Pharmacology Biochemistry & Behavior, Vol. 34, pp. 847–854. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Comparative Pharmacological and Biochemical Studies Between Butorphanol and Morphine

P. J. HORAN AND I. K. HO[1]

Department of Pharmacology and Toxicology, University of Mississippi Medical Center
2500 North State Street, Jackson, MS 39216

Received 17 January 1989

HORAN, P. J. AND I. K. HO. *Comparative pharmacological and biochemical studies between butorphanol and morphine.* PHARMACOL BIOCHEM BEHAV **34**(4) 847–854, 1989.—A number of in vivo and in vitro studies were undertaken to compare the pharmacological and biochemical effects of the partial agonist, butorphanol, with that of morphine. Both compounds were equipotent antinociceptive agents in the rat tail withdrawal test. In the acetic acid writhing test butorphanol had approximately 3.5 times the antiwrithing activity on a molar basis than morphine. In a study of the effects of these compounds on body temperature, butorphanol as well as morphine produced hyperthermia after acute dosing. Additionally, butorphanol produced a profound diuresis and decrease in urine osmolality after acute administration. In contrast, morphine produced an antidiuresis throughout most of the study period with no significant changes in urine osmolality from control. Butorphanol administration had no effect on respiratory rate, while morphine markedly decreased respiratory rate. In in vitro radioligand displacement studies, butorphanol was a potent competitor against ^{3}H-DAGO, ^{3}H-DPDPE, and ^{3}H(−)-EKC binding, exhibiting 3, 10, and 30 times more activity, respectively, than morphine. Both compounds were weak inhibitors of ^{3}H-(+)-SKF 10047 binding, yielding IC_{50} values of in excess of 1 μM. The results indicate that butorphanol has multiple actions on the opioid receptor system, and shares similarities as well as differences in its mechanism(s) of actions with morphine.

Butorphanol Morphine Pharmacological responses Opioid receptors

BUTORPHANOL (17-cyclobutylmethyl-3,14-dihydroxymorphinan) has been reported to be a mixed "agonist/antagonist" analgesic (14,32) belonging to a group of morphine surrogates known as morphinans. Butorphanol is a completely synthetic compound, as opium alkaloids are not required as precursors for its synthesis (27).

In comparison to the prototypical narcotic analgesic, morphine, butorphanol exhibits some pharmacological similarities as well as differences. Both compounds are antinociceptive. As with other mixed "agonist/antagonist" analgesics, butorphanol was reported to be more potent than morphine in the mouse phenylquinone-induced writhing test, while exhibiting weak activity in the rat tail flick test (32).

Butorphanol shares the miotogenic and respiratory depressant actions of morphine (18, 29, 32). In addition, both compounds decrease gastrointestinal transit (35). Butorphanol's dose-effect relationship on these latter 3 parameters is lost upon administration of larger dosages, a phenomenon common to other morphine surrogates exhibiting mixed "agonist/antagonist" activity (26).

Another characteristic butorphanol shares with other compounds that are mixed " agonist/antagonists" is the production of diuresis following its administration (22). In contrast, morphine administration typically produces an antidiuresis (13).

The antagonistic component of butorphanol has been reported in mice made dependent on morphine (32). This effect has also been explored in man (18,34). However, this aspect of butorphanol's pharmacology (i.e., butorphanol-precipitated withdrawal) may also have a limited action or "ceiling" effect in comparison to the standard opiate antagonist, naloxone (32).

Radioligand displacement studies suggest that butorphanol may have actions at the mu, delta, and kappa opioid receptor types (4,21). In addition, behavioral studies and clinical reports suggest that butorphanol may also bind to the opioid sigma receptor (18,36).

Due to a lack of comprehensive studies documenting the pharmacology and molecular actions of butorphanol tartrate, the following experiments were conducted. These studies have employed a correlative approach to an understanding of this compound's actions of the opioid receptor system, and for comparative purposes include morphine as a positive control or "reference agent." In these studies, a variety of pharmacological endpoints known to be associated with the opioid receptor system have been combined with in vitro radioligand displacement studies in order to more thoroughly assess the mechanism(s) of action of butorphanol.

[1]Requests for reprints should be addressed to Dr. I. K. Ho.

METHOD

Animals and Chemicals

Male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 226–250 g were used in in vivo experiments, and animals weighing 176–200 g were used in radioligand displacement assays. Animals were kept on a 12/12-hour light-dark cycle and had free access to food and water. Naloxone HCl, pentazocine HCl, DAGO (Tyr-D-Ala-Gly-MePhe-Gly-ol) and DPDPE ([D-penicillamine^2,D-penicillamine^5]enkephalin) were obtained through Sigma Chemical (St. Louis, MO). ^{3}H-DAGO (33.8 Ci/mmol), ^{3}H-DPDPE (43 Ci/mmol), ^{3}H-(−)-EKC (ethylketocyclazocine) (27 Ci/mmol), and ^{3}H-(+)-SKF 10047 (N-allyl normetazocine) (25.6 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Butorphanol tartrate was a generous gift of the Bristol-Myers Corp. (Syracuse, NY). Morphine sulfate was obtained from Mallincrodkt Chemical Works (St. Louis, MO).

Antinociceptive Assays

Rat tail withdrawal test. Following dosing with morphine (2.6, 5.7, 7.9, or 10.5 μmols/kg, SC) or butorphanol (3.1, 5.2, 10, 12.6 and 21 μmols/kg, SC), animals were tested according to the method of Janssen *et al.* (17). Prior to dosing, the rats were placed into Plexiglas restrainers, the distal 4 cm of the tail immersed into a hot water bath maintained at 55°C, and their latency to tail withdrawal evaluated. Any animal having a latency to tail withdrawal of greater than 6 seconds was excluded from the study in order to eliminate any false positive antinociceptive scores. The animals were then reintroduced into their restrainers, and any animal whose tail withdrawal reaction time was greater than 6 seconds was considered antinociceptive (or a responder). At each dose, the percent response was evaluated (i.e., number of responders/number of animals tested at each dosage level) at 15, 30, 45, 60, 90 and 120 minutes after morphine or butorphanol challenge. The ED_{50}'s and 95% confidence intervals were determined via the method of Litchfield and Wilcoxon (23). Mean baseline latencies and standard errors were 1.80 ± 0.59 and 1.83 ± 0.60 for morphine- and butorphanol-treated animals, respectively. Each animal was dosed only once with either butorphanol or morphine, and 6 animals were used per dosage level.

Acetic acid writhing test. In this test, the method of Hayashi and Takemori (12) was used (with minor modification). Rats were dosed with morphine, 0.13, 0.26, 0.66, 0.86, 1.1, 1.3 and 1.6 μmols/kg, SC or butorphanol 0.05, 0.1, 0.16, 0.17, 0.21, 0.25 and 0.33 μmols/kg, SC.

Fifteen minutes later the animals were given 0.6% acetic acid, 10 ml/kg, IP. Ten minutes following the administration of acetic acid, the animals were individually sequestered and observed for the presence of a writhe (defined as a characteristic stretching of the hind limbs and/or constriction of the abdominal musculature) 25–35 minutes after dosing with morphine or butorphanol. Animals that did not writhe were considered antinociceptive, or responders. The percent response (number of responders/number in dosing group) was evaluated at each dose, and the ED_{50}'s and 95% confidence intervals determined by the method of Litchfield and Wilcoxon (23). Each animal was dosed only once with either butorphanol or morphine, and from 5 to 7 animals were used per dosage level.

Urine Ouput and Water Consumption

For 3 days prior to dosing with morphine or butorphanol, animals were individually housed in stainless steel metabolism cages (Hazelton Systems, Inc., Aberdeen, MD) in order to acclimate the rats to their testing environment. Following this predosing acclimation procedure, animals were then administered saline (1 ml/kg, SC), morphine (48 μmols/kg, SC) or butorphanol (12 or 48 μmols/kg, SC). The urine volume and water consumption of each animal were then measured at 1, 2, 4, 6 and 8 hours following dosing. Food was withheld from the animals on the night prior to dosing in order to reduce variability in both urine output and osmolality measurements. The median and semi-interquartile distances were determined for each group at each time point, and the data were expressed as either the cumulative amount of urine collected or water consumed over time.

Urine Osmolality

The urine osmolality of each animal was determined using a μ Osmette Osmometer (Precision Systems, Inc., Natick, MA) from the mean of 2 independent measurements of an aliquot obtained from the total volume of urine collected at 8 hours after dosing. In order to minimize evaporation of the samples throughout the 8-hour collection period, a small amount (1 ml) or paraffin oil (EM Science, Cherry Hill, NJ) was added to the collection tubes prior to the beginning of the experiment. The results were expressed as mosmols/kg of water, and the mean and standard error were determined for each dosage group.

Rectal Temperature

Rectal temperature was determined using a Thermocouple Digital Thermometer (Atkins Technical, Gainesville, FL) inserted to a depth of 4.5 centimeters. The rats were then given butorphanol (12 or 48 μmols/kg, SC), morphine (48 μmols/kg, SC), or saline (1 ml/kg, SC), lightly restrained by the tail in their home cage, and the rectal temperature determined at 0.5, 1, 1.5, 2, 4, 6 and 8 hours after dosing. Temperatures were determined from 1000 to 1800 hours at an ambient temperature of 22.2 ± 0.1°C. In order to establish baseline measurements, rectal temperature was evaluated at the above time points for 3 days preceding dosing.

Data were expressed as change from baseline (Δ T°C), which in order to provide a more stable value, was constructed from the average of the last 2 days of predosing temperature determinations. The mean baseline rectal temperatures (°C) with corresponding standard errors for each time point were 37.56 ± 0.05 (0.5 hr); 37.45 ± 0.05 (1 hr); 37.55 ± 0.05 (1.5 hr); 37.5 ± 0.05 (2 hr); 37.57 ± 0.07 (4 hr); 37.35 ± 0.04 (6 hr) and 37.28 ± 0.05 (8 hr).

Respiratory Rate

Respiratory rate was measured using an RM-80 respirometer (Columbus Instruments, Columbus, OH) set to a gain of 1.5. Each animal was acclimated to the device for 30 minutes to allow for a stable baseline reading, at which time respiratory rate was measured for two 5-minute intervals. The mean of these 2 predosing baseline measurements was defined as the basal respiratory rate. The animals were then removed from the device, and dosed with either saline (1 ml/kg, SC), morphine (48 μmols/kg, SC) or butorphanol (12 of 48 μmols/kg, SC).

The animals were then allowed to reacclimate to the respirometer for an additional 30 minutes, and two 5-minute measurements of respiratory rate were obtained. These values were averaged, and the results expressed as percent of basal respiratory rate (posttreatment rate/baseline rate). The experiments were conducted from 0800 to 2000 hours at an ambient temperature of 21.6 ± 0.4°C.

One treatment group was tested per day. Median baseline respiratory rates per minute with corresponding semi-interquartile distances for each respective treatment group prior to dosing were

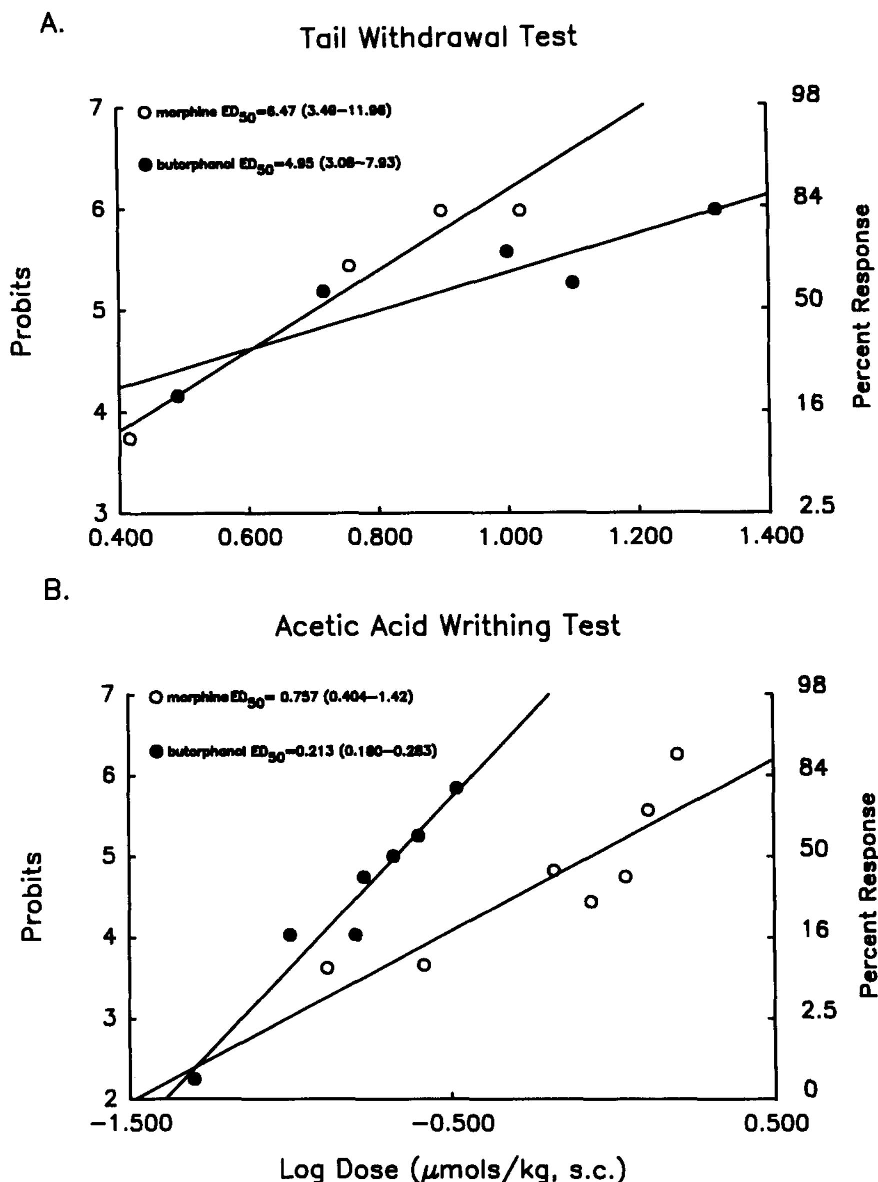

FIG. 1. (A) Antinociceptive effect of butorphanol and morphine in the warm water tail withdrawal test. (B) Antinociceptive effect of butorphanol and morphine in the acetic acid writhing test. Between 5 to 8 animals were used per dose of morphine or butorphanol. ED_{50} values with 95% confidence limits are presented in μmoles/kg of butorphanol or morphine.

169 ± 23.7 (saline); 166.7 ± 8.9 (morphine); 137.1 ± 19.8 (butorphanol 48 μmols/kg); and 146.3 ± 32.6 (butorphanol 12 μmols/kg).

Membrane Preparation

Following decapitation and removal of the brain from the skull, the cerebellum was dissected by the method of Glowinski and Iversen (10) and discarded (with the exception of brain tissue prepared for use in opioid sigma receptor assays, where cerebella were left intact). The brain tissue was then homogenized in 10 w/v of ice cold 0.32 M sucrose, and centrifuged at 1,000 × g for 10 minutes to remove cellular nuclei and debris.

The supernatant was then centrifuged at 20,000 × g for 20 minutes to obtain the crude P_2 fraction. The P_2 fractions were then incubated in 50 mM Tris HCl, pH 7.7, at 37°C for 20 minutes to facilitate the degradation of endogenous inhibitors (39), followed by extensive washing (5 times) in 10 w/v Tris buffer. After washing, the synaptic membranes were resuspended in 2 w/v 50 mM Tris HCl, and stored at −70°C until used for binding assays. Protein concentrations were determined by the method of Lowry *et al.* (24).

Radioligand Displacement Studies

Various concentrations of morphine (0.1 to 5000 nM) or butorphanol (0.1 to 100 nM) were incubated with ^{3}H-DAGO (1 nM), ^{3}H-DPDPE (5 nM), or ^{3}H-(−)-EKC (4 nM) for displace-

TABLE 1

EFFECT OF BUTORPHANOL OR MORPHINE ON RECTAL TEMPERATURE

Time (hr)	Treatment Group: Saline	Morphine: 48	Butorphanol: 12	Butorphanol: 48
0.5	−0.11 ± 0.19	0.38 ± 0.18†	0.61 ± 0.19*	0.74 ± 0.15*
1.0	0.00 ± 0.01	0.54 ± 0.12*	1.11 ± 0.19*	0.71 ± 0.11*§
1.5	−0.20 ± 0.07	0.82 ± 0.13*	1.10 ± 0.19*	0.86 ± 0.18*
2.0	−0.26 ± 0.10	0.42 ± 0.31	1.04 ± 0.15*§	1.18 ± 0.13*§
4.0	−0.21 ± 0.05	1.12 ± 0.30*	0.02 ± 0.10§†	0.43 ± 0.16‡
6.0	−0.28 ± 0.06	0.58 ± 0.16*	0.31 ± 0.90*	0.79 ± 0.14*
8.0	0.00 ± 0.09	0.50 ± 0.19	0.20 ± 0.06	0.38 ± 0.17

Mean rectal temperatures with corresponding standard errors obtained from saline-treated, 1 ml/kg (n = 7); morphine-treated, 48 μmols/kg (n = 8); or butorphanol-treated, 12 or 48 μmols/kg (n = 8) at 0.5, 1.0, 1.5, 2.0, 6.0 or 8 hours postdosing.

* $p<0.01$ vs. saline; † $p<0.05$ vs. saline; ‡ $p<0.01$ vs. morphine; § $p<0.05$ vs. morphine.

ment studies at the mu, delta, and kappa opioid receptor types, respectively. In these experiments, synaptic membranes from whole rat brain minus cerebellum were used. Protein concentrations were 0.4, 0.8, and 1.0 mg/ml for assays employing ^{3}H-DAGO, ^{3}H-DPDPE, and ^{3}H-(−)-EKC, respectively. Incubation times were 60 minutes for assays utilizing ^{3}H-DAGO and ^{3}H-(−)-EKC, and 120 minutes for those involving ^{3}H-DPDPE. Nonspecific binding was determined by that which was displaceable with 1 μM naloxone for ^{3}H-DAGO binding, and 10 μM naloxone for ^{3}H-DPDPE and ^{3}H-(−)-EKC binding. Unlabeled DAGO (100 nM) and DPDPE (100 nM) were included in incubations containing ^{3}H-(−)-EKC to block binding of this radioligand to mu and delta opioid receptors, respectively.

In investigations of potency at the opioid sigma receptor, morphine or butorphanol was incubated in 5 mM Tris HCl, pH 8.0 at 25°C for 45 minutes with 2 nM ^{3}H-(+)-SKF 10047. Incubation volumes were 1 ml. Membranes, at a final concentration of 1 mg/ml from whole rat brain (including cerebellum), were used in these assays. Nonspecific binding was defined as that displaceable by 10 μM pentazocine.

Following incubation, membranes were rapidly filtered over Whatman GF/B filters using a cell harvester (model M-24R, Brandell Corp., Gaithersburg, MD), and washed twice with 5 ml of ice-cold 50 mM Tris buffer.

To attenuate binding of the radioligands to the filter paper, the filters were presoaked in 0.1% polyethyleneamine (Sigma Chemical, St. Louis, MO). The filters were then transferred into polyethylene scintillation vials, and the radioactivity allowed to extract overnight in 10 ml of Safety-Solve (Research Products International Corp., Mount Prospect, IL). Radioactivity was determined via liquid scintillation spectrometry (Beckman LS 1800) at an efficiency of 60%. The IC_{50} values of morphine or butorphanol in these experiments were obtained by using the iterative curve fitting computer program EDBA (26). At least 9 concentrations of morphine or butorphanol were used per displacement curve, and the mean IC_{50} value and standard error were determined from the average of at least 3 experiments.

Statistics

To satisfy homogenity of variance assumptions, urine osmolality and rectal temperature data were subjected to square root transformation and analyzed via one-way ANOVA. Where overall group effects were observed, intergroup comparisons were performed using the Newman-Keuls test (41). Cumulative urine output, water consumption and respiratory data were compared via the Kruskal-Wallis test using the computer program Systat (Evanston, IL).

When significance was indicated, intergroup comparisons were made using a distribution free multiple comparison test (7). IC_{50} values were compared using a Student's t-test for grouped data. The dose-response curves for the tail flick test and acetic acid writhing assay were tested for parallelism by comparing the slopes of the regression lines of these curves using a Student's t-test (41). Baseline tail flick latencies were compared using a Student's t-test (41).

RESULTS

Antinociceptive Actions of Butorphanol and Morphine

In the rat tail withdrawal test, butorphanol was equipotent with morphine, yielding ED_{50} values of 6.47 (3.49–11.96) and 4.95 (3.08–7.93) μmols/kg, SC, respectively. Slopes of the regression lines and correlation coefficients for this test were 3.90 and .944 for the morphine dose-response curve, and 1.87 and .826 for the butorphanol dose-response curve. The slopes of these curves did not significantly differ, $t(5) = 2.398$. In contrast, the antiwrithing activity of butorphanol was approximately 3.5 times that of morphine. In this assay, the ED_{50} of butorphanol was 0.21 (0.16–0.28) μmols/kg, SC, while that of morphine was 0.75 (0.40–1.41) μmols/kg, SC (Fig. 1). The slopes and correlation coefficients of these dose-response curves were 2.11 and .771 (morphine-treated) and 4.16 and .951 (butorphanol-treated). The slopes of these lines differed significantly, $t(10) = 2.775$, $p<0.05$.

Effect of Butorphanol and Morphine on Rectal Temperature

Morphine (48 μmols/kg, SC) and butorphanol (12 and 48 μmols/kg, SC) markedly elevated rectal temperature with a time to peak effect of approximately 0.5 hours postdosing for animals receiving morphine and the higher dose of butorphanol (48 μmols/kg), and 1 hour for those receiving the lower dose of butorphanol (12 μmols/kg). Rectal temperature in all drug-treated groups was elevated essentially throughout the entire time course of the study up to 6 hours postdosing. Apart from the time to onset of peak effect, there were no consistent differences in rectal temperature found between animals dosed with either dose of butorphanol or morphine (Table 1).

The Influence of Butorphanol and Morphine on Urine Output and Water Consumption

At 2 and 4 hours following butorphanol effect administration

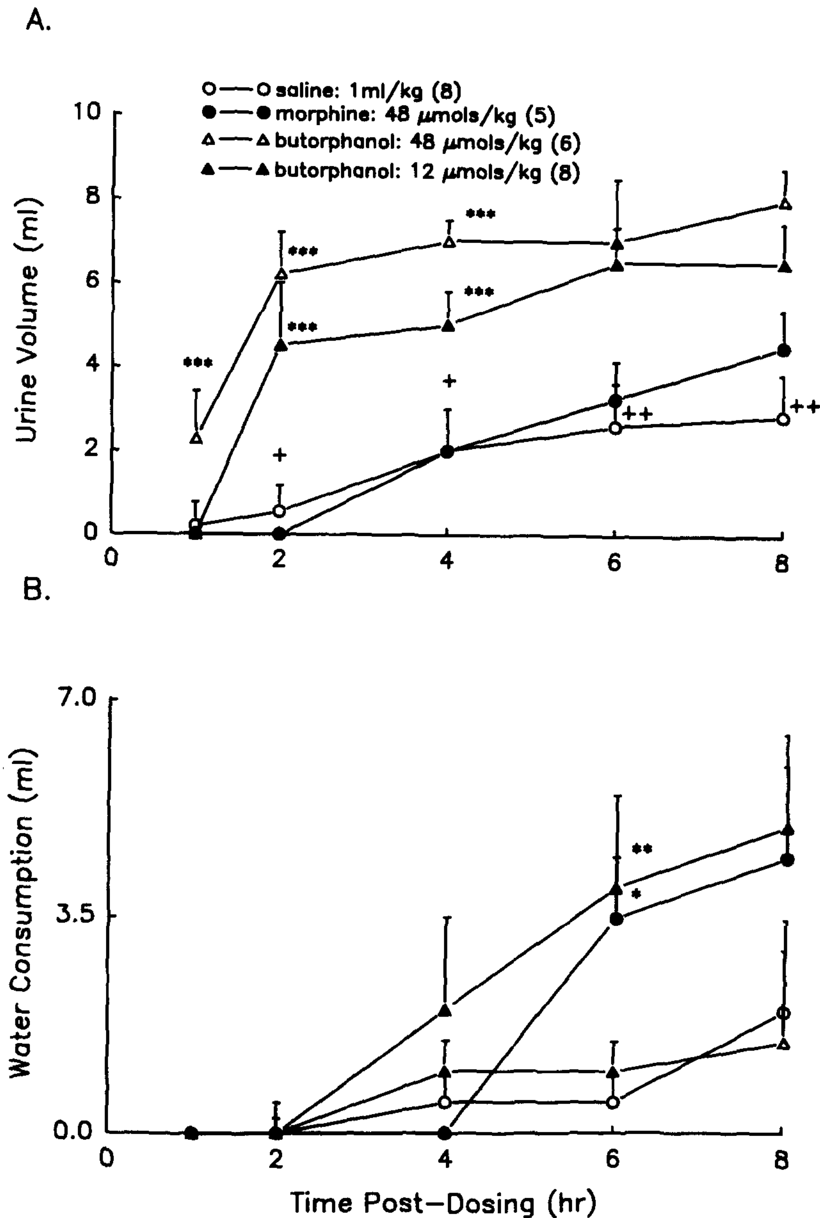

FIG. 2. (A) The effect of butorphanol or morphine on urine output. (B) The effect of butorphanol or morphine on water consumption. The data represent cumulative urine output or water consumption determined throughout the 8-hour course of the study, and represent the median and semi-interquartile distances for each group. $***p<0.01$ vs. morphine; $**p<0.01$ vs. saline; $*p<0.025$ vs. saline; $^{++}p<0.025$ vs. butorphanol 12 and 48 μmols/kg; $^{+}p<0.05$ vs. butorphanol 48 μmols/kg.

(12 or 48 μmols/kg, SC), the volume of urine collected was markedly increased compared to animals receiving morphine (48 μmols/kg, SC). Rats receiving the higher dose of butorphanol (48 μmols/kg) excreted a significantly higher amount of urine than those dosed with saline from 2 hours after dosing throughout the remainder of the study. This was not observed in rats receiving the lower dose of butorphanol (12 μmols/kg) until 6 and 8 hours after dosing. In rats dosed with morphine, urine excretion was virtually eliminated for 2 hours postdosing, however, a "rebound" phenomenon was observed at 4 hours after dosing in this group. After this time, cumulative urine output surpassed that of the saline-treated animals, but was not statistically significant (Fig. 2).

Throughout the course of this study, no statistical difference in water consumption could be seen in animals dosed with the higher dose of butorphanol (48 μmols/kg) from those given morphine or the lower dose of butorphanol. However, water consumption increased markedly at 6 hours following dosing in animals receiving the lower dose of butorphanol (12 μmols/kg), and was significantly different from that of saline-treated animals at 6 hours postdosing. At this time water consumption also increased in animals dosed with morphine, corresponding to the increase in urine output seen at this time, and was significantly different from

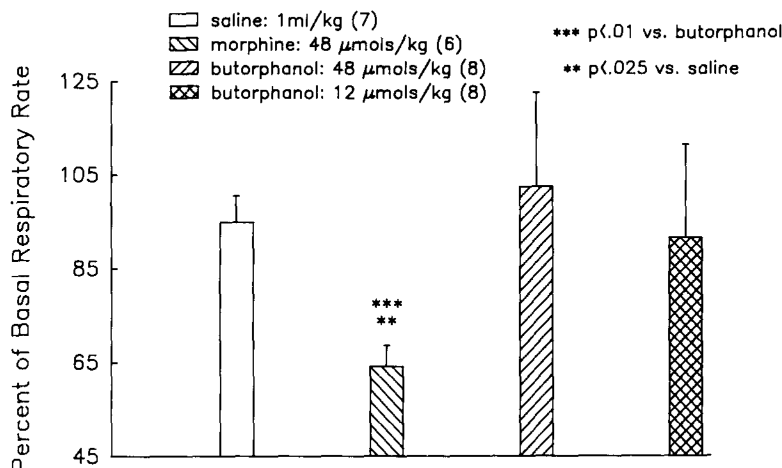

FIG. 3. The effects of butorphanol or morphine on respiratory rate. Number in parentheses indicate the number of animals per treatment group. Respiratory rate was determined from the average of 2 five-minute readings 35 to 40 minutes after dosing with saline, morphine or butorphanol. ***$p<0.01$ vs. butorphanol 12 or 48 μmols/kg; **$p<0.025$ vs. saline.

the saline-treated group.

Effect of Butorphanol and Morphine on Urine Osmolality

Both doses of butorphanol (12 and 48 μmols/kg) caused a marked reduction in urine osmolality at 8 hours postdosing compared to those animals given saline or morphine ($p<0.01$). The decrease in urine osmolality seen after butorphanol administration was larger than that produced by morphine, which was also statistically significant from saline-treated animals ($p<0.05$) (Table 2).

Effect of Butorphanol and Morphine on Respiratory Rate

Morphine (48 μmols/kg, SC) produced a marked depression of respiratory rate which was approximately 60% of the respiratory rate of saline-treated animals. In contrast, both doses of butorphanol had no statistically significant effect on respiratory rate when compared to saline-treated animals, though was significantly different from morphine-treated animals ($p<0.01$) (Fig. 3).

Effect of Butorphanol and Morphine on Opioid Radioligand Displacement

In rat whole brain minus cerebellum, butorphanol was a potent ligand at mu, delta, and kappa opioid receptors, respectively. The IC_{50}'s of this compound in displacement assays utilizing ^{3}H-DAGO, ^{3}H-DPDPE, and ^{3}H-(−)-EKC were 0.95, 12.3, and 4.88 nM, respectively. In contrast, the IC_{50} values obtained against these ligands with morphine were 3.08, 118, and 127.1 nM, respectively. Both compounds exhibited poor affinity for the opioid sigma receptor, with IC_{50} values greater than 1 μM in homogenates of rat whole brain with cerebellum (Table 3).

TABLE 2

URINE OSMOLALITY IN RATS DOSED WITH BUTORPHANOL OR MORPHINE

Treatment	mosmols/kg Water
Saline: 1 ml/kg	887 ± 138 (7)
Morphine: 48 μmols/kg	645 ± 170†‡ (7)
Butorphanol: 12 μmols/kg	178 ± 55* (5)
Butorphanol: 48 μmols/kg	249 ± 33* (6)

*$p<0.01$ vs. saline; †$p<0.05$ vs. saline; ‡$p<0.01$ vs. butorphanol 12 or 48 μmols/kg.

Number in parentheses represents the number of animals per dosing group.

Median values with semi-interquartile distances obtained from the cumulative amount of urine collected over the 8-hour study period.

DISCUSSION

These results demonstrate that butorphanol exhibits some pharmacological similarities as well as differences in comparison to the standard narcotic analgesic, morphine. Furthermore, these differences and similarities further clarify butorphanol's actions in the opioid receptor system. In the rat tail withdrawal test used in this study, morphine and butorphanol were equipotent antinociceptive agents. In contrast, butorphanol had approximately 3.5 times the antiwrithing activity of morphine in the acetic acid writhing test. An antinociceptive profile such as that seen in our studies with butorphanol is exemplary of opiates that exhibit "mixed" agonist-antagonist activity (42,45). From these antinociceptive data, it would appear that butorphanol possesses activity at all 3 opioid receptor types. The mu and delta opioid receptors are implicated in antinociceptive responsed to thermal stimuli (15,44). In the writhing test, activation of mu, delta and kappa opioid

TABLE 3

IC_{50} VALUES (nM) OF MORPHINE AND BUTORPHANOL AGAINST ^{3}H-DAGO (1 nM), ^{3}H-DPDPE (5 nM), ^{3}H-(−)-EKC (4 nM) AND ^{3}H-(+)-SKF 10047 (2 nM)

	Morphine	Butorphanol
^{3}H-DAGO	3.08 ± 0.46	0.95 ± 0.03*
^{3}H-DPDPE	118 ± 11.1	12.3 ± 1.23*
^{3}H-(−)-EKC	127.1 ± 10.92	4.88 ± 0.486*
^{3}H-(+)-SKF 10047	>1 μM	>1 μM

*Significantly different from morphine ($p<0.01$).

IC_{50} values represent the mean and standard error of at least 3 separate determinations.

receptors appear to elicit antinociception (33,40). In these experiments, morphine and butorphanol were equipotent in the rat tail withdrawal test. Additionally, the regression lines of the dose-response curves were parallel, suggesting that butorphanol's actions at the mu opioid receptor are responsible for its analgesic actions in this test.

Conversely, butorphanol had 3.5 times the antiwrithing activity of morphine, and the regression lines of these dose-response curves were not parallel implying a different interaction of butorphanol with the opioid receptor system, which is probably with the delta or kappa receptor.

Butorphanol administration resulted in hyperthermia, as did morphine. Moreover, there was no statistical difference in the magnitude of this hyperthermic response between either dose of butorphanol used in this study or morphine. In addition, these thermic responses to morphine and butorphanol were monophasic throughout the 8-hour course of this study. These results infer that the site(s) of action of butorphanol in the production of hyperthermia may be similar to morphine. Recent studies (8,9) have attempted to clarify the thermic responses to opioid receptor activation. Following the administration of compounds specific for various opioid receptors, it appears that the mu and kappa opioid receptors (at least in the rat) are involved in the hyper- and hypothermic responses, respectively, to opioid and opiate administration. More recently, the delta opioid receptor appears may not be involved in these responses (1) as evaluated via intracerebroventricular administration of DPDPE, a highly specific delta agonist (28). Therefore, it would appear that butorphanol's actions at the mu opioid receptor are responsible for its hyperthermic actions.

Butorphanol administration also resulted in a profound diuresis, with an accompanying decrease in urine osmolality. In contrast, morphine administration virtually eliminated urine excretion for 4 hours postdosing, with an apparent, although statistically insignificant, rebound in urine excretion at 6 and 8 hours after dosing.

The antidiuretic effect of morphine may in part be mediated via stimulation of vasopressin secretion (6), which is most likely mu opioid receptor-mediated (13). Conversely, opiate- and opioid-mediated diuresis is thought to be a result of vasopressin inhibition through kappa opioid receptor activation (11,22). These data would suggest that butorphanol is an agonist at the kappa opioid receptor. Alternatively, butorphanol may have mixed actions, with its actions at the mu opioid receptor attenuating its kappa-mediated actions (13). Nonetheless, it would appear that butorphanol's diuretic effect and consequential reduction in urine osmolality is mediated via its kappa opioid receptor actions.

Water consumption was not significantly increased until 6 hours post dosing, and then only in rats receiving either the lower dose of butorphanol (12 μmols/kg) or morphine. Additionally, this effect was obviated at 8 hours after dosing, as the water consumption of the animals receiving the higher dose of butorphanol (48 μmols/kg) or saline increased. Therefore, these results indicate that butorphanol's diuretic effect was independent of water consumption and perhaps an inconsistent response to volume depletion induced by these agents.

Butorphanol administration (12 or 48 μmols/kg, SC) did not affect respiratory rate when compared to saline-treated animals. In contrast, morphine administration markedly depressed respiratory rate when compared to saline- and butorphanol-treated animals. These data would suggest that butorphanol possesses less efficacy at the opioid site(s) mediating reduction of respiratory rate.

Opiate- and opioid-mediated respiratory depression appears to be mediated by both mu and delta opioid receptors (30,46). Perhaps butorphanol is a partial agonist at both of these sites, having little efficacy in reducing respiratory rate. Alternatively, butorphanol may have effects at delta opioid receptors which may modify its mu opioid receptor-mediated actions on respiratory rate. Such mu/delta opioid receptor interactions have been demonstrated in vivo (44).

In in vitro radioligand displacement studies, butorphanol is a potent ligand for mu, delta, and kappa opioid receptors, being 3, 10 and 30 times more potent at these sites, respectively, than morphine. Unfortunately, the nature of butorphanol's interaction with these opioid receptors cannot be ascertained from these data. Perhaps experiments utilizing the "sodium effect" (31) which appears to be operative at the mu, delta and kappa opiate receptor types (2,19) would be useful in discriminating the nature of butorphanol's actions at these receptors.

Butorphanol, as well as morphine appear to possess little affinity for the opioid sigma receptor. This is consistent with postulated structural requirements of opiates for sigma receptor binding (21). Of the morphine surrogates, those with highest apparent affinity for this site are the class of compounds known as benzomorphans, which lack the "C" ring of their parent compound, morphine. Butorphanol, a morphinan, possesses this structural component and as with other compounds in this classification, such as levorphanol, exhibits poor affinity for this site (21). Behavioral drug discrimination studies in vivo also suggest that butorphanol has little affinity for this site (37,47).

Taken in toto, these data indicate that butorphanol has multiple actions through the opioid receptor system, which are most likely mu, delta and kappa receptor-mediated. However, the nature of butorphanol's opioid receptor interactions cannot be determined from these data. It would be of interest to characterize the agonistic and antagonistic actions of this compound at these sites, especially in terms of butorphanol's potential to induce tolerance and dependence relative to morphine. This is of importance as at the present time, butorphanol is not classified as a controlled substance (DEA, personal communication) and reports have surfaced in the literature documenting both the diversion and abuse of this compound (3, 5, 16).

ACKNOWLEDGEMENT

This study was partially supported by grant DA05828 from the National Institute on Drug Abuse.

REFERENCES

1. Adler, M. W.; Geller, E. B.; Roscow, C. E.; Cochin, J. The opioid system and temperature regulation. Annu. Rev. Pharmacol. Toxicol. 28:429–49; 1988.
2. Appelmans, N.; Carroll, J. A.; Rance, M. J.; Simon, E. J.; Traynor, J. R. Sodium ions increase the binding of the antagonist ICI 174864 to the δ-opiate receptor. Neuropeptides 7:139–143; 1986.
3. Brown, G. R. Stadol dependence: Another case. JAMA 254:910; 1985.
4. Chang, K. J.; Hazum, E.; Cuatrecasas, P. Novel opiate binding sites for benzomorphan drugs. Proc. Natl. Acad. Sci. USA 78:4141–4145; 1983.
5. Evans, W. S.; Bowen, J. N.; Giordano, F.; Clark, B. A case of Stadol dependence. JAMA 253:2191–2192; 1985.
6. Firemark, H. M.; Weitzman, R. E. Effects of β-endorphin, morphine and naloxone on arginine vasopressin secretion and the electroencephalogram. Neuroscience 4:1895–1902; 1979.
7. Gad, S.; Weil, C. S. Statistics and experimental design for toxicologists. Cardwell, NJ: Telford Press; 1972.
8. Geller, E. B.; Hawk, C.; Keinath, S. H.; Tallarida, R. J.; Adler, M. W. Subclasses of opioids based on body temperature change in rats: Acute subcutaneous administration. J. Pharmacol. Exp. Ther. 225: 391–398; 1983.

9. Geller, E. B.; Rowan, C. H.; Adler, M. Temperature effects of opioids in rats: Intracerebroventricular administration. Pharmacol. Biochem. Behav. 24:1761–1765; 1986.
10. Glowinski, J.; Iversen, L. L. Regional studies of catecholamines in the rat brian—I. The disposition of [^{3}H][norepinephrine, [^{3}H]dopamine and [^{3}H]dopa in various regions of the brain. J. Neurochem. 13: 655–669; 1966.
11. Goldstein, A. Biology and chemistry of the dynorphin peptides. In: Gross, E.; Meinhofer, J., eds. The peptides. vol. 6. New York: Academic Press; 1984:95–145.
12. Hayashi, G.; Takemori, A. E. The type of analgesic-receptor interaction involved in certain analgesic assays. Eur. J. Pharmacol. 16:63–66; 1971.
13. Hayes, A. G.; Skingle, M.; Tyers, M. B. Evaluation of the receptor selectivities of opioid drugs by investigating the block of their effect on urine output by β-funaltrexamine. J. Pharmacol. Exp. Ther. 240:984–988; 1987.
14. Heel, R. C.; Brogden, B. N.; Speight, T. M.; Avery, G. S. Butorphanol: A review of its pharmacologic properties and therapeutic efficacy. Drugs 16:473–505; 1978.
15. Heyman, J. S.; Mulazney, S. A.; Mosberg, H. I.; Porecca, F. Opioid δ-receptor involvement in supraspinal and spinal antinociception in mice. Brain Res. 420:100–108; 1987.
16. Hoover, R. C.; Williams, R. B. Survey of butorphanol and nalbuphine diversion in U.S. hospitals. Am. J. Hosp. Pharm. 42:1111–1113; 1985.
17. Janssen, P. A. J.; Niemegeers, C. J. E.; Dony, J. G. H. The inhibitor effect of fentanyl and other morphine-like analgesics on the warm water induced tail withdrawal reflex in rats. Arzneimittelforschung 13:502–507; 1963.
18. Jasinski, D. R.; Griffith, J. D.; Pernick, J. S.; Clark, S. C. Progress report from the clinical pharmacology section of the addiction research center. In: Thirty-Seventh Annual Meeting, Committee on Drug Dependence: National Academy of Sciences; 1973:828–839.
19. Kosterlitz, H. W.; Paterson, S. J.; Robson, L. E. Modulation of binding at opioid receptors by mono- and divalent cations and by cholinergic compounds. J. Recept. Res. 8:363–373; 1988.
20. Lahti, R. A.; Mickelson, M. N.; McCall, J. M.; VonVoightlander, P. F. ^{3}H-U-69593: A highly selective ligand for the kappa opioid receptor. Eur. J. Pharmacol. 109:218–224; 1985.
21. Largent, B. L.; Wikstrom, H.; Gundlach, A. L.; Snyder, S. H. Structural determinants of σ receptor affinity. Mol. Pharmacol. 32:772–784; 1988.
22. Leander, J. D.; Hart, J. C.; Zerbe, R. L. Kappa agonist-induced diuresis: Evidence for stereoselectivity, strain differences, independence of hydration variables and a result of decreased plasma vasopressin levels. J. Pharmacol. Exp. Ther. 242:33–39; 1987.
23. Litchfield, J. T.; Wilcoxon, F. A simplified method of evaluating dose effect experiments. J. Pharmacol. Exp. Ther. 96:99–113; 1949.
24. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement wih the Folin phenol reagent. J. Biol. Chem. 193: 265–275; 1951.
25. McPherson, G. A. A practical computer-based approach to the analysis of radioligand binding experiments. Comput. Prog. Biomed. 17:107–114; 1983.
26. Martin, W. R. Pharmacology of opioids. Pharmacol. Rev. 35: 283–323; 1984.
27. Monkovic, I.; Conway, T. T.; Wong, H.; Perron, T. G.; Pachter, I. J.; Beileau, B. Total synthesis and pharmacological activities on N-substituted 3-14-dihydroxymorphinans. Int. J. Am. Chem. Soc. 95:7910–7911; 1973.
28. Mosberg, H. I.; Hurst, R.; Hruby, V. J.; Gee, K.; Yamamura, H. I.; Galligan, J. J.; Burks, T. F. Bis-penicillamine enkephalins possess highly improved selectivity toward delta opioid receptors. Proc. Natl. Acad. Sci. USA 80:5871–5874; 1983.
29. Nagashima, H.; Karmanian, A.; Mabunay, R.; Radna, P.; Ang, M.; Foldes, F. F. Respiratory and circulatory effects of intravenous butorphanol and morphine. Clin. Pharmacol. Ther. 19:738–745; 1976.
30. Pazos, A.; Florez, J. Interaction of naloxone with μ and δ agonists on the respiration of rats. Eur. J. Pharmacol. 87:309–314; 1983.
31. Pert, C. B.; Pasternak, G.; Snyder, S. H. Opiate agonists and antagonists discriminated by receptor binding in brain. Science 182:1359–1361; 1973.
32. Pircio, A. W.; Glyes, J. A.; Cavanagh, R. L.; Buyniski, J. P.; Bieiwagen, M. E. The pharmacology of butorphanol, a 3,14 dihydroxy narcotic antagonist analgesic. Arch. Int. Pharmacodyn. 220: 231–257; 1976.
33. Porreca, F.; Mosberg, H. I.; Omnas, J. R.; Burks, T. F.; Cowan, A. Supraspinal and spinal potency of selective opioid agonists in the mouse writhing test. J. Pharmacol. Exp. Ther. 240:890–894; 1987.
34. Preston, K. L.; Bigelow, G. E.; Liebson, I. A. Butorphanol-precipitated withdrawal in opioid-dependent human volunteers. J. Pharmacol. Exp. Ther. 246:441–448; 1988.
35. Roebel, L. E.; Cavanagh, R. L.; Buyniski, J. P. Comparative gastrointestinal and biliary effects of morphine and butorphanol (Stadol). J. Med. 10:225–238; 1979.
36. Schaefer, G. J.; Holtzman, S. G. Discriminative effects of cyclazocine in the squirrel monkey. J. Pharmacol. Exp. Ther. 205:291–301; 1978.
37. Shannon, H. E. Pharmacological analysis of the phencyclidine-like discriminative stimulus properties of narcotic derivatives in rats. J. Pharmacol. Exp. Ther. 222:146–151; 1982.
38. Shannon, H. E.; Holtzman, S. G. Further evaluation of the discriminative effects of morphine in the rat. J. Pharmacol. Exp. Ther. 201:55–66; 1977.
39. Simantov, R.; Snowman, A. M.; Snyder, S. H. Temperature and ionic influences on opiate receptor binding. Mol. Pharmacol. 12: 977–986; 1976.
40. Takemori, A. E.; Portoghese, P. S. Evidence for the interaction of morphine with kappa and delta opioid receptors to induce analgesia in β-funaltrexamine-treated mice. J. Pharmacol. Exp. Ther. 243:91–94; 1987.
41. Tallarida, R. J.; Murray, R. B. Manual of pharmacologic calculations with computer programs. 2nd ed. New York: Springer-Verlag; 1986.
42. Tyers, M. B. A classification of opiate receptors that mediate antinociception in animals. Br. J. Pharmacol. 69:503–512; 1980.
43. Vaught, J. L.; Mathiasen, J. R.; Raffa, R. B. Examination of the involvement of supraspinal and spinal mu and delta opioid receptors in analgesia using the mu receptor deficient CXBK mouse. J. Pharmacol. Exp. Ther. 245:13–16; 1988.
44. Vaught, J. L.; Rothman, R. B.; Westfall, T. C. Mu and delta receptors: Their role in analgesia and in the differential effects of opioid peptides on analgesia. Life Sci. 30:1443–1445; 1982.
45. Ward, S. J.; Takemori, A. E. Relative involvement of mu, kappa and delta receptor mechanisms in opiate-mediated antinociception in mice. J. Pharmacol. Exp. Ther. 224:525–530; 1983.
46. Ward, S. J.; Takemori, A. E. Determination of the relative involvement of mu-opioid receptors in opioid-induced depression of respiratory rate by use of beta-funaltrexamine. Eur. J. Pharmacol. 87:1–6; 1983.
47. White, J. M.; Holtzman, S. G. Further characterization of the three-choice morphine, cyclazocine and saline discrimination paradigm: Opioids with agonist and antagonist properties. J. Pharmacol. Exp. Ther. 224:95–99; 1983.