) (4) were potentiated by about the same degree as that of peripherally administered morphine. Subsequent studies have revealed that tolerance to the analgesic effects of morphine fails to alter restraint-induced potentiation of morphine-induced analgesia in rats (12). However, potentiation of opioid analgesia is attenuated by habituation to restraint and varies as a function of the interval between restraint and the time of analgesic testing (6).

Two important aspects of restraint stress potentiation of opioid-induced analgesia remain unknown: 1) the mechanism(s) of action responsible for restraint stress potentiation and 2) the type(s) of opioid receptor(s) involved. The primary objective of the present experiments was to gain some insight into these issues by examining the degree to which restraint alters the duration and magnitude of the antinociceptive action of several opioid agonists. In light of the existence of several types of opioid receptors [for review see (7)], the possibility exists that more than one receptor type is involved in restraint stress potentiation of opioid analgesia.

The drugs tested in experiments on restraint-induced potentiation of analgesia usually have been restricted to μ-selective agonists, morphine, methadone, and DAGO (1,4). DADLE has also been tested, to examine the possible involvement of δ receptors (4). However, DADLE is now known to have a low order of δ versus μ selectivity (15). Although restraint stress potentiated the analgesic effect of DADLE, the potentiation can be attributed to the μ receptor activity of DADLE (4).

[1]Requests for reprints should be addressed to Daniel J. Calcagnetti at his present address: Northeastern Ohio University College of Medicine, Pharmacology Department, Rootsville, OH 44272-9989.
[2]Present address: ATSDR/DHAC, Mailstop E32, 1600 Clifton Road, Atlanta, GA 30333.

TABLE 1

AGONISTS TESTED, DOSES, ROUTE OF ADMINISTRATION, RECEPTOR SELECTIVITY, AND ED_{50} IN RESTRAINED AND UNRESTRAINED RATS

Drug	Doses and Route Tested	Receptor Activity	ED_{50} (95% confidence limit) mg/kg or μg/rat(†) Restrained	Unrestrained
Fentanyl	(0.01-0.08 mg/kg, SC)	Full mu agonist	0.023 (0.019–0.027)	0.04 (0.024–0.064)
Meperidine	(3.0–30 mg/kg, SC)	Full mu agonist	11.5 (8.9–14.8)	29.5 (22.4–38.9)
Buprenorphine	(0.1–1.0 mg/kg, SC)	Partial mu agonist	0.21 (0.05–0.79)	*
Profadol	(1.0–10 mg/kg, SC)	Partial mu agonist	6.3 (5.25–7.58)	9.3 (7.4–10.2)
Pentazocine	(3.0–30 mg/kg, SC)	Partial mu/kappa agonist	*	*
Butorphanol	(0.3–3.0 mg/kg, SC)	Partial mu/kappa agonist	2.8 (1.8–4.5)	*
Nalbuphine	(3.0–30 mg/kg, SC)	Partial mu agonist	*	*
DPDPE	(10–100 μg/rat, ICV)	Full delta agonist	46.8 (31.6–69.2)†	*
U50488H	(1.0–10 mg/kg, SC)	Full kappa agonist	*	*
EKC	(0.1–1.0 mg/kg, SC)	Full kappa (mu) agonist	0.48 (0.42–0.55)	*
Morphine (12)	(1.0–8.0 mg/kg, SC)	Full agonist	2.39 (1.64–3.14)†	4.14 (2.78–5.5)
Morphine (3)	(0.03–10 μg/rat, ICV)	Full agonist	0.8 (0.5–1.4)†	4.4 (2.1–8.9)
DAGO (4)	(0.01–0.3 μg/rat, ICV)	Full mu agonist	0.03 (0.01–0.07)†	0.2 (0.1–0.3)
DADLE (4)	(1.0–10 μg/rat, ICV)	Full delta/mu agonist	1.2 (0.4–3.4)†	5.4 (2.8–10.7)

*Drug failed to produce 50% of effect.

Therefore, the purpose of the present study was to test drugs that are highly selective for μ, δ or κ receptors. In addition, the importance of intrinsic activity was evaluated by testing drugs that are partial agonists at the μ and/or κ receptors. Fentanyl (13), meperidine, [D-Pen^2, D-Pen^5] enkephalin [DPDPE, (17)] and U50488H (20) served as full and selective opioid agonists at μ, δ and κ receptors respectively. Ethylketocyclazocine (EKC) was also selected as a full κ agonist, although it is known to bind equally well to κ as μ receptors (15). Buprenorphine, pentazocine, profadol, butorphanol and nalbuphine were selected because of their mixed agonist-antagonist activity with varying degrees of intrinsic activity at the μ and κ receptors (11, 13, 18). Dose-response and time-course curves were generated for each agonist and the effective dose that produced 50% of the maximum response (ED_{50}) was calculated.

METHOD

Subjects

Adult male rats of Sprague-Dawley descent (SASCO/King, Omaha, NE) served as subjects. Their body weight ranged between 320–420 g at the time of testing. Subjects were group housed [3 per cage supplied with food (Purina 5001) and tap water] and maintained in a colony room (12:12 hr light:dark cycle with dark onset at 1800 hr) maintained at 20–24°C. Testing took place in a room separate from the colony and was conducted during the latter half of the light cycle, between 1300–1600 hr.

Surgery

Rats tested with DPDPE and nalbuphine were anesthetized using 100 mg/kg of ketamine hydrochloride. Under aseptic conditions, a stainless steel outer guide cannula (22 gauge, Plastic Products, Roanoke, VA) was stereotaxically implanted into the right lateral ventricle (coordinates: 0.5 mm posterior to bregma, 1.5 mm lateral to midline, and 3.2 mm ventral to the surface of the dura with the skull level between lambda and bregma). Cannula patency was verified behaviorally by measuring water intake after ICV injection of angiotensin II (10 ng/5 μl), a potent dipsogen (3). Subjects were tested with angiotensin II 5 days before the start of tail-flick testing and at the conclusion of our experiments. The testing of cannula patency with angiotensin II was separated by 5 days prior to analgesia testing to minimize any possible affect of angiotensin II on opioid analgesia. Data from animals failing to drink at least 4 ml of water in 10 min after angiotensin II injection were excluded from the analyses.

Procedure

Subjects were randomly assigned to one of two groups: 1) unrestrained and 2) restrained. Habituation to restraint consisted of 1 hr of immobility in Plexiglas cylinders (5 to 6 cm in diameter) on 5 consecutive days. All cylinders were sealed with a size 12 solid rubber stopper that had a slice removed such that the tail was freely mobile. During this time all NS-treated rats were handled.

Drug injection and testing of restraint stress subjects were performed with the subjects in the cylinders (rats receiving ICV injections were removed from the cylinders for only 2 min and returned). Drug injection and tail-flick testing was conducted twice weekly until every dose of a selected drug had been administered. Testing was then initiated with another compound. No single group of subjects received more than 3 different drugs. The subjects receiving U50488H, DPDPE, butorphanol, EKC and pentazocine were drug naive. All drug doses were randomly administered. This procedure conformed to a factorial design with 4 or 5 levels of dose and 2 treatment levels (restrained and unrestrained) with testing trials (6 samples) as a repeated measure.

Drugs and Injection

The drugs tested were buprenorphine hydrochloride (HCl) (National Institute on Drug Abuse, Rockville, MD), pentazocine and EKC, each as the free base (Sterling-Winthrop Research Institute, Rensselaer, NY), profadol HCl (Parke-Davis, Division of Warner-Lambert, Morris Plains, NJ), butorphanol tartrate (Bristol Laboratories, Evansville, IN), nalbuphine HCl (Du Pont

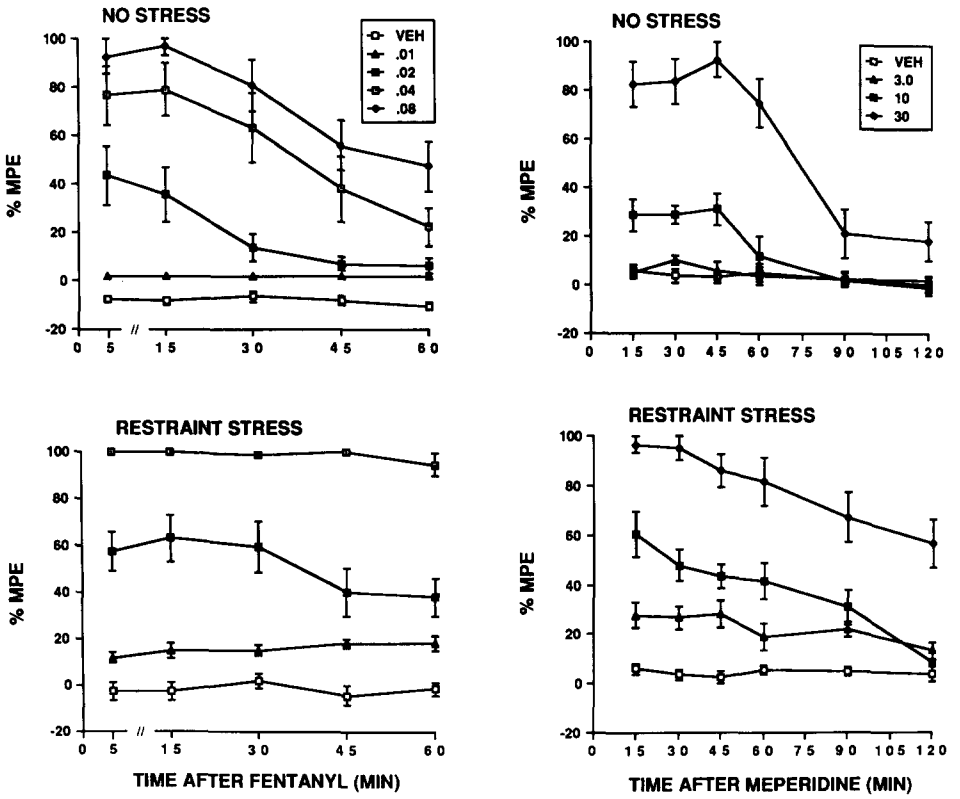

FIG. 1. Time-course illustrating restraint stress-induced potentiation of the analgesic effect of the mu opioid agonists, fentanyl and meperidine. Drug vehicle or one of the indicated drug doses (mg/kg) was injected subcutaneously at time 0; tail-flick latency was measured at the indicated times after injection. Each point is the mean % MPE (n = 6–12 per point). Vertical lines indicate ± 1 SEM, and are absent if the SEM is less than the radius of the point.

Pharmaceuticals, Wilmington, DE), fentanyl citrate (McNeil Pharmaceuticals, Springhouse, PA), meperidine HCl (Penick Corp., Lindhurst, NJ), DPDPE (Bachem Inc., Torrance, CA) and trans-3,4-dichloro-N-methyl-N-12 [2(1-pyrrolidinyl) cyclohexyl] benzeneacetamide, methanesulfonate, hydrate (U50488H, Upjohn Pharmaceuticals, Kalamazoo, MI), as summarized in Table 1.

All doses are expressed in terms of the free base. Drugs given subcutaneously (SC) were administered in a volume of 1.0 ml/kg. EKC, pentazocine, and buprenorphine were dissolved in 8.5% lactic acid and the pH was raised to between 4–5 with the addition of 1 N sodium hydroxide. DPDPE was dissolved in sterile distilled water and brought into solution with a few drops of acetic acid; the pH was raised to 6.5 with the addition of NaOH. All other drugs were dissolved in 0.9% saline. In every case the appropriate vehicle (VEH) was employed as the control injection.

ICV injections were performed by backloading an internal cannula (28 gauge) up a length of PE 20 tubing which was connected to a 25 μl gas-tight Hamilton microsyringe. ICV injections were given in a volume of 10 μl at a rate of 2 μl/5 sec. The tip of the internal cannula was cut to extend 0.5 mm beyond the guide cannula. The inner cannula was left in place for at least 15 sec after the drug injection in order to allow for pressure equilibrium. The injection system was checked for possible occlusion after each injection to assure positive drug delivery throughout the injection procedures.

Analgesic Testing

Analgesia testing was conducted as described by D'Amour and Smith (10), with modifications (14). Each rat was either hand held in a towel or remained in the restraint tube while its tail was placed in a "v"-shaped copper covered groove housing a photocell. A beam of radiant heat (from a 100-watt, 20-V, high amperage bulb), situated 18 cm above the tail, was aimed at a fixed point on the tail (about 5.0 cm from the tip) using an ellipsoidal reflector. An automated device recorded the interval (in 10ths of a sec) that lapsed between the onset of the beam and the movement of the tail, which terminated the beam. A cut-off time of 6.0 sec was set to minimize tissue damage.

Subjects were wrapped in a towel and underwent 3 predrug trials conducted about 5 min apart. The last two trials were averaged and served as the baseline measure for each subject. Within 5 min after baseline testing, subjects received either SC or ICV injections of drug; time-course testing began 15 min thereafter. Tail-flick latencies were recorded 15, 30, 45, 60, 90 and 120 min after drug injection except for fentanyl which, because of its short time-course of effects, was tested at 5, 15, 30, 45 and 60 min. Analgesia data are expressed as the mean percent maximum possible effect (% MPE) according to the following formula:

$$\%\ \text{MPE} = \frac{\text{Drug Latency} - \text{Predrug Latency}}{\text{6-Sec (cut-off time)} - \text{Predrug Latency}} \times 100.$$

Data Analysis

Dose-effect curves were derived by computing the area under the corresponding % MPE-15–120 min time-course curve for each treatment using the trapezoidal rule (19). The ED_{50} was calculated

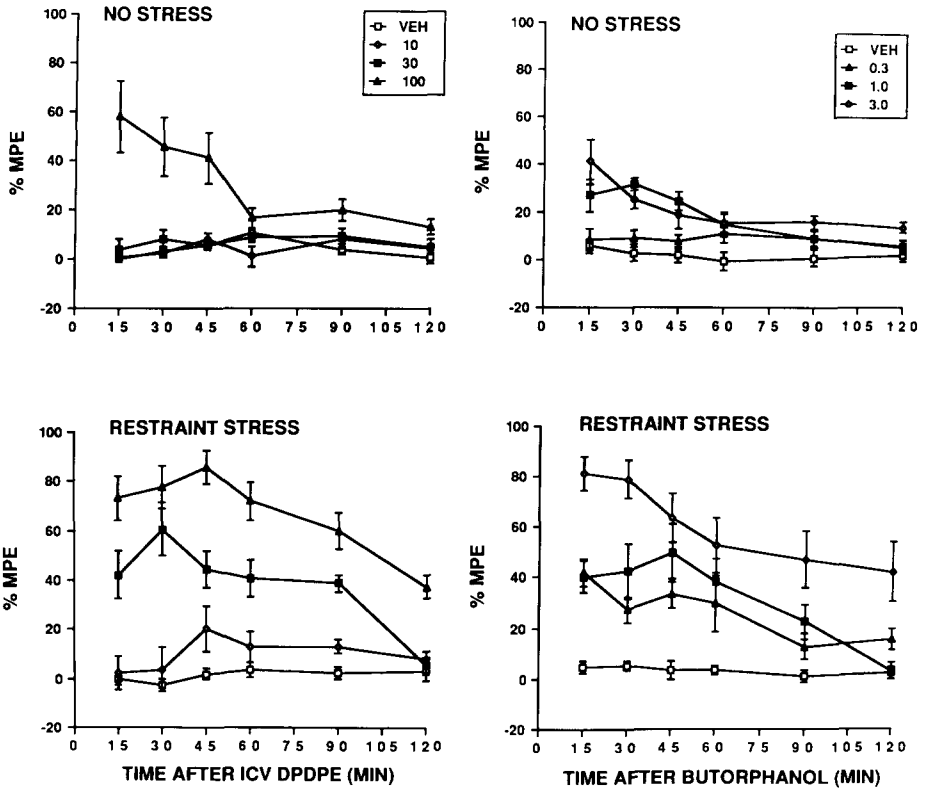

FIG. 2. Time-course illustrating restraint stress-induced potentiation of the analgesic effect of the delta opioid agonist, DPDPE, and the mixed agonist/antagonist, butorphanol. Drug vehicle or one of the indicated doses of butorphanol (mg/kg) was injected subcutaneously at time 0. DPDPE was administered intracerebroventricularly and the doses are shown as μg/rat.

by simple linear regression of the area of analgesia values per each subject and averaged. The mean (95% confidence limits) ED_{50}, route of administration and doses of each agonist tested are shown on Table 1. Statistical analysis for differences were performed on the dose-effect curves by two-way analysis of variance (ANOVA). In one experiment (ICV administered nalbuphine) unrestrained and restrained groups were tested for differences using a two-tailed, unpaired *t*-test. The level of probability for statistical significance was set at $p<0.05$.

RESULTS

Restraint stress treatment increased the duration of action of all agonists tested except nalbuphine. For example, the % MPE of fentanyl (0.04 mg/kg) at 60 min was about 30% in unrestrained rats whereas in restrained rats, the % MPE at this dose remained above 90%. Likewise, the % MPE for unrestrained rats given butorphanol (3.0 mg/kg) was about 20% at 90 min, whereas restrained rats at 90 min remained at about 40%. Figures 1 and 2 illustrate 4 representative series of time-course curves of tail-flick latencies [expressed as mean % MPE (±SEM)] for unrestrained (top) and restrained (bottom) rats. Subjects received four or five doses of fentanyl, meperidine, butorphanol or DPDPE (n = 6–12 per point). These figures illustrate that the magnitude of effect was increased by restraint. For example, the analgesic effect of 0.4 mg/kg of fentanyl and 3.0 mg/kg of butorphanol in unrestrained rats were increased 2–3-fold in restrained rats at the 60-min point.

These time-course figures also illustrate the observation that some of the opioids we tested did not return to baseline values within the time of the last test. The area under the curve for these opioids is actually greater than reported. Thus, there was a tendency to underestimate the actions of opioids with longer duration of action. We did not extend our time course measurements as there is a limit to the number of times a subject can be tested repeatedly using the radiant heat tail-flick assay in order to minimize tissue damage.

Figures 3–5 show the dose-effect curves (expressed as the mean area of analgesia) for unrestrained and restrained rats as derived from the % MPE-time-course curves for all 10 opioid agonists. ANOVAs of predrug baseline scores did not reliably differ for any treatment groups, F's<3.7, *p*'s>0.1. ANOVAs were conducted using area data from restrained and unrestrained treatment groups. ANOVA revealed significant differences for treatment with every drug tested, F's>5.7, *p*'s<0.04. ANOVA also revealed significant differences for dose, F's>5.9, $p<0.005$. These results show restrained subjects displayed dose-related potentiation of antinociception and duration of effect compared to unrestrained subjects.

Nalbuphine was the only peripherally administered drug that failed to produce significant dose-related increases in tail-flick latency in restrained subjects, $F(2,22)=0.1$, $p=0.8$. Whereas peripheral administration of 3.0–30 mg/kg of nalbuphine failed to produce analgesia in either treatment group, ICV administration of a single 100 μg dose of nalbuphine significantly increased tail-flick latency in restrained rats (mean area = 4494, SEM = 675) to 8 times that of unrestrained rats (mean area = 516, SEM = 213), unpaired two-tailed *t*-test yielded, $t(18)=-5.1$, $p<0.001$ (data not shown).

Buprenorphine was unique in that it displayed an inverted

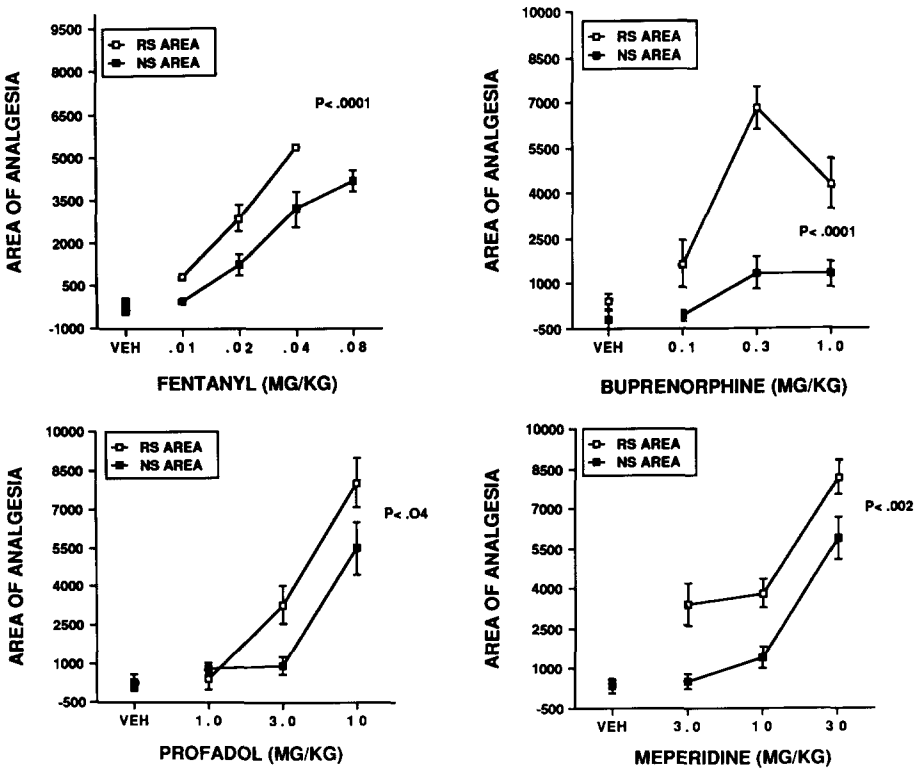

FIG. 3. Displays the dose-effect curves [expressed as the area of analgesia (time − % MPE)] for restraint stress- (RS area) and no stress- (NS area) treated rats injected with one of four mu opioid agonists, fentanyl, profadol, buprenorphine and meperidine. Areas were derived from time-course curve data. Each point represents the mean (± SEM) of 6–12 subjects. "VEH" signifies the vehicle control injection.

U-shaped dose-response curve in restrained subjects. Peak effect was found using the middle dose; the highest dose tested produced a lower % MPE, perhaps indicative of its agonist-antagonist activity. A similar biphasic dose-response curve for buprenorphine was generated in a tail-withdrawal test in rats (8). All other curves were linear in shape indicating that restraint resulted in a shift of the dose-effect curves to the left and, in some cases, upward.

DISCUSSION

The magnitude and duration of analgesia produced by all opioid agonists tested were found to be dose-dependently increased in restrained rats compared to unrestrained rats except for peripherally administered nalbuphine. Two hypotheses may account for the lack of effect with nalbuphine. First, perhaps our selections of peripheral doses were too low and that testing with a higher peripheral dose of nalbuphine may have resulted in observable analgesia. Second, in order for opioids to produce their effects, it is necessary for a ligand to induce a conformational change in the receptor following binding (13). Whereas full agonists like fentanyl have high intrinsic activity, agonist-antagonists, such as buprenorphine and pentazocine, induce moderate to low conformational change. Nalbuphine is known to have the lowest intrinsic activity and highest antagonist activity of all compounds we tested (13). Therefore, it is possible that the low intrinsic activity/high antagonist activity of nalbuphine at the μ receptor can account for its lack of effect. However, when administered directly into the brain, it is clear that nalbuphine (100 μg/rat, ICV) can produce analgesia in restrained subjects (this dose yielded an area of analgesia at 4500). Comparing all of the drugs tested in this and previous studies, potentiation seems to be greater with ICV administration (4–8-fold) than with SC administration (1.5–3-fold). The significance of this observation is unclear.

The other relatively μ receptor preferring ligands, fentanyl, meperidine, buprenorphine and profadol, showed the highest degree of potentiation (1.5–3-fold) in restrained rats as well as a moderate degree of analgesia in unrestrained rats regardless of intrinsic activity (e.g., fentanyl versus profadol). Restrained rats receiving EKC showed significant potentiation whereas the analgesia in unrestrained rats failed to reach 50% of maximum effect. These results could indicate that κ receptor-mediated analgesia is capable of being potentiated by restraint stress. However, because the more κ selective agonist U50488H failed to display a similar degree of potentiation, it seems more likely that the potentiation of EKC was due to its effects at μ receptors because EKC binds equally well to μ receptors and this μ component may have contributed to potentiated analgesia (15). Like U50488H, pentazocine did produce analgesia in restrained rats that was significantly higher than unrestrained rats, however, the degree of analgesia was rather minimal and did not produce 50% of the effect given either treatment.

Our results demonstrate that activation of δ receptors by a moderate dose of DPDPE (30 μg) failed to produce significant analgesia in unrestrained rats. These findings are not unexpected as prior research has established that ICV administration of DPDPE (10–100 μg) failed to produce analgesia in rats using the hot-water (55°C) tail-withdrawal assay (16). The stimulus properties of radiant heat as opposed to hot water do not seem to differ significantly.

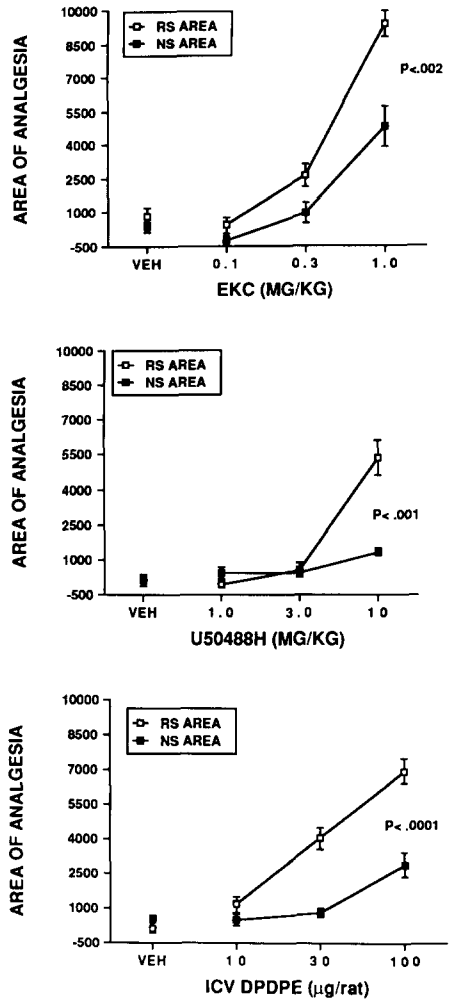

FIG. 4. Displays the dose-effect curves [expressed as the area of analgesia (time − % MPE)] for restraint stress- (RS area) and no stress- (NS area) treated rats injected subcutaneously with kappa opioid agonists, ethylketocyclazocine (EKC) and U50488H, or injected intracerebroventricularly with the delta receptor selective agonist DPDPE. Areas were derived from time-course data.

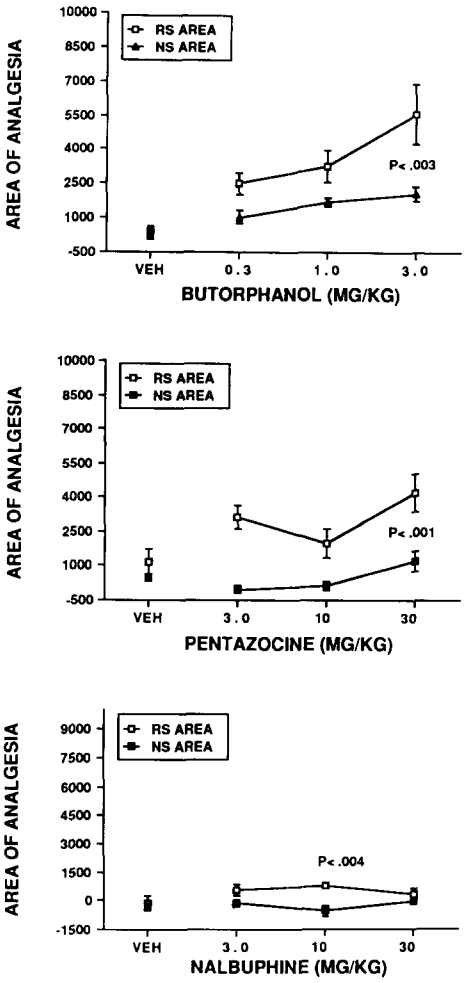

FIG. 5. Displays the dose-effect curves (expressed as the area of analgesia) for rats injected with one of three mixed agonist/antagonists, butorphanol, pentazocine and nalbuphine. Areas were derived from time-course data.

Most importantly, the ICV administration of DPDPE (30 μg) into restrained rats dose-dependently produced significant potentiation of the magnitude and duration of antinociception. This is perhaps the first demonstration that ICV administered DPDPE increased tail-flick latency in rats. The involvement of δ receptors in restraint stress alteration of DPDPE-induced antinociception is not entirely unexpected because restraint exposure has already been reported to alter δ receptor-mediated analgesia (5) and to increase the number of δ receptors in the striatum as well (21). The highest dose of DPDPE (100 μg) tested resulted in analgesia regardless of treatment. Given this high dose of DPDPE, it is likely that opioid receptors other than δ were activated and contributed to the increased tail-flick latency.

One parsimonious explanation of our results is that the μ receptor is of primary importance in restraint stress potentiation of analgesia. First, of all the agonists and agonist-antagonists tested, ligands known to exert a high degree of intrinsic activity at μ receptors displayed the most consistent potentiation of the magnitude and duration of analgesia. Second, drugs with low affinity and/or low intrinsic activity at the μ receptor (pentazocine, butorphanol and nalbuphine) showed little activity.

Last, although DPDPE has been considered a highly selective agonist for receptors, this conclusion has been based primarily on the strength of binding data. Cowan and Murray have offered evidence that the in vivo selectivity of DPDPE remains questionable (9). Therefore, we cannot exclude the possibility that activity seen with DPDPE can be attributed to activation of the μ receptor. Given that DPDPE shows a 300-fold selectivity for δ versus μ receptors, 100 μg is equivalent to a 0.3 μg dose at μ receptors; a 0.3 μg dose of DAGO is sufficient to produce an analgesic effect of 8000 % MPE-min in restrained rats (4). Potentiated analgesia was observed with U50488H-treated subjects only at a very high dose and may also be attributed to a μ receptor component. We conclude that μ receptors play a primary role in restraint stress potentiation of full agonist and agonist-antagonist-induced analgesia; however, our results do not rule out the possibility that δ and κ receptor types are also involved in this phenomenon.

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