DEPENDENCE STUDIES OF NEW COMPOUNDS IN THE RHESUS MONKEY, RAT AND MOUSE (2001)

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All compounds, except (γ)-hydroxybutyric acid, caffeine, lobeline and agmatine were unknown to us when submitted by the Biological Coordinator, Dr. Andrew Coop of University of Maryland, School of Pharmacy. These studies were conducted under the auspices of the Drug Evaluation Committee in association with the College on Problems of Drug Dependence. See summary of new data in Table l.

Dependence-Liability Studies in Rhesus Monkeys

Substitution-for-Morphine (SDS) Test. Male and female rhesus monkeys (M. mulatta) weighing 2.5-7.5 kg were used, and they received 3 mg/kg, s.c., of morphine·SO_4 every 6 hr. All the animals had received morphine for at least 3 months and were maximally dependent on morphine (Seevers and Deneau 1963). A minimal 2-week recuperation period was allowed between tests. At least 3 monkeys/dose were used. The assay (Aceto and co-workers, 1977 and 1978) was initiated by a subcutaneous injection of the test drug or control substances (morphine and vehicle) into animals in a group that had not received morphine for 14-15 hr and showed definite signs of withdrawal. Each animal was randomly chosen to receive one of the following treatments: a) a dose of the compound under investigation; b) morphine control, 3.0 mg/kg; and c) vehicle control, 1 ml/kg. The animals were scored for suppression of withdrawal signs during a 2.5-hr observation period. The observer was "blind" regarding the choice of treatments. At the end of the study, the data were grouped according to dose and drug. The mean cumulative score ± SEM was calculated and the data illustrated in figure form. If indicated, the data were analyzed using the Kruskal-Wallis ANOVA and post hoc Mann-Whitney U-Tests.

Precipitated-Withdrawal (PPT-W) Test. This evaluation was done under the same conditions as described above, except that the animals were administered a test compound 2-3 hr after the last dose of morphine. These animals were not in withdrawal. Naloxone·HCl (0.05 mg/kg, s.c.) served as the positive control.

Primary-Physical-Dependence (PPD) Study. Drug-naive monkeys were medicated with drug, using escalating dose regimens, periodically challenged with naloxone or placed in abrupt withdrawal. They were observed for overt behavioral signs during drug administration and when they were challenged with the antagonist, naloxone, or abruptly withdrawn from the drug.

Rat-Infusion Studies

The continuous-infusion method was reported by Teiger (1974) and certain modifications are indicated as follows. Rats were anesthetized after which each was fitted with a specially

prepared cannula, which was passed subcutaneously from the nape of the neck to the lateral side of the lower abdomen and then inserted into the peritoneal cavity. The cannula was anchored at both ends with silk sutures and attached to a flow-through swivel mechanism, which allowed the animal to move about in the cage and eat and drink normally. The swivel was connected to a syringe, which was attached to a syringe pump. The animals received 7-10 ml of solution every 24 hr.

SEE TABLE 1

AND

SEE TABLE 1 (CONTINUED)

Substitution-for-Morphine (SM) Test. The rats received morphine·SO_4 (50 mg/kg/24 hr on the first day, 100 mg/kg/24 hr on the second day, and 200 mg/kg/24 hr from days 3 and 4). Then, a test drug was substituted for 2 days. The morphine controls received an infusion of sterile water for injection. The animals were observed for changes in body weight and for behavioral-withdrawal signs for 0.5 hr at 6, 24, 48, 72 and/or 96 hr after stopping the infusion of morphine.

Primary-Physical-Dependence (PPD) Study. The rats received test compound, as specified above, for 4-6 days and then, were placed in abrupt withdrawal and observed for overt behavioral signs.

Mouse-Antinociception Tests

Male mice, weighing 20-30 g, were used. All drugs were dissolved in distilled water or in the vehicle indicated and injected subcutaneously (s.c.). At least three doses were tested, and 6-10 animals per dose were used. When applicable, ED50's were calculated by using computerized probit analysis. The results obtained with reference compounds are summarized in Table 2. Occasionally, when requested, drugs were given orally (p.o.) or intravenously (i.v.) and the pretreatment times are indicated in the text.

Tail-Flick (TF) and (TF vs M) Assays. The procedure and modifications were described (D'Amour and Smith 1941 and Dewey *et al*. 1970 and 1971) in the literature. Briefly, the mouse's tail was placed in a groove, which contained a slit under which was located a photoelectric cell. When the heat source of noxious stimulus was turned on, the heat focused on the tail, and the animal responded by flicking its tail out of the groove. Thus, light passed though the slit and activated the photocell, which, in turn, stopped the recording timer. The heat source was adjusted to produce tail flick of 2-4 sec under control conditions. Mice were injected with drug or vehicle and tested 20 min later. In three assays for antagonism of the antinociceptive effect, the potential antagonists were administered 10 min before the agonist, and evaluation occurred 20 min later.

Phenylquinone Abdominal-Stretching (PPQ) Assay. The procedure was reported previously (Pearl and Harris, 1966). The mice were injected with test drug and 10 min later received 2.0 mg/kg intraperitoneally (i.p.) of a freshly prepared paraphenylquinone (PPQ) solution. The mice were then placed in cages in groups of two each. Ten min after the PPQ injection, the total number of stretches per group were counted over a 1-min period. A stretch was characterized by an elongation of the mouse's body, development of tension in the abdominal muscles, and extension of the forelimbs. The antinociceptive response was expressed as the percent inhibition of the PPQ-induced stretching response.

Hot-Plate (HP) Assay. The method was also reported previously (Eddy and Leimbach, 1953 and Atwell and Jacobson, 1978). The hot plate was held at 55°C. Mice were placed on the hot plate and activity was scored if the animal jumped or licked its paws after a delay of 5 sec or more, but no more than 30 sec beyond the control time.

Table 2

Comparative Data (ED50, mg/kg s.c.) [95% C.L.] of Selected Standards in 4 Mouse Agonist-Antagonist Tests

Drug	**Tail-Flick**	**Tail-Flick Antagonist**	**Phenylquinone**	**Hot-Plate**
Pentazocine	15% at 10.0	18	1.7 (12-26)	13% at 30.0 (1.0-2.5)
Cyclazocine	17% at l.0[a]	0.03 (0.02-0.78)	0.01 (0.005-0.03)	25% at 9.0
Nalorphine·HCl	None at 10.0	2.6 (0.7-1.0)	0.6 (0.03-1.44)	13% at 30.0
Naloxone·HCl	None at 10.0	0.04 (0.0-0.09)	No Activity	- - - -
Naltrexone·HCl	None at 10.0	0.007 (.002-0.02)	No Activity	- - - -
Morphine·S0$_4$[b]	1.92 (0.89-4.14)	Inactive	0.4[b] (0.2-0.8)	0.85 (0.39-1.86)
Codeine·P0$_4$	- - - -Inactive	8.25 (5.12-13.29)	6.4 (2.4-16.8)	
Meperidine·HC1	8.37 (4.59-15.27)	Inactive (1.18-11.7)	- - - -	4.6

[a]Mice were ataxic at 3.0 and 10.0 mg/kg but there was no further increase in reaction time
[b]ICR - Harlan-Sprague-Dawley Inc.

Calculation of Apparent pA_2. Using the tail-flick or PPQ assay, the apparent pA_2 and 95% confidence limits were calculated using Schild and constrained plots as described in Tallarida and Murray (Manual of Pharmacologic Calculations with Computer Programs, 2nd ed., Springer Verlag, NY, 1987).

Briefly, mice were pretreated with vehicle or various doses of antagonist followed 10 min later by an injection of agonist. The mice were tested 30 min after receiving the antagonist. Dose-response lines for antinociception were plotted using at least 3 doses of each opioid agonist in the presence of vehicle or one of the selected doses of antagonist. ED5Os were estimated according to the method of Litchfield and Wilcoxon (J. Pharmacol. Exp. Ther., 96, 399, 1949). Each dose ratio (x) was calculated by dividing the ED50 of the opioid in the presence of a given dose of antagonist by that of the agonist alone. Log (x-l) was plotted against

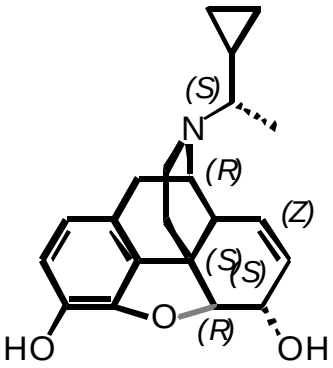
[a]Negative logarithm of the molar concentrations of antagonist required to produce a two-fold shift of the agonist dose-response curve to the right. Competitive antagonism can be assumed when slope = -1. pA_2 provides a measure of the relative potency and affinity of the antagonist. When the slope differs significantly from unity, this may indicate non-equilibrium conditions, interactions with multireceptors, receptor sensitization, precoupling mechanisms, or multiple drug properties. With a constrained plot, the slope of the regression line is restricted to slope of - 1.

Special Intracerebroventricular Tail-Flick and PPQ Assays. In order to develop an in-vivo agonist and antagonist model to correlate with the in-vitro binding data of the various opioid receptor types (mu, kappa and delta), we chose the mouse Tail-Flick and PPQ tests and a variety of routes of administration. The intracerebroventricular (i.c.v.) route was chosen to accommodate thee fact that no delta agonist is available which is active by peripheral routes of administration
the negative logarithm of the molar dose of the antagonist. At least 3 logs (x-l) were plotted. The pA_2 values for the antagonists were calculated from the point of intersection of the regression line with the abscissa. See Table 3 for summary of results.

Table 3. Apparent pA_2 values[a] using the mouse tail-flick assay

Treatment Antagonist/Agonist	Schild Plot pA_2 (95% C.L.) Slope	Constrained Plot pA_2 (95% C.L.)
1) Naloxone/Morphine	7.2 (7.0-7.4)-1.2	7.3 (7.1 - 7.6)
2) Naloxone/Sufentanil	7.0 (6.5 - 7.5)-1.0	7.0 (6.8 - 7.1)
3) Naloxone/Mirfentanil	7.6 (7.3 - 8.0)-0.7	7.2 (6.9 - 7.5)
4) Naloxone/NIH 10672 (Enadoline) (selective kappa agonist)	6.1 (5.6 - 6.6)-1.2	6.6 (6.3 - 7.0)
5) Naloxone/U-50,488 (kappa agonist)	6.6 (6.3 - 6.9)-1.1	6.2 (5.9 - 7.3)
6) Naloxone/(-)-Nicotine	5.3 (5.3-5.3)-0.5	-
7) Nalmefene/Morphine	8.0 (7.6 - 8.3)-1.1	8.0 (7.7 - 7.6)
8) Naltrexone/Morphine	7.7 (4.9 - 10.5)-0.8	7.6 (7.1 - 8.3)
9) (-)-Quadazocine/Morphine	6.8 (6.7 - 7.0)-0.9	6.8 (6.1 - 7.6)
10) (-)-Quadazocine/Enadoline	6.2 (6.1 - 6.2)-1.7	6.7 (6.6 - 6.8)
11) nor BNI/Enadoline	6.5 (5.9 - 7.0)-1.3	6.6 (5.9 - 7.3)
12) Mecamylamine/(-)-Nicotine	6.6 (6.2 - 6.9)-0.9	-

NIH 10497 *N*-(1*R*-1-Cyclopropyl)ethylnormorphine hydrochloride



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 2.0 (0.6 – 6.6)[a]
2) TF vs. M – Inactive at 1, 10 and 30[a]
3) PPQ – 0.03 (0.01 – 0.2)[a]
1. 4) HP – NT

[a]Reported in NIDA Res. Monog. 95, 1989

Special Tests:

1) Naloxone vs ED80 of morphine in TF: 2.98 (1.19 – 7.48)
2) Opioid subtype testing
   a) β-FNA (i.c.v.) vs ED80 of NIH 10497 (s.c.) in TF: Inactive at 1, 10 and 30 μg/brain.
   b) nor-BNI (s.c.) vs ED80 of NIH 10497 (s.c.) in TF: AD50 = 11.17 (2.9 -42.9) mg/kg.
   c) Naltrindole (s.c.) vs ED80 of NIH 10497 (s.c.) in TF: 10% at 1, 0% at 10 and 23% at 30 mg/kg.

New Data

Table. Comparison of the antinocioceptive effects of three samples of NIH 10497 in the mouse tail-flick test.

Compound No.	Route of Administration	ED50
MCV 4558	s.c.	4.47 (0.51 – 39.08)
NIH10497A	s.c.	2.52 (0.67 – 9.47)
NIH 10497B	s.c.	1.67 (0.31 – 8.96)

MONKEY DATA (Reported in NIDA Monog. 95, 1989)
(SDS)

NIH 10497 substituted completely for morphine. The drug acted promptly and its duration of action was about 2 hr (see fig). In addition, this drug is slightly less potent than morphine. Many drug-related side effects were seen including body sag, jaw sag, slowing, staring, and salivation. The incidence of drowsiness was more than that observed in morphine-treated controls. [In this context, salivation suggested kappa agonist activity].

NIH 10497 (Continued)

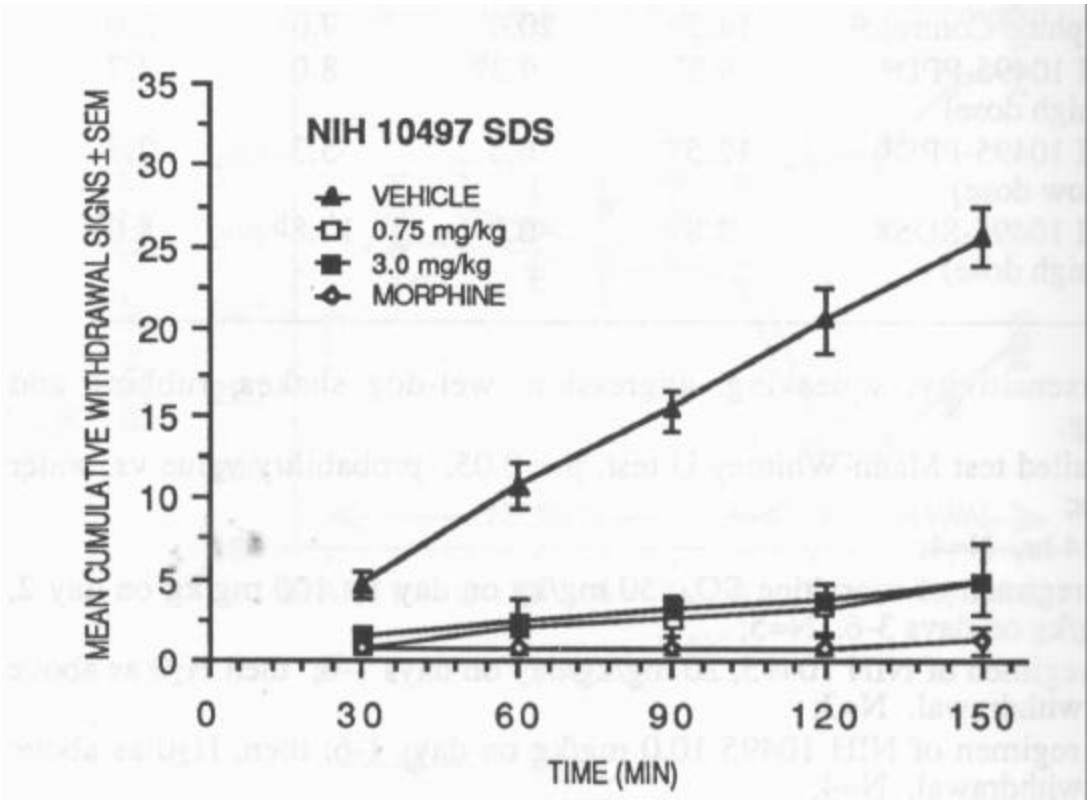


Fig NIH 10497-SDS. Results of study in which single doses of NIH 10497 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: Apparently, NIH 10497 is a selective kappa agonist in both species. Some weak delta-opioid receptor agonist activity was observed.

RAT CONTINUOUS INFUSION ASSAY (PPD) (Reported in NIDA Monog. 95, 1989)

Primary physical dependence study

Each rat was randomly allocated a treatment regimen. They were then assigned to a cage on a rack. The 6-day morphine dose regimen that was used by Teiger (1974) was modified by us and shortened to 4 days because studies in our laboratory indicated that the withdrawal syndromes were qualitatively and quantitatively similar. The dose regimen for morphine was 50 mg/kg day on the first day, 100 mg/kg day on day 2, and 200 mg/kg day on days 3 and 4. NIH 10497's low-dose regimen was the same as morphine. The high-dose regimen was double that of the low-dose regimen.

NIH 10497 (Continued)

Physiological and Behavioral Measurements

During the infusion of vehicle, NIH 10497, or morphine, the rats were weighed and observed daily for 1 hr for overt behavioral signs. Body weight was recorded daily. The sign wet-dog NIH shakes was quantified. Irritability was scored as proposed by Teiger (1974). Scoring for this sign was as follows: 0 (remains tame when touched and on being grasped and lifted); 1 (remains tame when touched and on being grasped and lifted makes only a feeble attempt to wiggle free); 2 (remains tame when touched but when grasped and lifted **claws, bites and** or vocalizes); and, 3 (reacts to initial touch by vocalizing and biting and to attempts to grasp it by rolling over on its back and clawing). All other behavioral signs were simply noted. A trained observer was blind regarding treatment assignments.

Statistical Analysis

The data from were combined and analyzed. Quantified data was assessed using a repeated measures ANOVA. One factor ANOVA was used to evaluate daily blocks of data. If overall significance was found, Fisher's LSD test was used for post-hoc comparisons. Scored data was analyzed using the nonparametric Kruskal-Wallis one-way ANOVA. Post hoc comparisons of nonparametric data were made using the Mann-Whitney U test. In all cases significance was set at the 95% level. The StatView statistical package (Brainpower, Inc., Agoura Hills, CA) was utilized for these analyses.

Drugs and Solutions

NIH 10497 was forwarded to us by Dr. A. Jacobson of NIH. Morphine sulfate was purchased from Mallinckrodt, Inc., St. Louis MO. All drugs were dissolved in distilled water and solutions were prepared daily.

Results

Body Weight Loss These results are displayed in Fig. 1. Two-factor repeated measures analysis of variance revealed significant differences among treatment groups ($F = 5.33$, $P = 0.0083$) and days ($F = 21.048$, $P = 0.001$). One factor analysis of body weights at the start of the experiment indicated no significant differences ($F = 0.172$, $P = 0.9142$). One factor ANOVAs for day 3($F = 3.718$, $P = 0.0306$) , day 4 ($F = 11.607$, $P = 0.0002$), day 5 ($F = 7.882$, $P = 0.0014$), day 6 ($F = 19.747$, $P = 0.0001$) day 7 ($F = 4.1$, $P = 0.0221$), and day 8 ($F = 25.349$, $P = 0.0001$) but not days 1 ($F = 1.899$, $P = 0.1959$), and 2 ($F = 2.175$, $P = 0.1263$) showed significant differences among treatments.

During the infusion of morphine the rats initially showed small increases in body weight during the first 2 days when compared to that of the vehicle controls; the gain was statistically significant on day 1. After morphine was abruptly withdrawn and vehicle substituted, there was a precipitous and significant loss of body weight during the first 24 hr (day 5) followed forty-

eight hr later (day 6), by an even greater loss. Although body weights appeared to be recovering during the rest of the experiment, weight loss was still significantly reduced compared to that of vehicle controls.

In sharp contrast with the results obtained with the morphine controls, body weight decreased in a dose-dependent manner in the rats treated with NIH 10497 during its administration and began recovering within 24 hours *after* it was abruptly discontinued. For the high-dose regimen of NIH 10497, weight loss was significant compared to the vehicle control group beginning on day by day 4. It is interesting that although weight loss was still significant compared to the vehicle group at the end of the experiment it was also significantly less than that of the morphine-treated group.

Wet-dog Shakes The results are depicted in Fig. 2. When examined for overall differences, 2-factor repeated measures analysis of variance indicated that differences among treatments were significant only for days ($F = 5.217$, $P = 0.001$). One factor ANOVAs for day 6 (48 hr post withdrawal) indicated a significant difference among treatments ($F = 3$, $P = 0578$). Comparison of the results of the morphine-treated group with those of the vehicle group was the only comparison among groups that was significant.

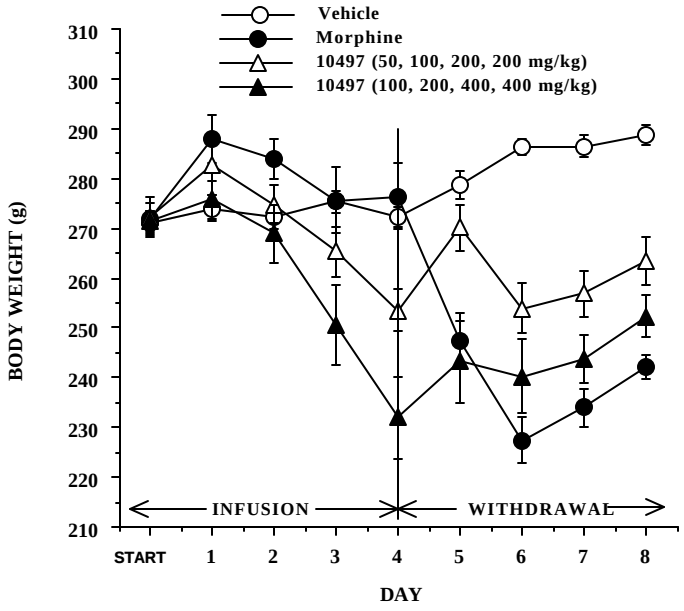


Fig.1. Rat PPD: Body Weight

Irritability Fig. 3 displays the results obtained with this sign. The critical value (based on 4 treatments and 3 degrees of freedom) for this experiment using Kruskal-Wallis ANOVA is $X^2_{0.05}$ (3) = 7.82 and H for day 5 was 0.357. Post hoc comparisons (Mann-Whitney one-tail test) on

this day indicated that only the results of the morphine-treated group showed a significant difference when compared to those of the vehicle group.

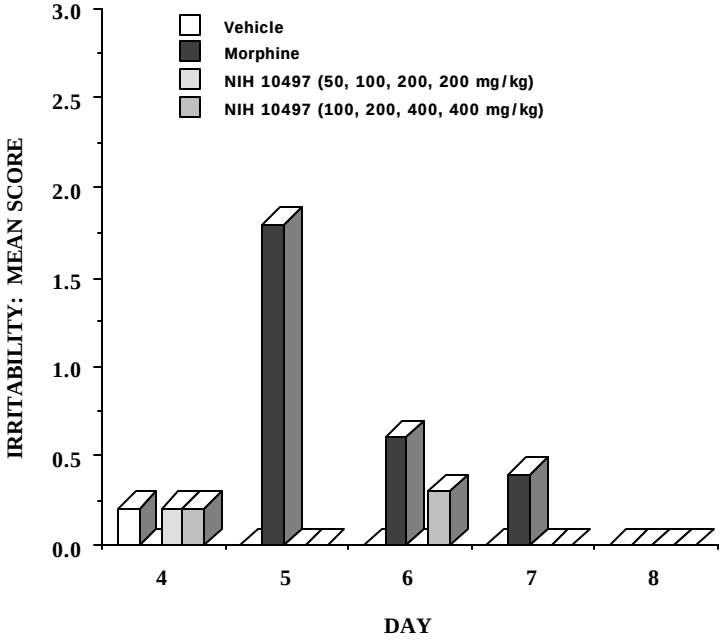


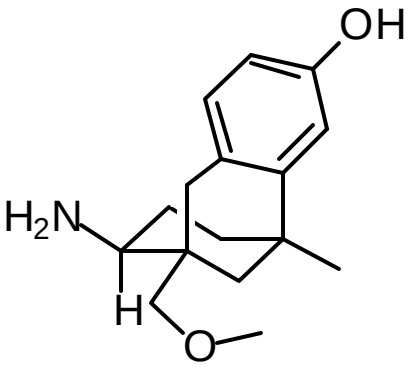
Fig. 3. Rat PPD: Irritability

Summary

At dose regimens approximately equal to and double that of morphine sulfate, NIH 10497 did not produce body weight loss, the most reliable index of physical dependence on morphine (Aceto 1990). Neither did it increase the degree of irritability in response to handling, another significant mu-opioid receptor agonist abstinence sign (Himmelsbach *et al*. 1935 and Aceto 1990). Finally, it did not express another important morphine-like abstinence sign designated wet-dog shakes. These results suggest that NIH 10497 is relatively free of mu-opioid induced physical dependence liability.

NIH 10497 has a novel profile of activity. It lacks mu-opioid receptor properties and has selective kappa-and weak delta-opioid receptor effects. Since it substituted for morphine in morphine-dependent monkeys and appears to be free of mu-opioid physical-dependence liability, it may prove to be useful in the pharmacotherapy of heroin-like abuse. However, species difference may have accounted for these results. Finally, because each sample had a different color, they were retested in the tail-flick test. The original sample was yellow and a TLC indicated contamination. Sample A was white and sample B was tan. Samples A and B did not show contamination when examined by TLC.

NIH 10945 (+)-(5*S*,8*S*,9*R*)-8-Amino-3-hydroxy-5,9-methano-9-(methoxymethyl)-5-methylbenzocyclooctene



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)
1) TF - 0% at 1, 17% at 10 and 3% at 30[a]
2) TF vs M - Inactive at 1, 10 and 30[a]
3) PPQ - 3.75 (1.62 - 8.64)[a]
4) HP - 25% at 1, 38% at 10 and 38% at 30[a]

[a]Vehicle was 10% hydroxypropyl-β-cyclodextrin in water.

New Mouse Data

Special: Naloxone vs ED80 of NIH 10945 in PPQ: AD50 = 2.63 (0.99 - 6.98)

MONKEY DATA
(SDS)

As shown in the fig below, NIH 10945 reduced the number of withdrawal signs at both doses. Curiously the lower dose appeared more effective in that regard. However, the drug did not substitute completely for morphine in withdrawn morphine-dependent monkeys. Vehicle was 10% hydroxypropyl-β-cyclodextrin in sterile water.

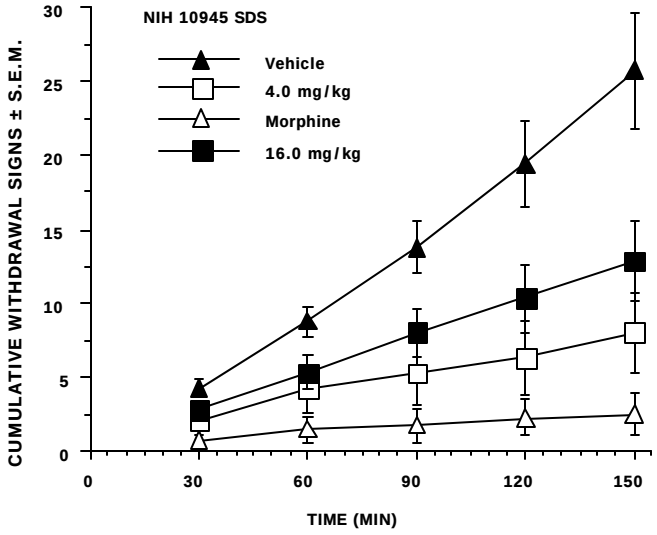
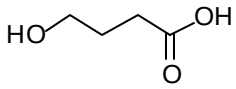


Fig NIH 10945. Results of study in which single doses of NIH 10945 were substituted for morphine in morphine-dependent monkeys in withdrawal.

NIH 10945 (Continued)

Comment: The compound does not have robust opioid activity. The naloxone AD_{50} in the PPQ test suggests weak heterogenous opioid effects. Because of the relatively large AD_{50}, most likely the delta-opioid component predominates or the opioid effects are indirect.

NIH 10947 Gamma-Hydroxybutyric Acid, Sodium Salt



Gamma-Hydroxybutyric Acid (GHBA), a precursor and metabolite of gamma-aminobutyric acid, which has been used in Europe, as a general anesthetic and hypnotic, as an aid in childbirth, in the treatment of alcoholism and in anxiety attendant with detoxication from cocaine and amphetamines, depression and other conditions, has also gained popularity as a fashionable recreational drug. Because little is known about its interaction with opioids, this study was initiated. GHBA, per se, (at 30, 60, 80, and 100 mg/kg s.c.) had little effect on the normal reaction time in the tail-flick test. When these doses of GHBA were co-administered with the ED25 of morphine, dose-related synergism was observed. In mice made completely tolerant to morphine antinociceptively (25 mg/kg.s.c., 4 times a day for 4 days), GHBA (60 mg/kg s.c.) in combination with M morphine partially restored antinociception. Naloxone (1 mg/kg s.c.) nearly abolished this effect.

Table 1. Antinociceptive Effects of GHB in the Mouse Tail Flick and Paraphenylquinone Assays[a]

Test	Route of Administration	Pretreatment Time	ED50 or AD50 (95% C.L.) (mg/kg or % Change)
Tail-flick	i.v.	20 min	Inactive at 60 and 52% at 120
"	s.c.	20 min	Inactive at 1, 10, 30 and 60
"	p.o.	20 min	Inactive at 60 and 51% at 120
"	p.o.	1 hr	Inactive at 60 and 120
PPQ	i.v.	20 min	30.88 (15.34 - 62.17)

[a]Reported in NIDA Monograph 179, p 363, 1999.

Table 2. GHB effects in the tail-flick test by the i.c.v. and oral route and orally in combination with 12% alcohol

Tail-flick	Route of Administration	Treatment	Comment and ED50 or % Change
"	i.c.v (μg/brain)	GHB (mg/kg)	16% at 1, 7% at 10 and 15% at 30 μg/kg
"	p.o.	GHB in 12% alcohol	21% at 30, 32% at 100 mg/kg. At 30 mg/kg mice were ataxic within 5 min of dosing. At 100 mg/kg mice were ataxic and two mice were anesthetized. One regained its righting reflex after 40 min. The other remained anesthetized until it was euthanized.
"	p.o.	12% alcohol controls	Mice were sedated and showed 25% increase in latency

MONKEY DATA (Reported in NIDA Monograph 2000, In Press)
(SDS)

The results suggest an inverse dose-response relationship (see figure) for GHB regarding attenuation of withdrawal signs in withdrawn morphine-dependent monkeys. Statistical analysis of the data obtained at 150 min, revealed by Kruskal-Wallis one-way analysis of variance, predict highly significant differences among treatment regimens ($H=23.26$, χ^2 0.005 = 16.75). The Mann-Whitney U test was used to assess between-treatment comparisons. The results indicated that all treatment regimens, except the GHBA 120 mg/kg and GHBA 240 mg/kg, were significantly different from vehicle (at U = 6 or less, P = 0.05 or less). In addition, all GHBA-treated group-withdrawal scores were significantly higher than those in morphine-treated monkeys (U= 6 or less, P = 0.01 or less). Finally. The scores of the low-dose GHB group (7.5 mg/kg) were significantly less than the scores of the highest dose GHBA

NIH 10947 (Continued)

group (U = 0, P = 0.014), as were those of the 30 mg/kg GHBA group (U = 3, P = 0.014). Both the 60 mg/kg-treated GHBA group scores and the 120 mg/kg-treated GHBA group scores were lower than those of the 240 mg/kg-treated GHBA group. However, the differences only approached significance at P = 0.056.

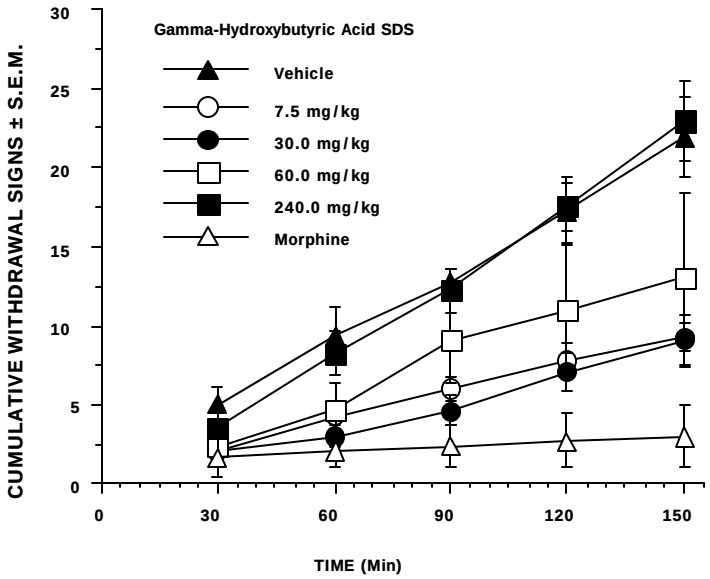


Fig NIH 10947-SDS. Results of study in which single doses of NIH 10947 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: In the mouse and monkey, the results suggest interesting interactions of GHBA with the opioid system which deserve further investigation. In combination with alcohol, GHB produces anesthesia of long duration. Additional studies at lower doses are planned.

NEW DATA

Rat Continuous Infusion Assays

a) Combined four-day primary physical dependence and 2-day substitution for morphine study.

The method has been reported in the literature (Aceto *et al.*, Eur. J. Pharmacol. 307, 2000). Each rat was randomly allocated a treatment regimen. They were then randomly assigned to a cage on a rack. Five treatment regimens consisting of 5-6 rats per regimen were studied. During the 4-day infusion, one group served as the vehicle-vehicle group (8 ml, i.p./24hr.), another group received GHB (50, 100, 200 and 200 mg/kg, i.p./24 hr on days 1 through 4 respectively) and 3 other groups received morphine (same dose regimen as GHB). Then, at the end of 4 days, GHB

was abruptly withdrawn and vehicle was substituted in one group. Morphine was also abruptly withdrawn in one of the groups receiving morphine and vehicle was substituted. Finally, 2 dose regimens of GHB (100 or 200 mg/kg,i.p./24hr) were substituted for morphine in the remaining morphine-treated groups. In effect, we conducted a primary physical dependence study and a substitution for morphine study.

Body weight was noted daily, the sign designated wet-dog shakes was quantified. Irritability was scored as follows: 0 (remains tame when touched and on being grasped and lifted); 1 (remains tame when touched and makes only a feeble attempt to wiggle free; 2 (remains tame when touched but when grasped and lifted bites and or vocalizes; and 3 (reacts to initial touch by vocalizing and biting and to attempts to grasp it by rolling over on its back and clawing). All other behavioral signs were simply noted. A trained observer was blind regarding the treatment regimen.

Quantified data were assessed using repeated measures ANOVA. One factor ANOVA was used to evaluate each day. If overall significance was found, the Scheffe test was used for comparisons among means. Scored data were analyzed using Kruskal-Wallis one-way ANOVA (nonparametric test). The Mann-Whitney U test was used for post hoc comparisons. In all cases significance was set at $p = 0.05$ or less. The Stat View statistical package (Brainpower, Inc., Agoura Hills, CA) was utilized for these analyses. For simplicity sake, the data were separated and illustrated and analyzed according to the components designated abrupt withdrawal (PPD) and substitution for morphine (SM).

1.0 Body Weight

The results of GHB, morphine and vehicle on body weight are illustrated in Fig. 1 below. Repeated measures ANOVA indicated significant differences among days ($F = 17.149$, $p = .0001$) and for subjects versus treatments ($F = 8.718$, $p = .004$).

During the infusion, ANOVA revealed significant differences among treatments on days I ($F = 6.398$. $p = .0116$) and 2 ($F = 11.002$, $p = 0016$). Post hoc analysis showed that the morphine-vehicle controls increased their weight significantly compared to either the vehicle-vehicle group or the GHB-vehicle group. It should be noted that with this dose regimen morphine controls typically behave this way. On days 3 and 4 the F values were not significant indicating no significant differences in body weight among the treatment groups.

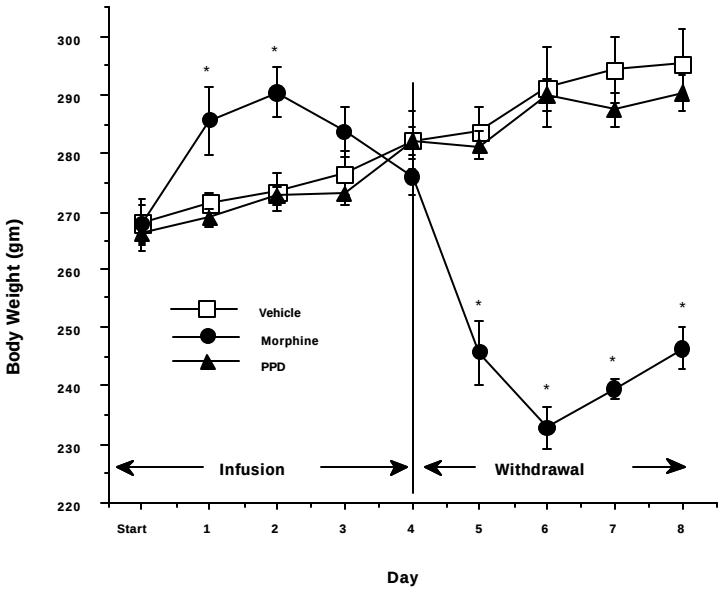


Fig. 1. PPD: Depicted results of the primary physical dependence study of GHB and morphine. *Significant at p = 0.05.

Dramatic changes occurred during the withdrawal. ANOVAs for days 5 and 6 indicated highly significant Fs (F = 25.632, p = .0001 and F = 51.344, p = .0001 respectively). However, post hoc comparisons indicated that only the rats in the morphine-vehicle group lost significant body weight compared to the vehicle-vehicle-treated group. ANOVAs for the final 2 days of the experiment also indicated significant differences among the treatment groups (F = 62.818, p = .0001 for day 7 and F = 37.519, p = .0001 for day 8). Again, compared to the vehicle-vehicle control group, only the morphine-vehicle-treated group showed a significant loss of body weight. However, the morphine group appeared to be recovering. The vehicle-vehicle and GHB-vehicle groups steadily gained weight throughout the study.

Body weight of the SM study is illustrated in Fig 2. Repeated measures ANOVA indicated significant differences among treatments (F = 7.683, p = .0021) and days (F = 177.627, p = .0001). ANOVA by day revealed no significant values at the start of the experiment (F = .002, p = .9999) and on day 1 (F = 2.357, p = .1058). On day 2 significant difference were evident among all treatments (F = 5.539, p = .0071); the morphine groups, had significantly increased weight compared to the vehicle group. However, ANOVA for days 3 and 4 showed that all groups were now equivalent regarding weight.(F = .576, p = .6381 and F = .884, p = .468 , respectively). Twenty-four and 48 hr (days 5 and 6) after morphine was withdrawn, neither vehicle nor GHB substitution (100 or 200 mg/kg/24 hr, prevented severe loss of body weight (F = 25.948, p = .0001 for day 5 and F = 42.633, p = .0001). Post hoc comparison with the vehicle-vehicle group confirmed this observation. Thus, GHB did not substitute for morphine. Although body weight remained depressed throughout the remainder of the experiment (F = 28.474, p =

.0001 on day 7 and F = 24.963, p = .0001 on day 8 and confirmed by post hoc analyses) recovery was evident.

(Kruskal-Wallis one-way analysis of variance revealed significant differences among treatment regimens (H = 14.505 for day 5 and 14.667 for day 6. The critical value of χ^2 0.05(2) = 5.99. Post hoc comparisons using the Mann- Whitney U test confirmed that only the morphine-vehicle group had significantly elevated scores on days 5 and 6 (24 and 48 hr post withdrawal).

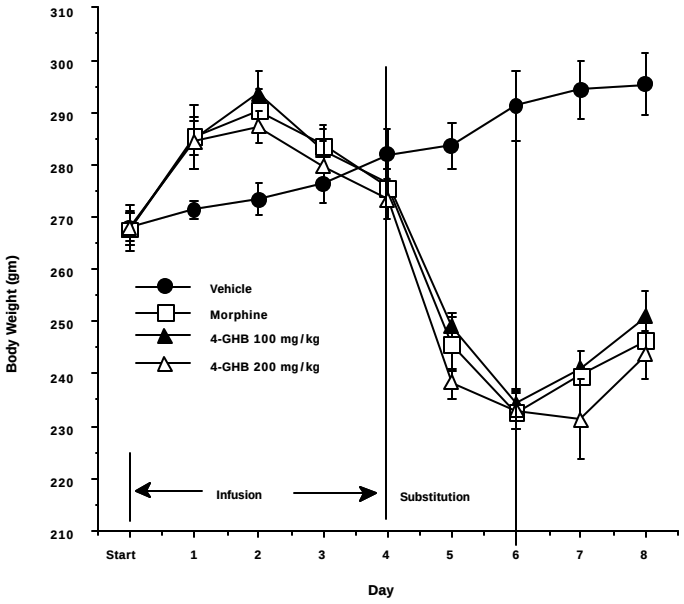


Fig. 2. Illustration of the effects of GHB on body weight either after abrupt withdrawal (end of day 4 to day 8) or when it was substituted for substituted for morphine (days 5 and 6).

2.0 Irritability

Irritability associated with morphine withdrawal was not blocked or attenuated after substituting either of the two dose regimens of GHB or vehicle for morphine (SM). (H = 11.236 for day 5,19.583 for day 6, and 20.778 for day 7, χ^2 0.05(3) = 7.82). Post hoc analyses indicated that all the rats receiving morphine had elevated irritability scores. Thus, GHB did not substitute for morphine.

3.0 Wet-Dog Shakes

Before abrupt withdrawal of GHB (day 4), ANOVA indicated no significant differences among treatments (F < 1). After withdrawal, F approached significance on day 5, (F = 2.426, p = .1272)

and was significant on day 6 (F = 11.114, p = 0015), p = .0421. The Scheffe test confirmed that the morphine-vehicle group displayed a significantly increased number of wet-dog shakes vis a vis the vehicle-vehicle group.

The incidence of wet-dog shakes was not significantly before GHB was substituted for morphine (day 4, F < 1). After substitution (day 5), ANOVA did not suggest significant differences among treatments (F = 1.716, p = .1995) However, at 48 hr after substitution (day 6) ANOVA indicated significant differences among treatments (F = 3.351, p = .0421). Unfortunately, Scheffe's test did not identify the treatment group(s) with increased scores.

B. Combined high-dose 5-day primary physical dependence (PPD) and precipitated-withdrawal study (PPt-W)

Four groups of 6-8 rats per group were prepared for infusion. Then, one group received vehicle (8 ml/24hr) for 5 days and then challenged with a single injection of vehicle (i.p.) Another group received vehicle by infusion as above and then challenged with a single injection of the GHB antagonist NCS-382 (50 mg/kg i.p.). Two groups each received a dose regimen of 600, 1200, 2400, 4800 and 4800 mg/kg, i.p./24hr of GHB for 5 days, in that order. Next, one group of GHB-treated rats was given a single dose of NCS-382 (i.p.) and the other was given vehicle (i.p.). The same experimental design outlined above was observed, In addition, behavioral observations were recorded I hr before and 1 hr after challenge with vehicle or NCS-382.

Body Weight

Repeated measures ANOVA indicated significant differences among days (F = 6.869, p = .0001) as well as a significant interaction days and body weight (F = 3.916, p = .0001).

The body-weight results are displayed in Fig. 3 below. As can be seen in the figure, the body weights of all the body weight of the treatment groups remained essentially the same until day 5: no significant changes were calculated: at the start, of the experiment (F = .208, p = .8901) or on days 1, 2, and 3 (F = .101, p = .9588, F = .053, p = .9836, and F = .471, p = .7051, respectively. On day 4, statistical significance was achieved. ANOVA revealed that F =2.564, p = .0783.

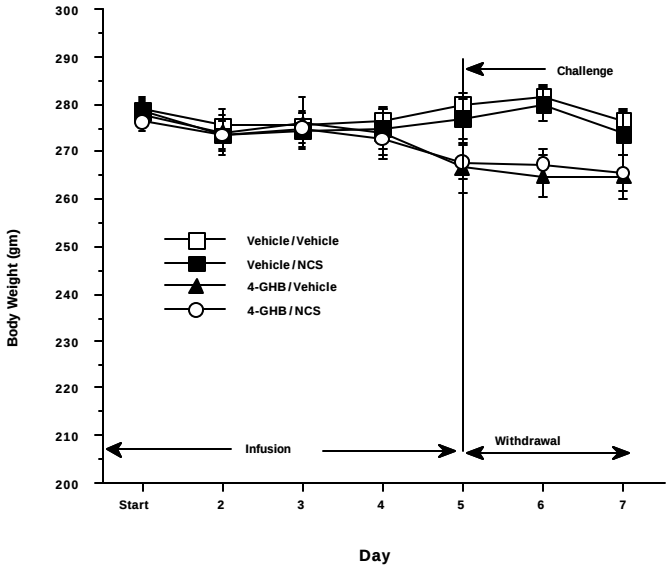


Fig. 3. Body weight changes before and after abrupt and precipitated withdrawal of GHB.

Apropos abrupt withdrawal, there is no evidence that this occurred since weight loss commenced before GHB was withdrawn. F was not significant ($F = 1.973$, $p = .449$). Concerning precipitated withdrawal, a statistically significant weight loss was evident on day 5 ($F = 5.432$, $p = .0054$). However, this weight loss was probably due to GHB since it occurred before the NCS-382 challenge.

2.0 Irritability

There was no evidence that the sign irritability was expressed either after abrupt withdrawal of GHB or after challenge with NCS-3382. All the scores were either 0 or 1.

3.0 Wet-Dog Shakes

Repeated measures analysis of the abrupt withdrawal and precipitated withdrawal data indicated no significant differences regarding the intervals 1 hr pre and post challenge, and 24 hr post challenge with regard to the sign wet-dog shakes (A) ($F = 2.314$, $p = .1014$) intervals (B) ($F = 2,41$, $p = .1006$), or their interaction (AB) ($F = 1.393$, $p = .2368$). Also, one-way ANOVA for intervals revealed no significant differences among treatments.

Summary and Conclusions

GHB was given continuously to rats using two dose regimens of 50, 100, 200 and 200 mg/kg /24 hrs for 4 days respectively and 600, 1200, 2400, 4800 and 4800 mg/kg/24 hr for 5 days, respectively.

NIH 10947 (Continued)

At the lower dose regimen, there was no effect on body weight either during its administration or after its abrupt withdrawal. Typical withdrawal signs designated irritability and wet-dog shakes were not observed. In addition, at doses of 200 mg/ kg/24 hr for 2 days GHB did not substitute for morphine in withdrawn dependent rats.

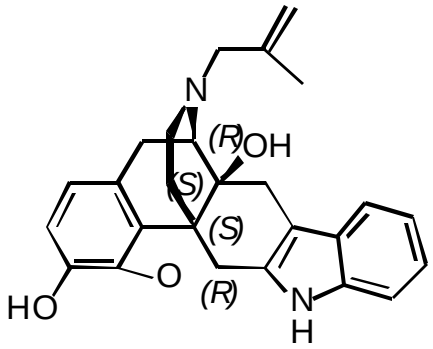
At the much higher dose regimen, GHB did not express signs of physical dependence either when it was abruptly discontinued or after challenge with a purported antagonist (NCS-382). A non statistically significant trend in body-weight loss began during its administration. After abrupt withdrawal or after challenge with NCS-382 the decline plateaued. This was the only overt sign of drug effect. The withdrawal signs irritability and wet-dog shakes were not noted.

We conclude that the physical dependence liability of GHB is very low.

General Comment

GHB interacts with the opioid system producing antinociceptive synergy with morphine and reversal of tolerance to morphine in mice. Depending on the dose, it attenuates withdrawal signs in morphine-dependent monkeys, Finally, it has very low toxicity and physical dependence capacity in rats. GHB may be useful in man in the pharmacotherapy of pain and dependence.

NIH 10978 *N*-(3-Methylallyl)noroxymorphindole



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 1% at 1, 0% at 10 and 3% at 30[a]
2) TF vs. M – 3% at 1, 31% at 10, 51% at 30 and 46% at 60[a]
3) PPQ – Inactive at 1, 7% at 10 and 30% at 30[a]
4) HP – Inactive at 1, 10 and 30[a]

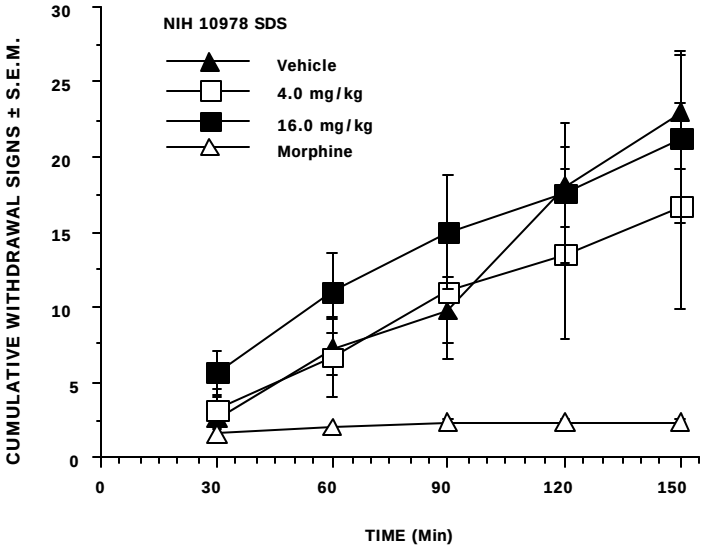
[a]Vehicle was 30% hydroxypropyl-β-cyclodextrin in water.

MONKEY DATA
(SDS)

The accompanying illustration indicates lack of significant effects by NIH 10978 in withdrawn morphine-dependent monkeys. It neither substitutes for morphine nor exacerbates withdrawal. Vehicle was 30% hydroxypropyl-β-cyclodextrin in water. Perhaps the drug was not sufficiently absorbed or had a delayed onset of action.

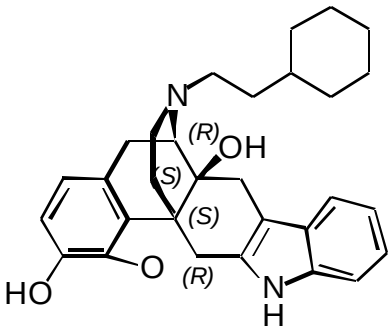
NIH 10978

Fig NIH 10978-SDS. Results of study in which single doses of NIH 10978 were substituted for morphine in morphine-dependent monkeys in withdrawal.



Comment: Studies in the mouse suggest weak mu-opioid receptor antagonist properties. Some non dose-related antinociceptive activity was observed in the PPQ test. Data from the substitution for morphine studies in monkeys suggest that NIH 10978 neither substitutes for morphine nor exacerbates withdrawal.

NIH 10979 *N*-Cyclohexylethylnoroxymorphindole.HCl



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 3.86 (2.66 - 5.59)[a,b]
2) TF vs. M – Inactive at 1, 10 and 30
3) PPQ – 0.39 (0.17 - 0.86)
4) HP – 2.42 (1.57 - 3.73)

[a]Straub tails and increased locomotor activity at 6.
[b]Increased locomotor activity at 4.

Special Test: Naloxone vs ED80 of NIH 10979 in TF test: AD50 = 0.1 (0.07 - 0.6)

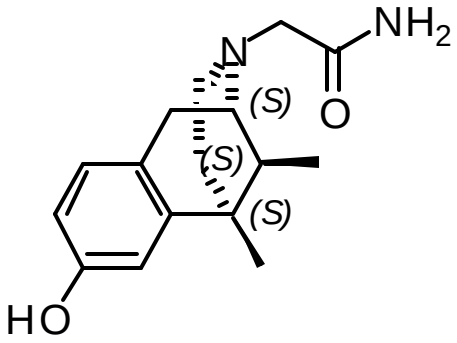
NIH 10979

MONKEY DATA
(SDS)

Not Tested.

Comment: The antinociceptive and behavioral profiles suggest opioid involvement. The high AD50 value with naloxone implicates multiple opioid-receptor agonist mechanisms.

NIH 10992 (+)-(1*S*,5*S*,9*S*)-2-Acetamido-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – Inactive at 1, 10 and 30[a]
2) TF vs. M – 0% at 1, 5% at 10 and 9% at 30[a]
3) PPQ – 4.05 (1.82 - 9.05)[a]
4) HP – Inactive at 1, 10 and 30.

[a]Vehicle was 5% hydroxypropyl-β-cyclodextrin in water.

MONKEY DATA
(SDS)

The data are not indicative of activity at 4 and 16 mg/kg. Thus, NIH 10992 (see figure) neither substituted for morphine nor exacerbated withdrawal. Vehicle was dilute HCl in water.

NIH 10992

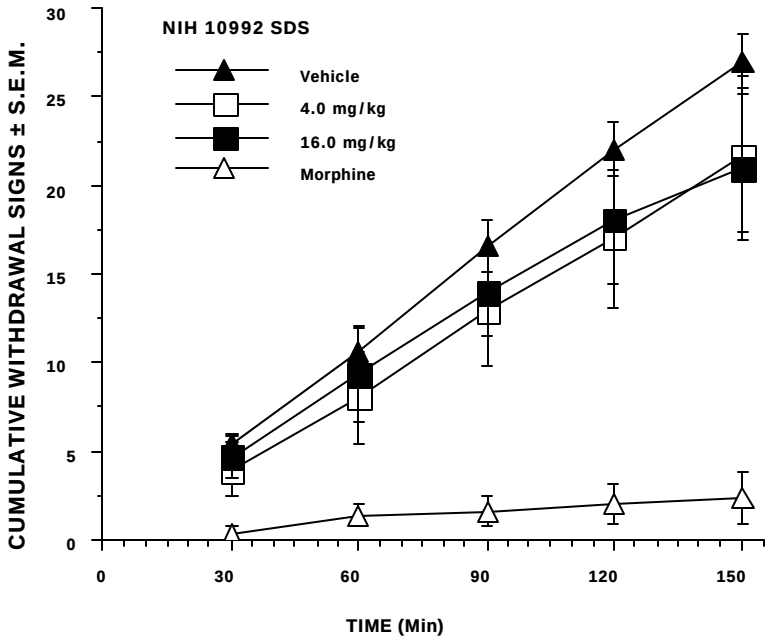
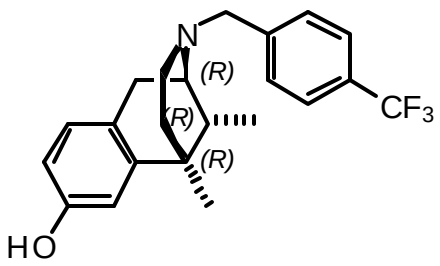


Fig NIH 10992-SDS. Results of study in which single doses of NIH 10992 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The results in mice and monkeys are not suggestive of remarkable opioid properties.

NIH 10994 (-)-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2'-hydroxy-2-(4-trifluoromethylbenzyl)-6,7-benzomorphan .oxalate



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 3% at 1, 0% at 10 and 8% at 30[a,b]
2) TF vs. M – Inactive at 1, 10 and 30[a,b]
3) PPQ – 27.40 (11.30 - 66.41)[a,b]
4) HP – Inactive at 1 and 10, 13% at 30[a,b]

[a]Vehicle was 5% hydroxypropyl-β-cyclodextrin in water.

MONKEY DATA
(SDS)

At doses of 4 and 16 mg/kg, NIH 10994 neither substituted for morphine nor exacerbated withdrawal. At the high dose, jaw sag was observed in one monkey. Drug supply was exhausted. Vehicle was 10% hydroxypropyl-β-cyclodextrin in water.

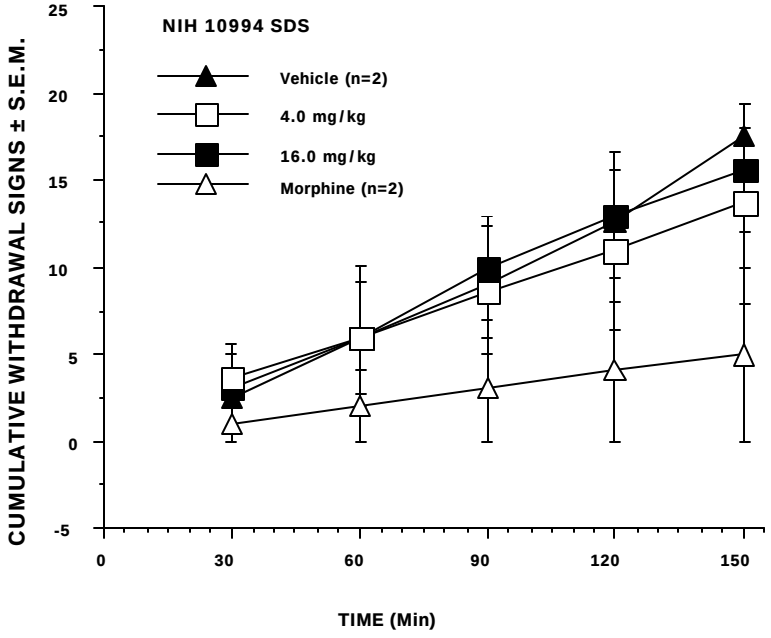
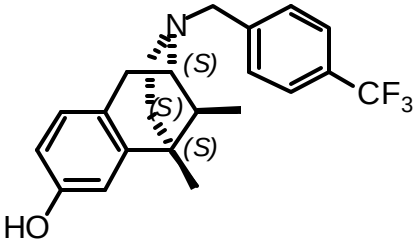


Fig NIH 10994-SDS. Results of study in which single doses of NIH 10994 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The results do not portend mu-opioid agonist or antagonist activity. Very weak antinociceptive action was noted in the PPQ test in mice.

NIH 10995 (+)-(1*S*,5*S*,9*S*)-5,9-Dimethyl-2'-hydroxy-2-(4-trifluoromethylbenzyl)-6,7-benzomorphan .oxalate



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 3% at 1, 1% at 10 and 7% at 30[a,b]
2) TF vs. M – Inactive at 1, 10 and 30[a,b]
3) PPQ – 32.81 (16.44 - 65.45)[a,b]
4) HP – Inactive at 1 and 10, 25% at 30[a,b]

[a]Vehicle was 5% hydroxypropyl-β-cyclodextrin in water.

MONKEY DATA
(SDS)

The results shown in the figure suggest an inverse dose response, i.e., the low dose attenuated withdrawal behavior more than the high dose. Most of the attenuation of withdrawal signs were associated with the reduction in the number of signs designated vocalization when the abdomen was palpated and decreased abdominal rigidity.

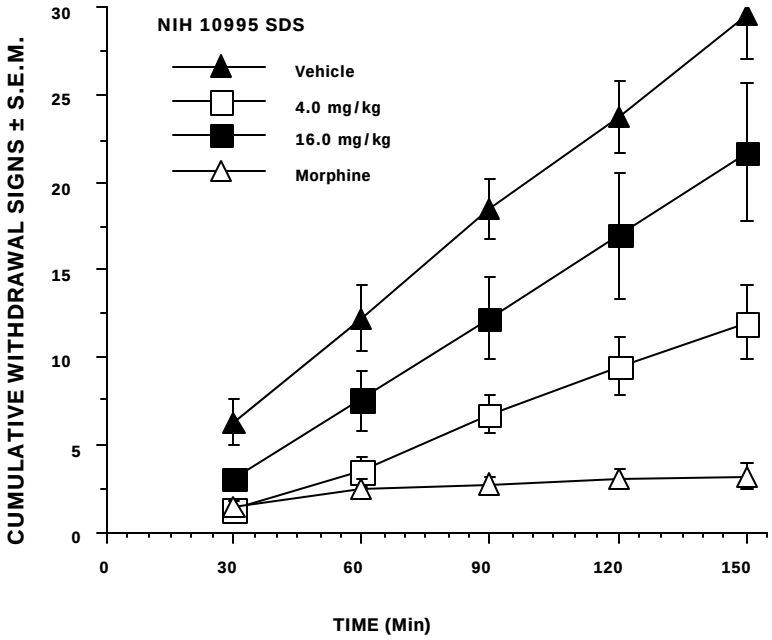
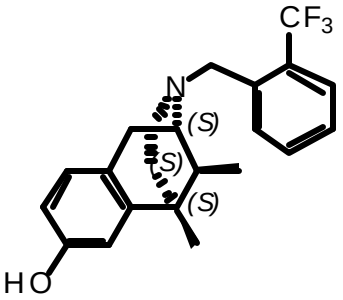


Fig NIH 10995-SDS. Results of study in which single doses of NIH 10995 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: Evidence for mu-opioid agonist activity is unimpressive.

NIH 11003 (+)-(1*S*,5*S*,9*S*)-5,9-Dimethyl-2'-hydroxy-2-(2-trifluoromethylbenzyl)-6,7-benzomorphan.HCl



MOUSE DATA - ED50 OR AD50
% C.L.) (mg/kg or % change)

1) TF – 4% at 1, 0% at 10 and 30[a]
2) TF vs. M – Inactive at 1, 10 and 30[a]
3) PPQ – Inactive at 1, 10 and 30[a]
4) HP – 0% at 1 and 10, 13% at 30[a]

[a] Vehicle was 5% hydroxypropyl-β-cyclodextrin in water.

MONKEY DATA
(SDS)

NIH 11003, at 4 and 16 mg/kg, was inactive in morphine-dependent monkeys in withdrawal. Vehicle was 10% hydroxypropyl-β-cyclodextrin in water

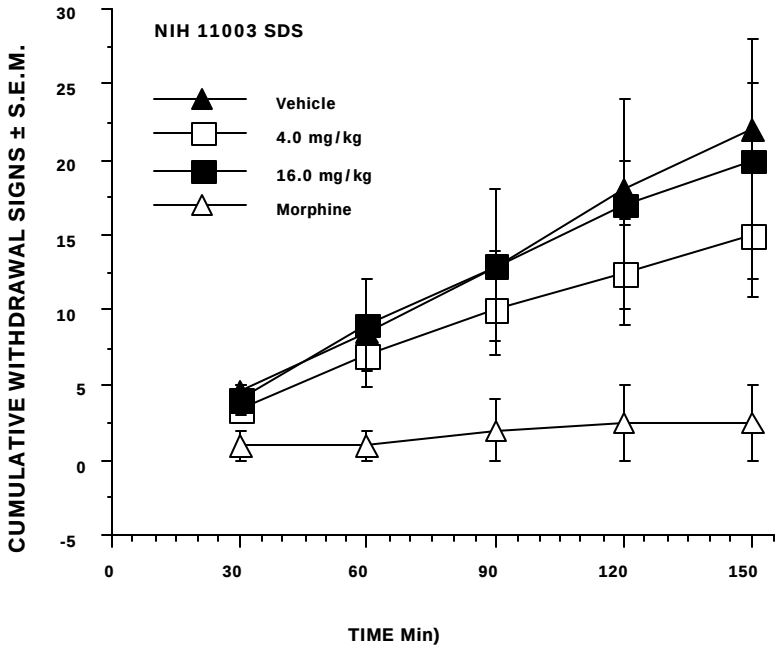
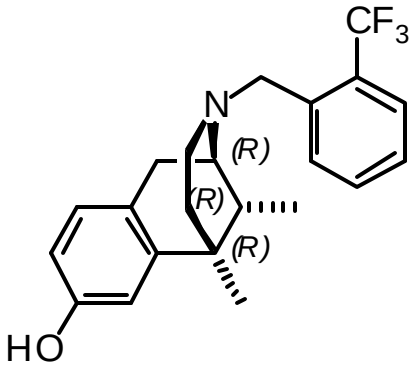


Fig NIH 11003-SDS. Results of study in which single doses of NIH 11003 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: NIH 11003 displays little or no activity in this battery of tests. It is probably devoid of mu-opioid activity.

NIH 11004 (-)-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2'-hydroxy-2-(2-trifluoromethylbenzyl)-6,7-benzomorphan.HCl



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – Inactive at 1, 10 and 30
2) TF vs. M – Inactive at 1, 10 and 30
3) PPQ – 0% at 1, 8% at 10 and 9% at 30
4) HP – Inactive at 1, 10 and 30

Vehicle was 5% hydroxypropyl-β-cyclodextrin in water.

MONKEY DATA
(SDS)

As illustrated in the accompanying figure, NIH 11004 did not substitute for morphine, attenuate or exacerbate withdrawal behavior. Vehicle was 10% hydroxypropyl-β-cyclodextrin in water.

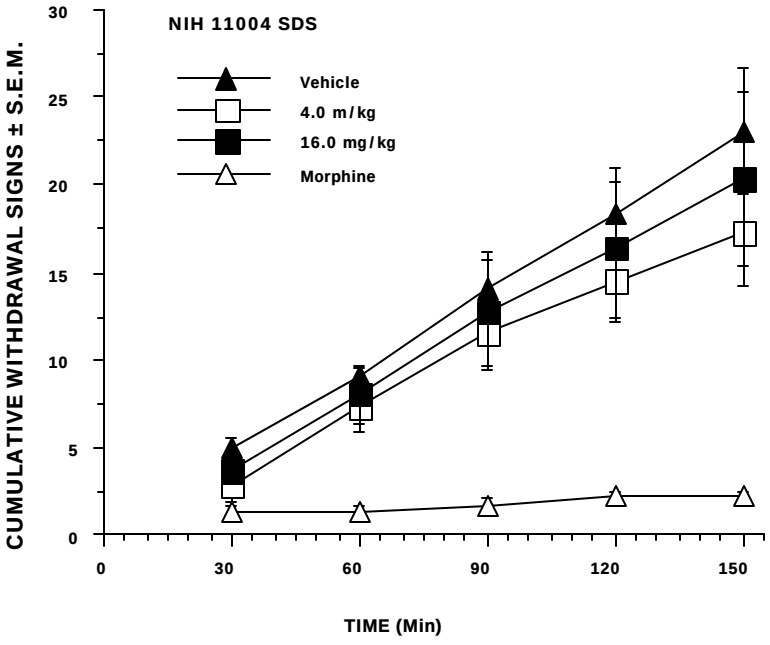
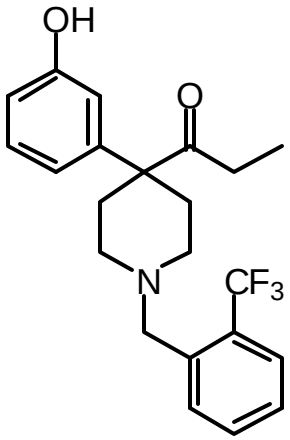


Fig NIH 11004-SDS. Results of study in which single doses of NIH 11004 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: Evidently NIH 11004 is devoid of opioid properties in these bioassays.

NIH 11005 4-(3-hydroxyphenyl)-4-(1-oxo-propyl)-1-(2-trifluoromethylbenzyl)piperidine.HCl



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – Inactive at 1, 10 and 30
2) TF vs. M – Inactive at 1, 10 and 30
3) PPQ – 14% at 10, 49% at 30 and 51% at 60
4) HP – 0% at 1 and 10, 13% at 30

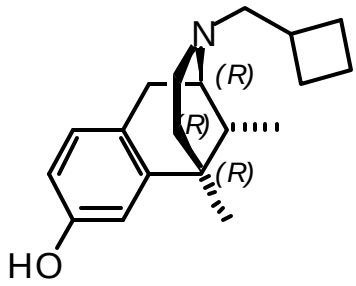
Vehicle: 5% hydroxypropyl-β-cyclodextrin

MONKEY DATA
(SDS)

Due to limited supplies, only the preliminary study was conducted. Does of 1, 2, 4 and 8 mg/kg were given at 15 min intervals, respectively. The cumulativive dose of 15 mg/kg was without effect. Vehicle was 10% hydroxypropyl-β-cyclodextrin in water.

Comment: NIH 11005 has weak, if any, antinociceptive properties in mice. The data do not indicate significant mu-opioid receptor activity.

NIH 11006 (-)-(1*R*,5*R*,9*R*)-2-Cyclobutylmethyl-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.HCl



MOUSE DATA - ED50 OR AD50
% C.L.) (mg/kg or % change)

1) TF – 0.26 (0.07 - 0.88)
2) TF vs. M – Inactive at 1, 10 and 30
3) PPQ – 0.04 (0.02 - 0.08)
4) HP – 0.12 (0.044 - 0.31)

Special Test: Naloxone AD50 vs ED80 of NIH 11006 in TF = 0.84 (0.35 - 2.0) mg/kg.

NIH 11006 (Continued)

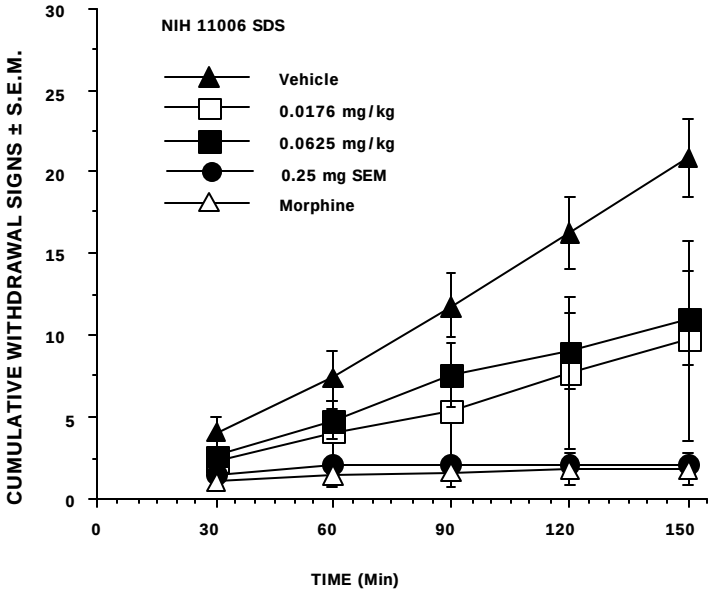
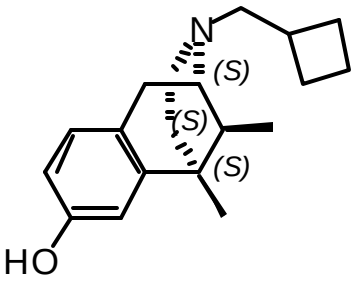


Fig NIH 11006 SDS. Results of study in which single doses of NIH 11006 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The relatively high naloxone AD50 in the mouse tail-flick test and the results in the monkeys suggest heterogeneous opioid activity with a strong kappa-opioid subtype component.

NIH 11007 (+)-(1S,5S,9S)-2-Cyclobutylmethyl-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.HCl



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – Inactive at 1, 10 and 30
2) TF vs. M – 0% at 1, 12% at 10 and 19% at 30
3) PPQ – 14% at 1, 3% at 10 and 14% at 30
4) HP – Inactive at 1, 10 and 30

NIH 11007 (Continued)

MONKEY DATA
(SDS)

These results should be considered as preliminary since the drug supply was exhausted before the experiment was completed. Attenuation of withdrawal was accompanied by ataxia and slowing at the high dose. Other behavioral signs observed at the 16 mg/kg included walking in circles, spinning while sitting and staggering during first 1/2 hour.

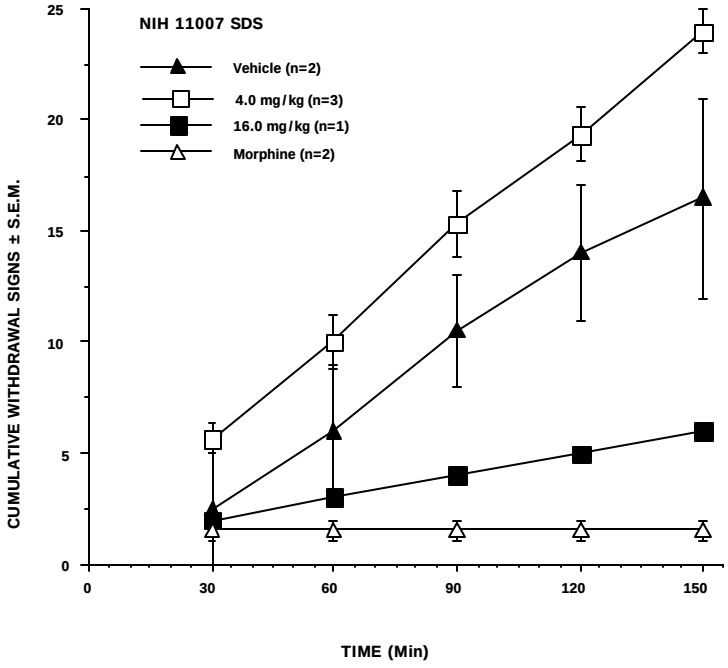
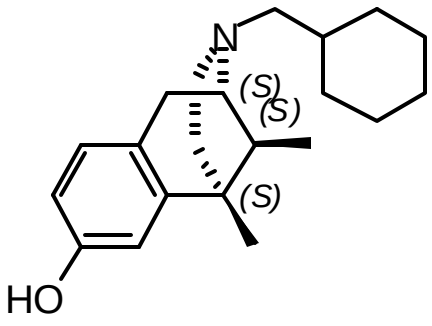


Fig NIH 11007-SDS. Results of study in which single doses of NIH 11007 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: NIH 11007 appears to be devoid of mu-opioid properties. However, CNS effects are prominent.

NIH 11011 (+)-(1*S*,5*S*,9*S*)-2-Cyclohexylmethyl-5,9-dimethyl-2'-hydroxy-6,7benzomorphan. HCl



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – Inactive at 1, 10 and 30
2) TF vs. M – 20% at 1, 1% at 10 and 7% at 30
3) PPQ – 17.57 (5.91 – 52.26)
4) HP – 0% at 1 and 10, 13% at 30

NIH 11011 (Continued)

MONKEY DATA
(SDS)

Drug supply was exhausted. A complete evaluation of NIH 11011 was precluded. Attenuation of withdrawal signs at 16 mg/kg was accompanied by jaw sag and ataxia. These behavioral signs and salivation were also noted at the lower dose.

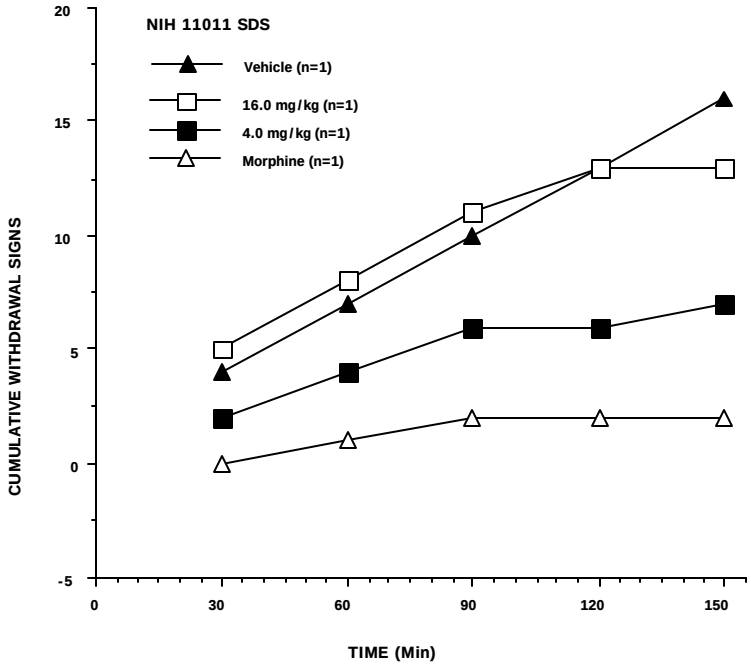
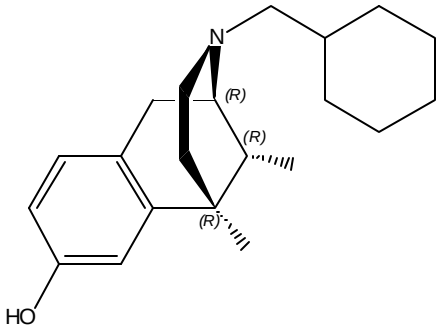


Fig NIH 11011-SDS. Results of study in which single doses of NIH 11011 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The profile of activity does not suggest mu opioid-receptor properties.

NIH 11012 (-)-(1*R*,5*R*,9*R*)-2-Cyclohexylmethyl-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan. HCl



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 3% at 1 and 10, 0% at 30[a]
2) TF vs. M – 3.70 (1.02 – 13.45)[a]
3) PPQ – 14.62 (3.12 – 68.3)[a,b]
4) HP – 0% at 1 and 10, 13% at 30[a]

[a]Vehicle was 4% Tween 80 in water.
[b]One mouse at 60 mg/kg was very lethargic and one mouse had convulsions that lasted to end of experiment.
[c]Vehicle was 5% hydroxypropyl-β-cyclodextrin in water.

Special Test:

5) Naltrindole (s.c.), (30 min pretreatment) vs NIH 11012 ED80 in the PPQ test: 15% at 1, and 13% at 10 and 30[c] mg/kg.

MONKEY DATA
(SDS)

At doses of 0.75 and 3.0 mg/kg, NIH 11012 did not substitute for morphine or exacerbate withdrawal. Jaw sag was noted at 3 mg/kg. One monkey who received 12 mg/kg had tremors followed by convulsions. Pentobarbital (30 mg/kg, i.p.) effectively terminated the convulsions.

NIH 11012 (Continued)

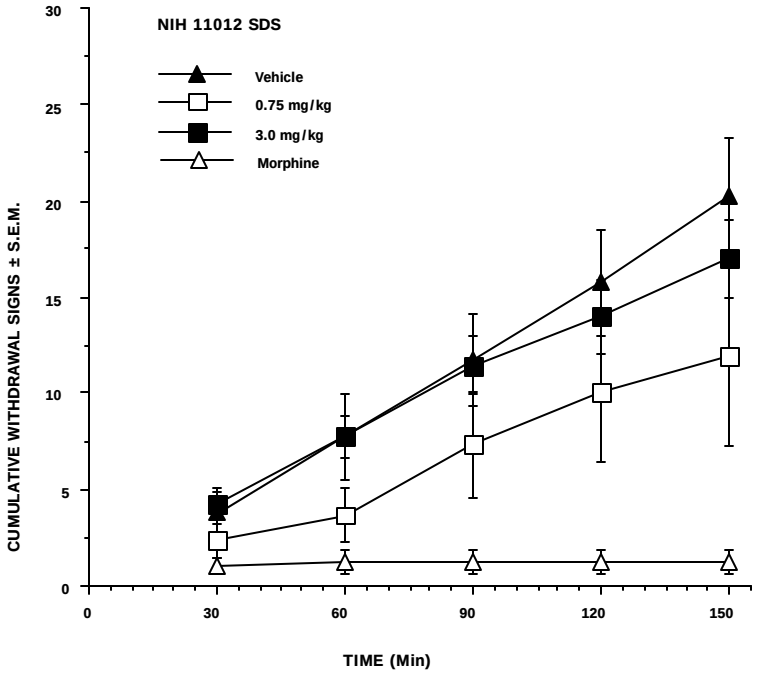
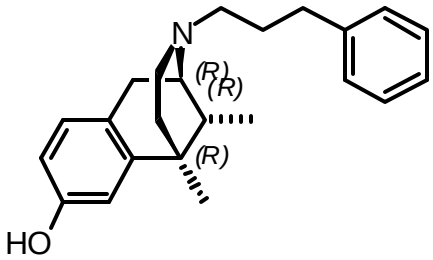


Fig NIH 11012-SDS. Results of study in which single doses of NIH 11012 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The results suggest that NIH 11012 has weak mu-opioid antagonist properties. Some antinociception was noted in the PPQ test which was not delta-opioid receptor related. The drug also produced convulsions in both species.

NIH 11013 (-)-(1*R*,5*R*,9*R*)-2-(3-Phenylpropyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan. HCl



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 8.86 (3.64 - 21.60)
2) TF vs. M – 14% at 1, and 0% at 10 and 30
3) PPQ – 4.59 (2.9 - 7.2)
4) HP – 9.63 (2.92 - 31.81)

Vehicle: 2 drops lactic acid in water.

NIH 11013 (Continued)

MONKEY DATA
(SDS)

As shown in the figure, NIH 11013 partly substituted for morphine at the high dose. At this dose the signs designated ataxia, jaw sag, slowing and eyelid ptosis were noted. Onset of action was prompt; however less than morphine's. In the preliminary study in one monkey, an accumulative dose of 7 mg/kg was associated with labored respiration.

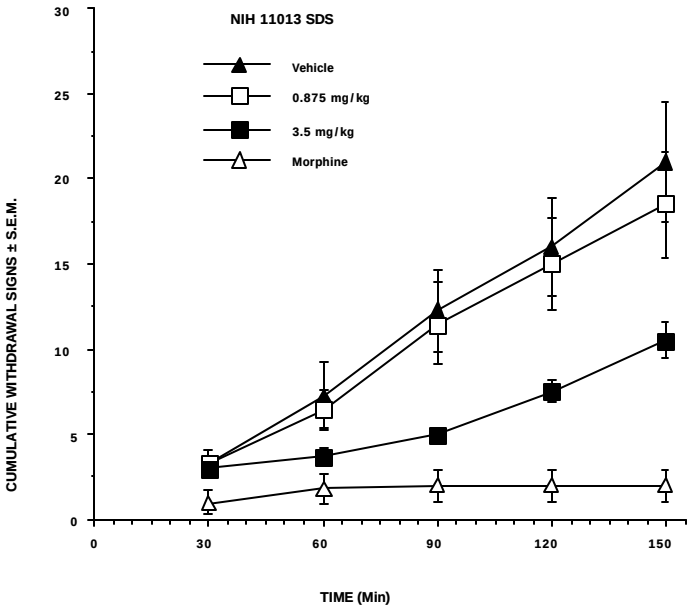
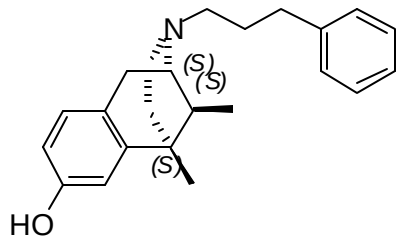


Fig NIH 11013-SDS. Results of study in which single doses of NIH 11013 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The profile of activity suggests heterogenous opioid and/or central nervous system effects.

NIH 11014 (+)-(1*S*,5*S*,9*S*)-2-(3-Phenylpropyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan. HCl



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 19.14 (11.57 – 31.66)[a]
2) TF vs. M – Inactive at 1, 10 and 30
3) PPQ – 1.42 (0.39 – 5.18)[b]
4) HP – 22.06 (16.81 – 28.94)[c]

[a] At 30 eyelid ptosis and immobility. Mice didn't move when touched.

[b] At 30 decreased locomotor activity (mice nearly immobile). Eyelid ptosis was also noted.

[c] At 30 decreased locomotor activity. Eyelid ptosis was noted at 15, 20 and 30.

MONKEY DATA
(SDS)

NIH 11014 produced a very weak non dose-related attenuation of withdrawal signs (see figure). At the high dose, jaw sag, salivation and eyelid ptosis were noted in one monkey. Drug supply was exhausted (n=2).

NIH 11014 (Continued)

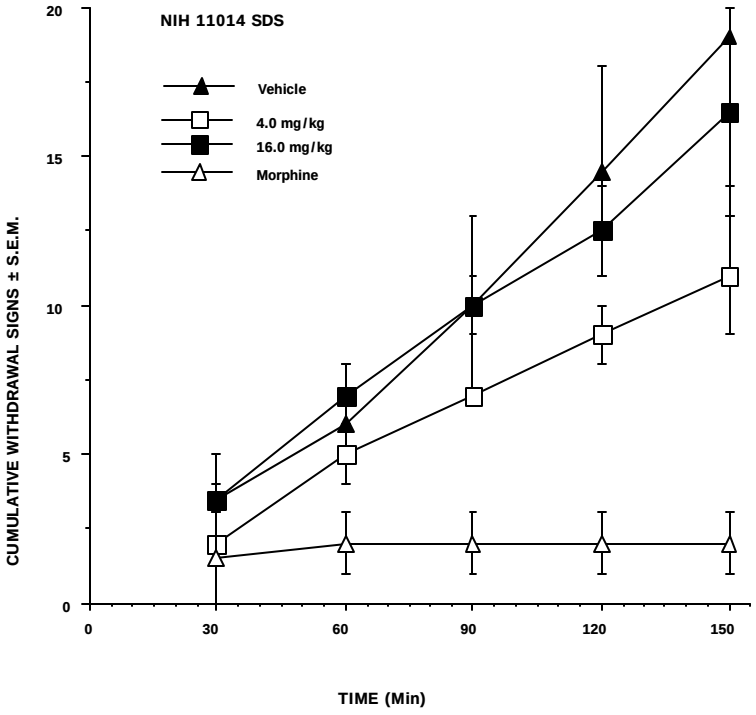
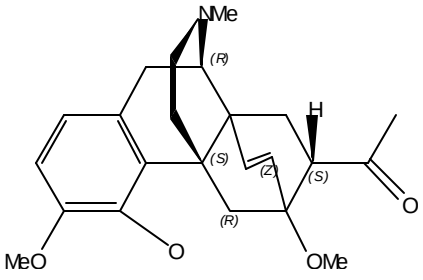


Fig NIH 11014 SDS. Results of study in which single doses of NIH 11014 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: Additional supplies might help clarify the profile of this drug. It appears to be a weak opioid compound and scant evidence suggests kappa-opioid activity and/or other CNS effects.

NIH 11015 (10631) Thevinone.oxalate



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 5.65 (2.89 – 11.08)
2) TF vs. M – 2% at 1, 13% at 10 and 0% at 30
3) PPQ – 2.36 (1.02 – 5.45)
4) HP – 4.49 (1.61 – 12.54)

NIH 11015 (Continued)

Special Tests: Naloxone (s.c.) vs ED80 of NIH11015 (s.c.) in TF: 0.02 (0.008 – 0.52)

Opioid subtype testing

a) β-FNA (i.c.v.) vs ED80 of NIH 11015 (s.c.) in TF: 0% at 0.1, 28% at 0.03, 26% at 0.1, 62% at 0.3, 72% at 1, 37% at 3, 65% at 10 and 69% at 30.
b) Nor-BNI (s.c.) vs ED80 of NIH 11015 (s.c.) in TF: 7% at 1, 13% at 3, 47% at 10, 56% at 30 and 23% at 60.
c) Naltrindole (s.c.) vs ED80 of NIH 11015 (s.c.) in TF: 6% at 3, 43% at 10, 47% at 10 and 36% at 30.

MONKEY DATA

(SDS)

Doses of 2 and 8 mg/kg completely substituted for morphine in morphine-dependent monkeys in spontaneous withdrawal. At the high dose, the signs scratching, ataxia, jaw and body sag and eyelid ptosis were observed. The drug acts promptly and duration of action was shorter than that of morphine. NIH 11015 appears to be as potent as morphine.

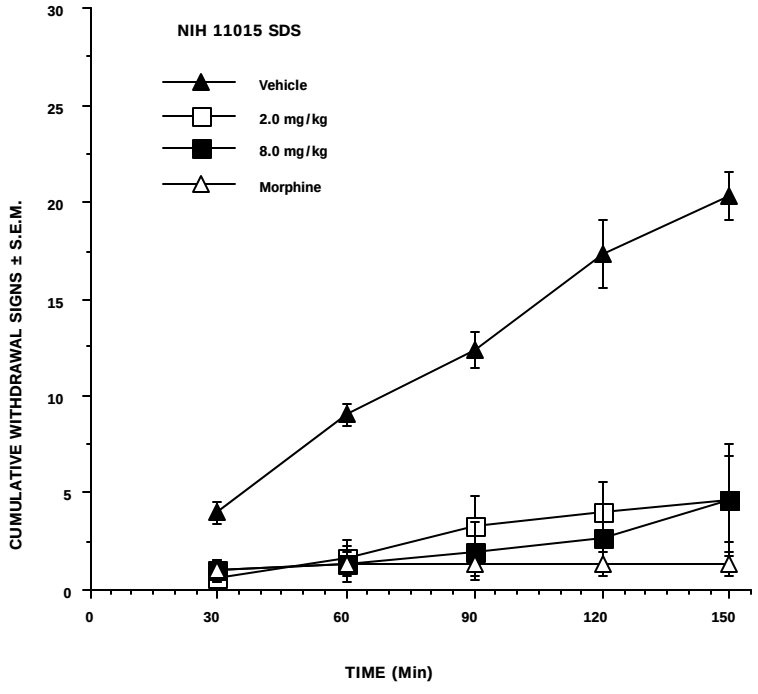
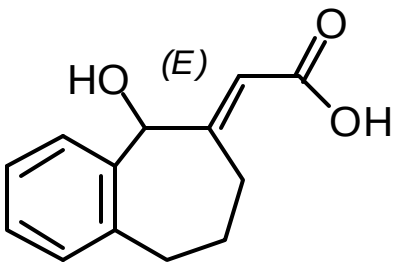


Fig NIH 11015-SDS. Results of study in which single doses of NIH 11015 were substituted for morphine in morphine-dependent monkeys in withdrawal.

NIH 11015 (Continued)

Comment: This compound has many characteristics in common with morphine. The naloxone AD50 suggested selective mu opioid-receptor properties; however, subtype testing indicated mu and very weak kappa- and delta-receptor interactions.

NIH 11016 (NCS-382, GHB antagonist)



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 0% at 1, 9.2% at 10 and 5.5% at 30
2) TF vs. M – 20% at 1, 5% at 10 and 0% at 30
3) PPQ – 2% at 1, 0% at 10 and 20 and 38% at 30
4) HP – 0% at 1 and 10, 12.5% at 30

MONKEY DATA
(SDS)

NIH 11016 produced a feeble exacerbation of withdrawal signs at the 4,16 and 32 mg doses (see Fig NIH 11016-SDS). Some jaw sag was noted at the high dose.

MONKEY DATA
(PPt-W)

As shown in the Fig (NIH 11016 PPt-W), it is evident that NIH 11016, at 32 mg/kg s.c., did not precipitate withdrawal in morphine-dependent monkeys. Because drug supply was exhausted, n = 2.

NIH 11016 (Continued)

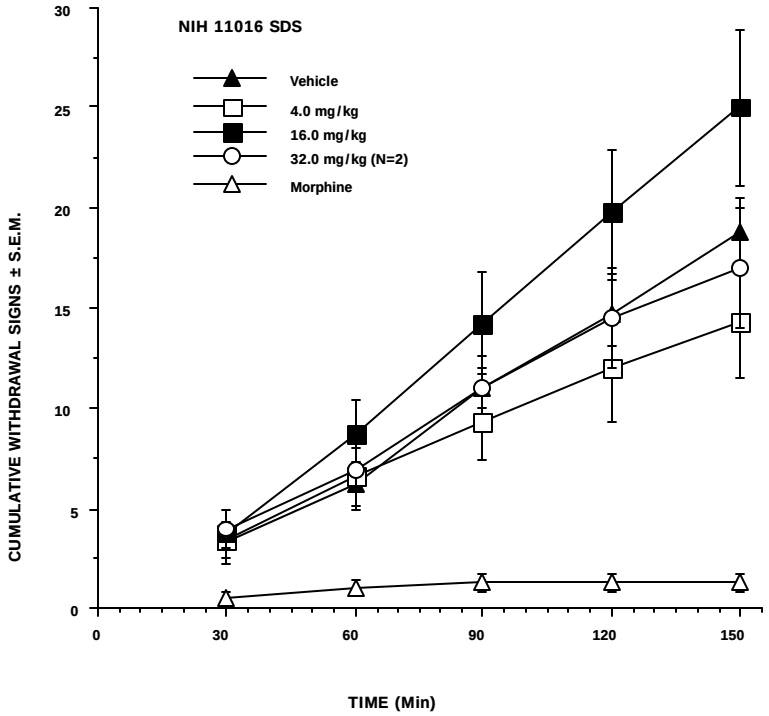


Fig NIH 11016-SDS. Results of study in which single doses of NIH 11016 were substituted for morphine in morphine-dependent monkeys in withdrawal.

NIH 11016 (Continued)

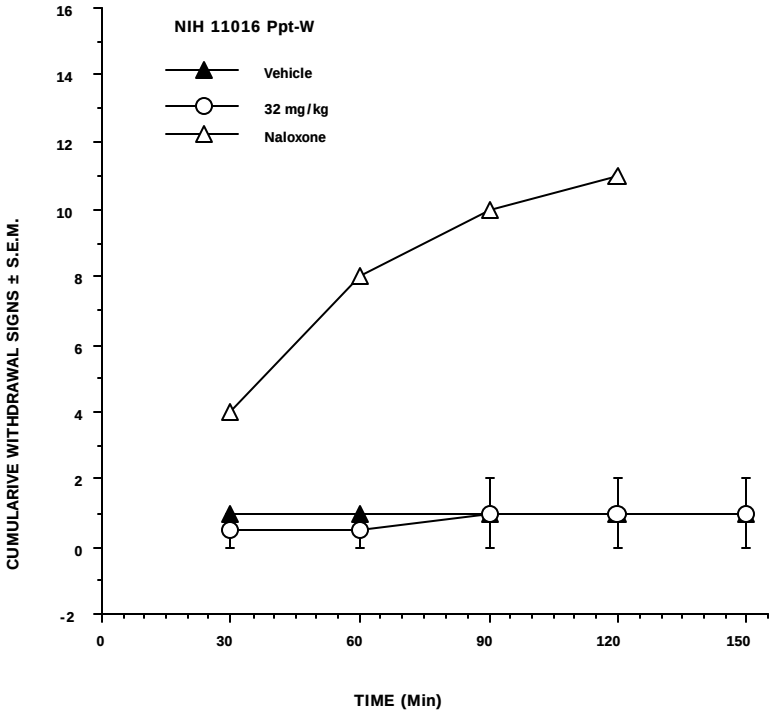
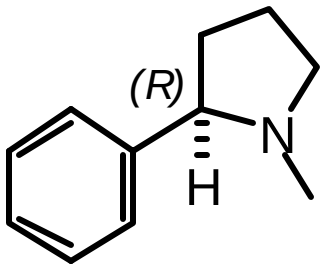


Fig NIH 11016-PPt-W. Results of study in which NIH 11016 was administered to morphine-dependent monkeys 2 hr after morphine.

Comment: Apparently, NIH 11016, a GHB antagonist, has little effect antinociceptively and neither substitutes for morphine nor acts like an opioid antagonist. Because drug supply was exhausted, no further work was done.

NIH 11017 (9801) (*R*)-(+)-Nicotine di-*d*-tartrate



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 0% at 1, 28% at 10 and 0% at 30
2) TF vs. M – 0% at 1, 13% at 10 and 0% at 30
3) PPQ – 10.23 (4.53 – 23.09)
4) HP – 25% at 1, 10 and 30

Special Test:

Table. Naltrindole vs ED80 of NIH 11017 in PPQ test.

Pretreatment Time		**Route**	% Antagonism
Naltrindole	NIH 11017		
30 min	20 min	s.c.	19% at 1, 36% at 15 and 20, 93% at 25 and 81% at 30
30 min[a]	20 min	s.c.	11% at 1, 28% and 9% at 10 and 11% at 30[b]

[a]Repeated.
[b]On each test the mice showed tension in their front paws with toes arched. Hind feet were slightly splayed.

MONKEY DATA
(SDS)

At low doses of 1.5 mg/kg, s.c., NIH 11017 reduced the number of withdrawal signs (see figure); the reduction was attributable to the relaxed abdominal muscles and failure to vocalize when the abdomen was palpated. However, at the high dose, 6.0 mg/kg, s.c., withdrawal seemed intensified due primarily to increased incidence of restlessness, tremors and retching. At this dose other signs were noted including salivation, jaw sag and eyelid ptosis. The drug has a dual action which may reflect multiple properties.

NIH 11017 (Continued)

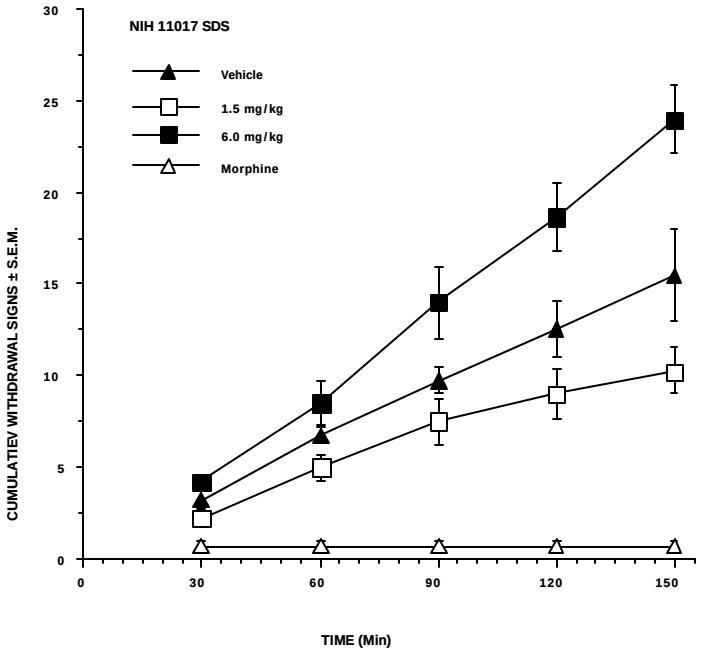
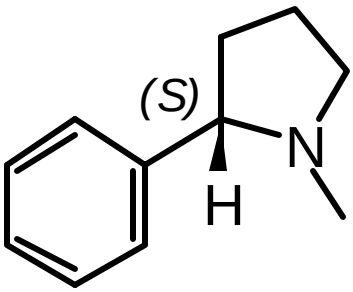


Fig NIH 11017 SDS. Results of study in which single doses of NIH 11017 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: Although NIH 11017 showed delta-opioid receptor agonist properties in mice, the dose response with naltrindole was erratic and precluded the determination of a meaningful AD50. The side effects probably played a role. In withdrawn monkeys, a rather complex dose-dependent profile of CNS peripheral effects was observed.

NIH 11018 (9733) (*S*)-(-)-Nicotine di-*l*-tartrate



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)
1) TF – 8.91 (3.35 – 23.67)[a]
2) TF vs. M – Inactive at 1, 10 and 30[b]
3) PPQ – 1.42 (0.44 – 4.61)[c]
4) HP – 16.92 (7.05 – 40.61)[d]

[a]All mice were sedated at 10, two convulsed at 30, all were jittery.
[b]All mice convulsed and two died at 30, two convulsed at 10.
[c]One mouse died and two mice convulsed at 10, mild sedation at 3.
[d]One mouse died and two mice convulsed at 30, One convulsed at 20, all were sedated at 10.

Special Test:

Table. Naltrindole vs NIH 11018 (25 mg/kg) in tail-flick test.

Pretreatment Time		**Route**	AD50 or % Antagonism
Naltrindole	NIH 11018		
30 min	20 min	s.c.	0% at 1, 2% at 10 and 0% at 30[a,b]

[a]One mouse died at 30.
[b]Naltrindole reduced the intensity of the convulsions and tremors induced by NIH 11018.

MONKEY DATA
(SDS)

As shown in the figure, at doses of 0.75 and 3.0 mg/kg, s.c., NIH 11018 appeared to exacerbate withdrawal signs, however, the apparent increase was due to an increased incidence of retching with both doses and at the high dose, a higher incidence of vocalization when the abdomen was palpated. In addition, the signs tremors, jaw sag and eyelid ptosis were noted.

NIH 11018 (Continued)

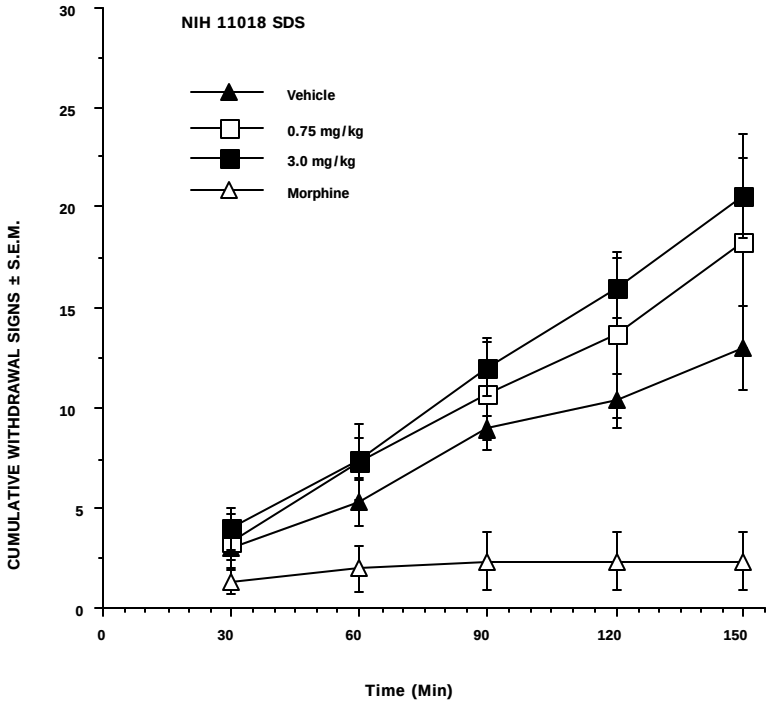
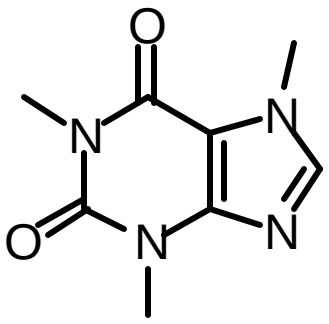


Fig NIH 11018-SDS. Results of study in which single doses of NIH 11018 were substituted for morphine in morphine-dependent monkeys in withdrawal

Comment: This compound has analgesic properties in mice that are not delta-opioid receptor related. The drug is a CNS stimulant in mice judging from the convulsions. In the monkey, NIH 11018 exacerbated withdrawal. However, the increase was associated with an increased incidence of retching and vocalization. The drug manifests many CNS stimulant and possibly peripheral effects.

NIH 11019 (10613) Caffeine tartrate



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 0% at 1 and 10, 8% at 30
2) TF vs M – 0% at 1, 2% at 10 and 0% at 30
3) PPQ – Inactive at 1, 10 and 30
4) HP – Inactive at 1, 10 and 30

NIH 11019 (Continued)

MONKEY DATA
(SDS)

As shown in the accompanying figure, NIH 11019 neither substituted for morphine nor exacerbated withdrawal at doses of 4 and 16 mg/kg.

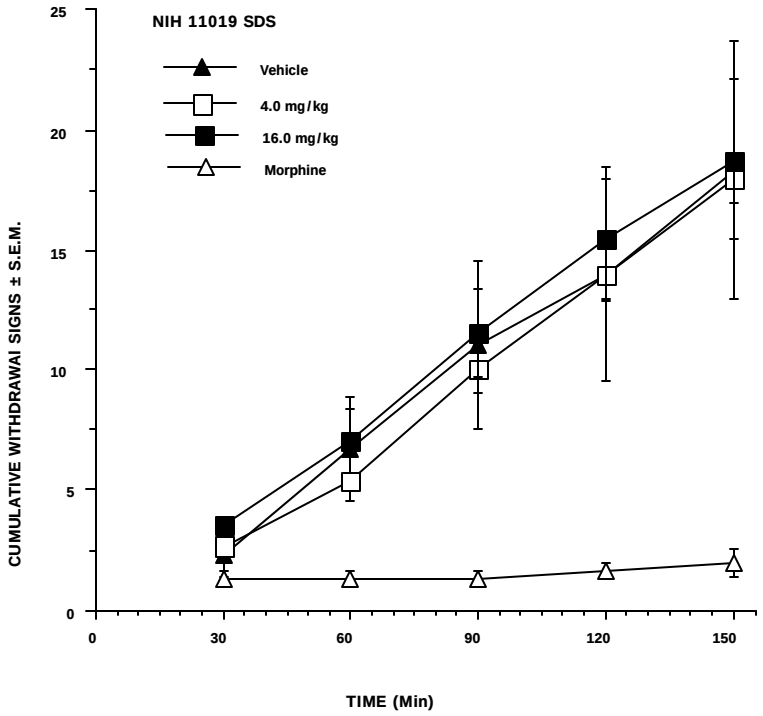
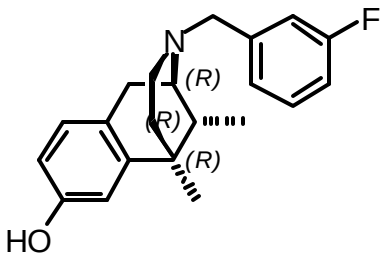


Fig NIH 11019-SDS. Results of study in which single doses of NIH 11019 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: NIH 11019 seems to be devoid of opioid properties.

NIH 11020 (-)-(1*R*,5*R*,9*R*)-5,9-dimethyl-2-(3-fluorobenzyl)-2'-hydroxy-6,7-benzomorphan.oxalate



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – Inactive at 1, 10 and 30
2) TF vs. M – 0% at 1, 12% at 10 and 3% at 30
3) PPQ – 26% at 1, 0% at 3, 49% at 10 and 27% at 30
4) HP – 13% at 1, 0% at 10 and 13% at 30

MONKEY DATA
(SDS)

The results illustrated in the accompanying figure indicate that at 4 and 16 mg/kg, NIH 11020 neither substituted for morphine nor exacerbated withdrawal.

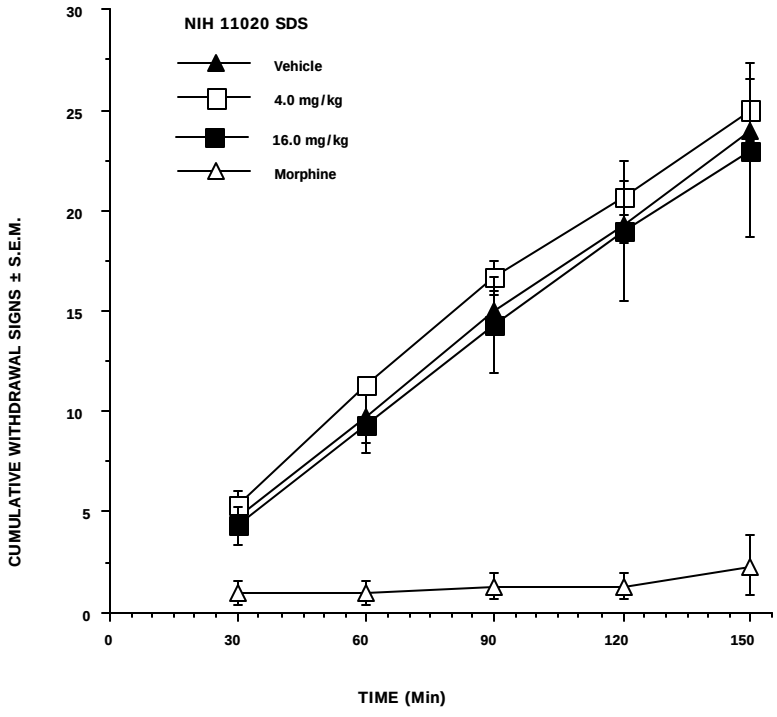
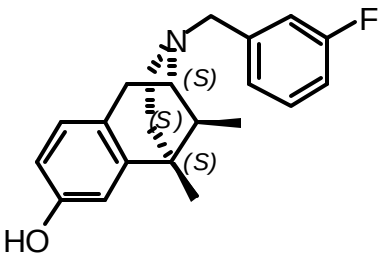


Fig NIH 11020 SDS. Results of study in which single doses of NIH 11020 were substituted for morphine in morphine-dependent monkeys in withdrawal

Comment: The results in mice and monkeys do not indicate opioid activity.

NIH 11021 (+)-(1*S*,5*S*,9*S*)-5,9-dimethyl-2-(3-fluorobenzyl)-2'-hydroxy-6,7-benzomorphan.oxalate



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 0% at 1 and 10, 2% at 30
2) TF vs. M – 8% at 1, 0% at 10 and 13% at 30
3) PPQ – 5% at 1, 23% at 10 and 26% at 30
4) HP – 0% at 1, 25% at 10 and 0% at 30

MONKEY DATA
(SDS)

At 4 and 16 mg/kg, NIH 11021 neither substituted for morphine nor exacerbated withdrawal. See accompanying illustrated data.

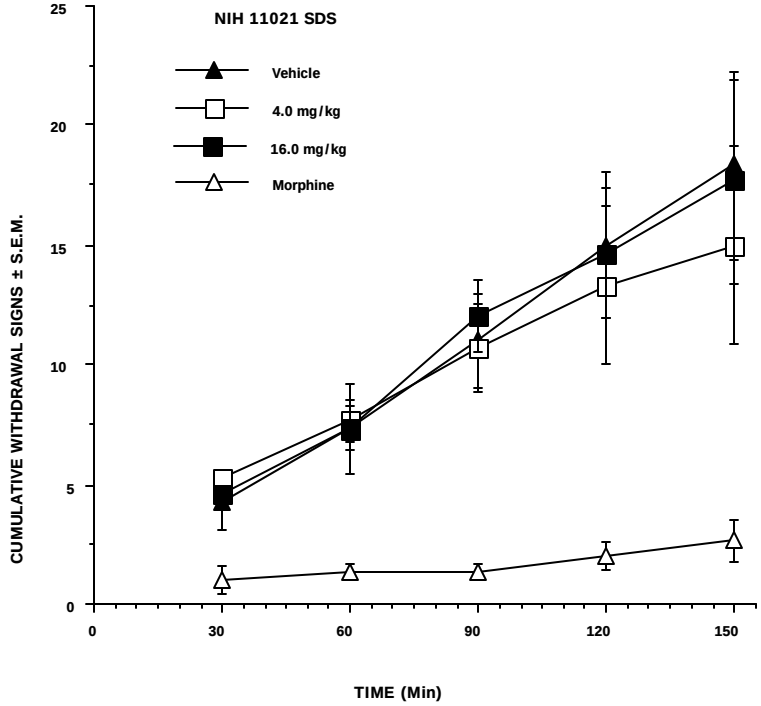
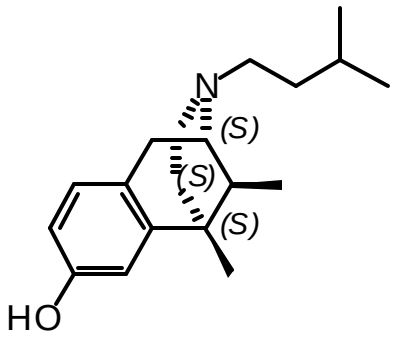


Fig NIH 11021 SDS. Results of study in which single doses of NIH 11021 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The results in mice and morphine-dependent rhesus monkey do not predict opioid properties for NIH 11021.

NIH 11022 (+)-(1*S*,5*S*,9*S*)-2-(3-Methylbutyl)- 5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.oxalate



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 1%at 1, 0% at 10 and 5% at 30[a]
2) TF vs. M – 0% at 1, 5% at 10 and 0% at 30[a]
3) PPQ – 19.36 (12.82 - 29.25[a]
4) HP – 13% at 1. 10 and 30[a]

[a]Vehicle was 5% hydroxypropyl-β-cyclodextrin in water.

MONKEY DATA
(SDS)

Because drug supply was exhausted, the data shown in the accompanying figure are those of one experiment. These limited data suggest that at 4 and 16 mg/kg, that NIH 11022 neither substituted for morphine nor exacerbated withdrawal.

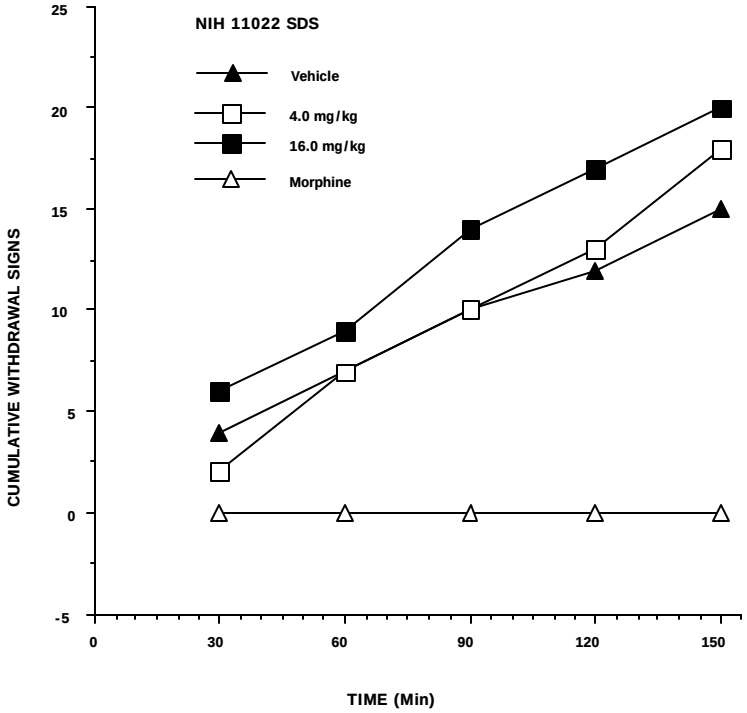
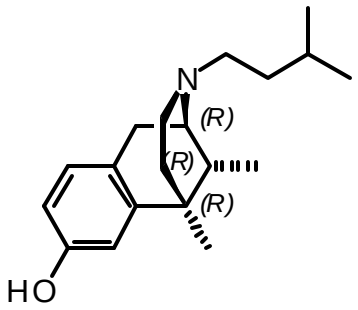


Fig NIH 11022 SDS. Results of study in which single doses of NIH 11022 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: NIH 11022 shows some weak activity in the PPQ test. The limited testing does not indicate a remarkable mu-opioid receptor interaction.

NIH 11023 (-)-(1*R*,5*R*,9*R*)-2-(3-Methylbutyl)- 5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.oxalate



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 2.5 (1.23 - 5.93)[a]
2) TF vs. M – 0% at 1 and 10, 12% at 30 [a,b]
3) PPQ – 0.39 (0.23 - 0.66)[a]
4) HP – 13% at 1, 0% at 10 and 13% at 30[a]

aVehicle was 5% hydroxypropyl-β-cyclodextrin.

MONKEY DATA
(SDS)

At doses of 2 and 8 mg/kg, NIH 11023 reduced the overall number of withdrawal signs but it did not substitute for morphine. At the high dose jaw sag, salivation and eyelid ptosis were observed. In addition one of the monkeys in the group convulsed 2 min after receiving the drug. Pentobarbital, 30 mg/kg i.p., effectively terminated the convulsion. Vehicle was 10% hydroxypropyl-β-cyclodextrin.

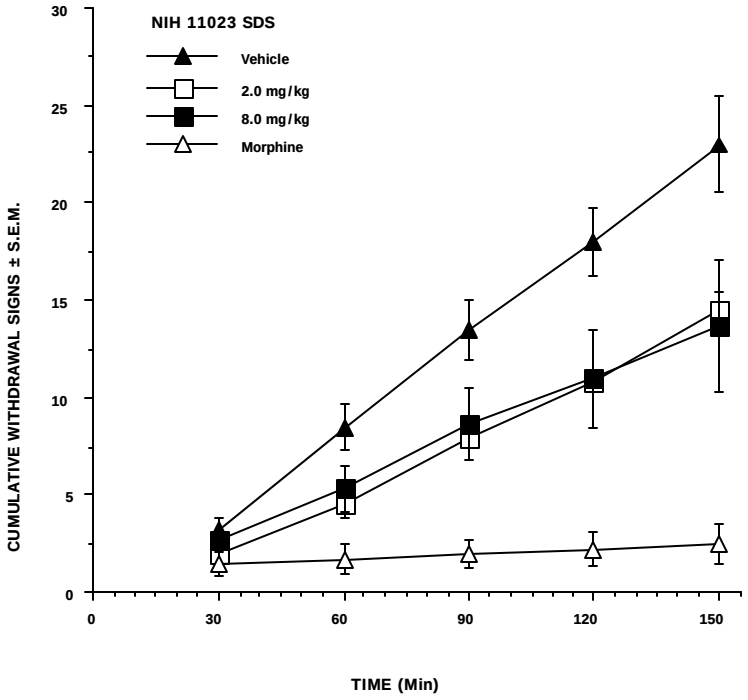
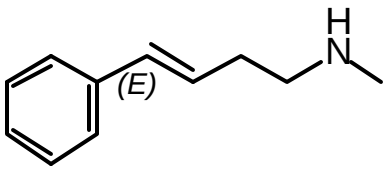


Fig NIH 11023 SDS. Results of study in which single doses of NIH 11023 were substituted for morphine in morphine-dependent monkeys in withdrawal

Comment The profile activity of this compound suggests heterogeneous opioid receptor properties (possibly kappa and delta opioid). Opioid subtype testing could resolve this issue.

NIH 11024 (10936) Metanicotine.oxalate



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 0% at 1, 3% at 10 and 0% at 30[a]
2) TF vs. M – Inactive at 1, 10 and 30[a]
3) PPQ – 0% at 1 and 10, 26% at 30[a]
4) HP – 0% at 1, 12.5% at 10 and 30[a]

Intravenous (5 min pretreatment time)
5) TF - 0% at 1 and 10, 14% 30[a]
[a]Clonic convulsions in 6/6 at 30. 3/6 died and 3/6 recovered 5min after injection.

MONKEY DATA
(SDS)

As shown in the accompanying figure, NIH 11024 neither substituted for morphine nor exacerbated withdrawal in the dose range of 4-16 mg/kg.. Some jaw sag was observed in 1/4 monkeys at the low and high doses. Slowing was noted in 1/4 monkeys at the high dose.

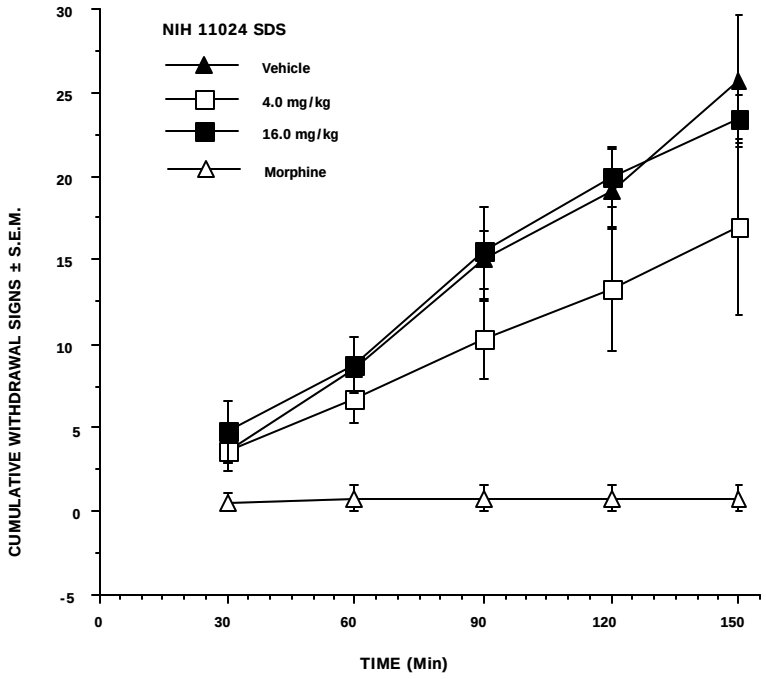
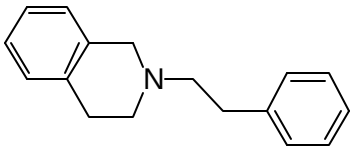


Fig NIH 11024 SDS. Results of study in which single doses of NIH 11024 were substituted for morphine in morphine-dependent monkeys in withdrawal

Comment: NIH 11024 did not display mu-opioid receptor agonist properties. In the monkey, perhaps it is a CNS depressant.

NIH 11025 2-(2-phenethyl)-1,2,3,4-tetrahydroisoquinoline.oxalate



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 5% at 1, 14% at 10 and 26% at 30
2) TF vs. M – inactive at 1, 10 and 30
3) PPQ – 10.5 (2.1 - 54.4)
4) HP – 12.5% at 1, 0% at 10 and 30

MONKEY DATA
(SDS)

At 4 and 16 mg/kg, some delayed attenuation of withdrawal signs was apparent with NIH 11025 (see accompanying figure).

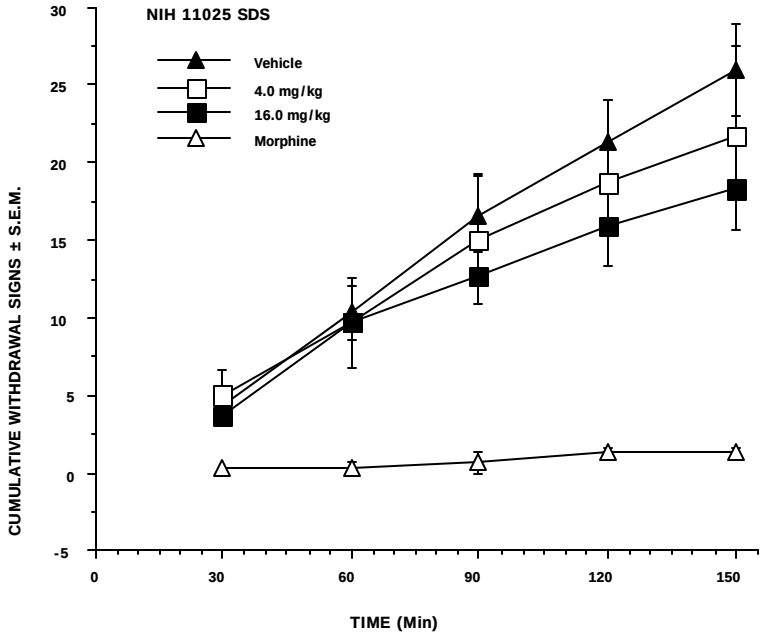
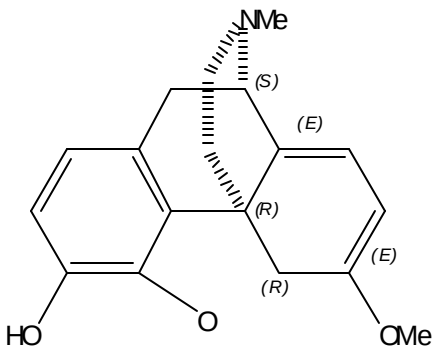


Fig NIH 11025-SDS. Results of study in which single doses of NIH 11025 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The results in mice and monkeys suggest some weak and/or delayed effects. Under conditions of these assays, NIH 11025 does not portend significant opioid effects.

NIH 11026 ((+)-Oripavine)



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 1% at 1, 20% at 10 and 29% at 30
2) TF vs. M – 7% at 1, 4% at 10 and 0% at 30
3) PPQ – 0.58 (0.14 - 2.36)
4) HP – 13% at 1, 0% at 10 and 38% at 30

Special Tests: Opioid subtype testing in PPQ test:

a) β-FNA (i.c.v., 4 hr pretreatment) vs ED80 of NIH 11026 (s.c., 20 min pretreatment) in PPQ test: 12% at 1, 21% at 3, 29% at 10 and 46% at 30 μg/brain.
b) nor-BNI (s.c., 2 hr pretreatment) vs ED80 of NIH 11026 (15 mg/kg, 20 min pretreatment) in PPQ: 0% at 1, 60% at 10 and 35% at 30.
c) Naltrindole (s.c., 30 min pretreatment) vs ED80 of NIH 11026, 20 min pretreatment) in PPQ: Inactive at 1, 10 and 30.

MONKEY DATA
(SDS)

NIH 11026 partly attenuated withdrawal signs in morphine-dependent monkeys. As shown in the figure, the effect was dose-related. Onset of action was prompt; however, the duration was short

NIH 11026 (Continued)

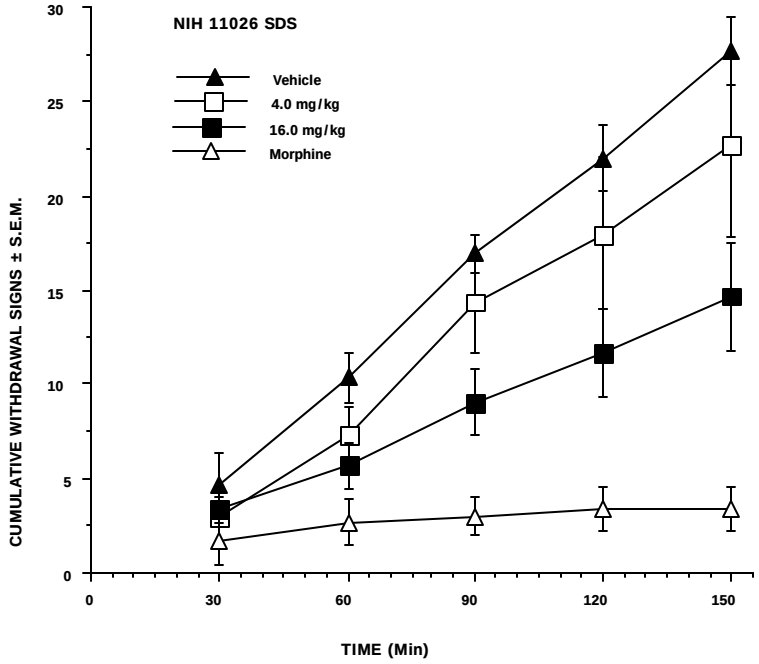
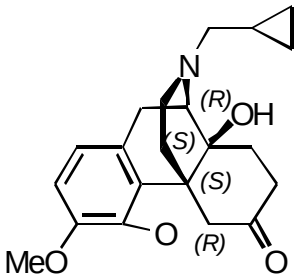


Fig NIH 11026-SDS. Results of study in which single doses of NIH 11026 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: NIH 11026 demonstrated very weak opioid activity, possibly of mu and kappa subtypes.

NIH 11028 (3-O-Methylnaltrexone.HCl)



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 1% at 1, 0% at 3 and 3% at 30
2) TF vs. M – 0.47 (0.30 – 0.72)
3) PPQ – NT
4) HP – 13% at 1 and 10, 0% at 30

NIH 11028 (Continued)

Special Tests: 1) NIH 11028 (p.o.) vs ED80 of morphine (s.c.) in TF: AD50 = 2.31 (1.73 – 3.09)
2) NIH 11028 (s.c., 6 hr pretreatment) vs ED80 of morphine (s.c.) in TF: 29% at 0.5, 9% at 1, 7% at 4 and 12% at 6.

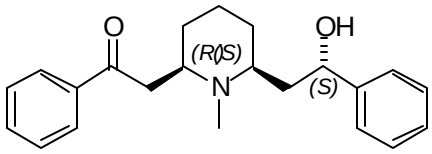
Note: Naloxone (p.o.) AD50 vs ED80 of morphine (s.c.): 1.44 (0.51 – 4.03)

MONKEY DATA
(SDS)

Not Tested.

Comment: Apparently, NIH 11028 is a non selective, orally active opioid antagonist which acts promptly. Its duration of action is less than 6 hr.

NIH 11034 (Lobeline)



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – 0% at 1, 5% at 10[a]
2) TF vs. M – Inactive at 1 and 10
3) PPQ – 3.01 (1.36 – 6.70)
4) HP – 0% at 1, 13% at 10 and 13% at 30[b]

[a]Five of six convulsed and died at 30 mg/kg. At 10 mg/kg there was decreased locomotor activity.
[b]Eight of eight convulsed at 30 mg/kg; four of eight died at this dose. At 10 mg/kg there was decreased locomotor activity.

Lobeline has been found to be a short-acting antagonist at the nicotine receptor in the nucleus accumbens (Benwell, M.E. and Balfour, D.V., Br. J. Pharmacol. 125, 1115-9 (2000)). Knowing of nicotine's interaction with opioids, we decided to evaluate this compound for similar properties.

MONKEY DATA
(SDS)

At doses of 1 and 4 mg/kg s.c., lobeline had little effect regarding attenuation of withdrawal (see figure). It did relax abdominal muscles but increased the incidence of retching.

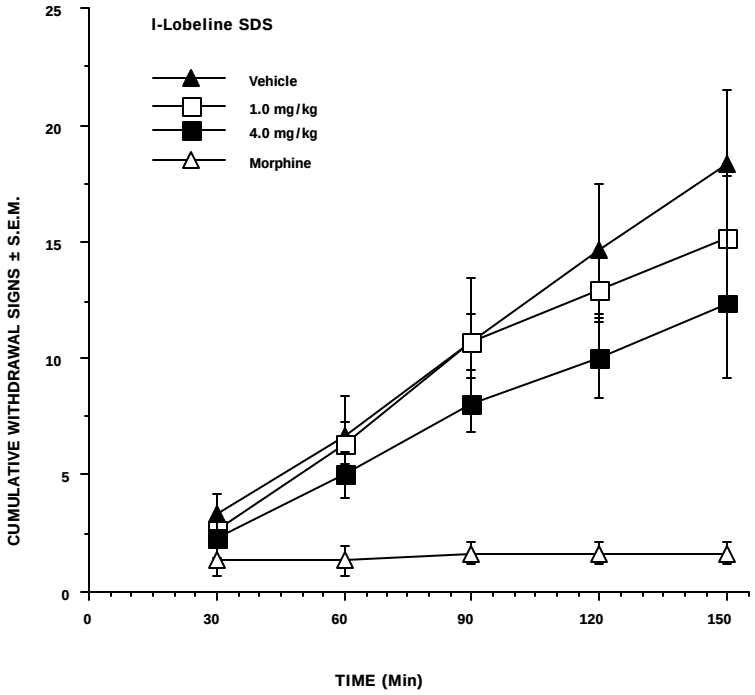
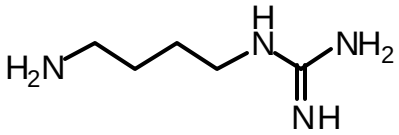


Fig L-Lobeline-SDS. Results of study in which single doses of lobeline were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: Lobeline resembles the action of nicotine regarding convulsions in mice and PPQ activity. In the monkey it also displayed nicotine's profile with reference to muscle relaxation and retching.

NIH 11035 (Agmatine Sulfate)



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – a) Inactive at l, 10 and 30[a] (s.c.)
   b) 2% at 1, 32% at 10 and 31% at 30 (μg/brain, i.c.v.)
2) TF vs. M – Inactive at 1, 10 and 30[a]
3) PPQ – a) 38% at 1, 40% at 10 and 0% at 30[a] (s.c.)
   b) 3% at 0.1, 13% at 0.3, 16% at 1, 50% at 3, 41% at 10, and 53% at 30 μg/brain (i.c.v.)
4) HP – 0% at 1, 20% at 10 and 38% at 30[a]

Evidence suggests that agmatine, an endogenous metabolite of L-arginine, is an important neurotransmitter in mammals. It binds to alpha$_2$-adrenoreceptor and imidazoline binding sites, blocks NMDA receptor channels and other cationic channels. It inhibits tolerance to and withdrawal from morphine and has been reported to block alcohol withdrawal in dependent rats (Reis, D.J. and Regunathan, S., Trends Pharmacol. Sci., 21,187-93 (2000) and Uzbay, I. T., Yesilyurt, O., Celik, T., Ergun, H. and Isimer, , A., Behav. Brain Res., 107, 153-9 (2000).

MONKEY DATA
(SDS)

The data are illustrated in Fig NIH 11035 SDS. Kruskal-Wallis ANOVA indicated significance (P = 0.05 or less) differences among the treatments at each time interval. Post hoc comparison using the Mann-Whitney U test revealed significant differences between morphine and each treatment group at each time interval. Agmatine, at the low dose of 6 mg/kg, showed a strong trend (p = 0.17 to 0.06) regarding attenuation of withdrawal signs at each time interval.

NIH 11035 (Continued)

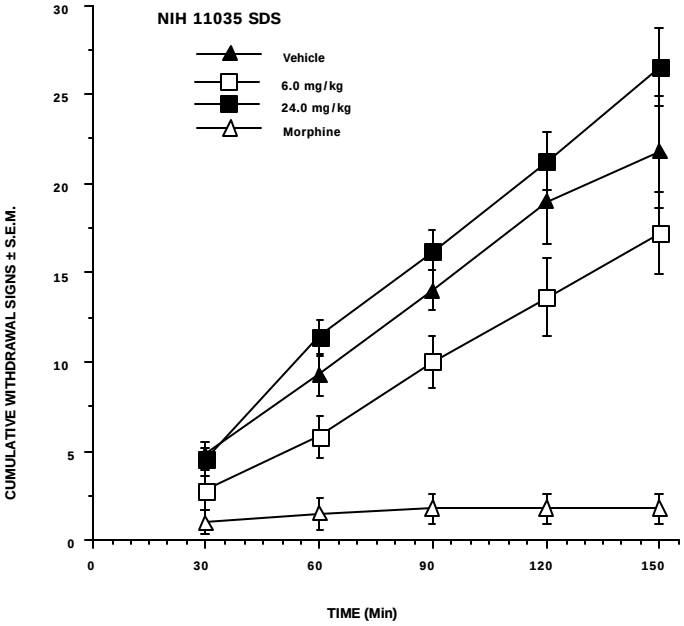
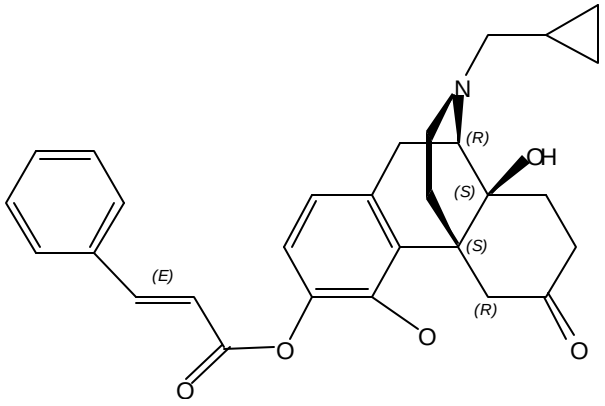


Fig NIH 11035 SDS. Results of study in which single doses of NIH 11035 were substituted for morphine in morphine-dependent monkeys in withdrawal

Comment: Agmatine may require more fine-tuning to characterize its interaction with the opioid system.

NIH 11037 (3-O-Cinnamoylnaltrexone.HCl)



MOUSE DATA - ED50 OR AD50
(95 % C.L.) (mg/kg or % change)

1) TF – Inactive at 1, 10 and 30[a]
2) TF vs. M – 0.013 (0.003 - 0.04) 30 min
3) PPQ – Inactive at 30[a]
4) HP – 13% at 30[a]

Special 4-hr pretreatment study (s.c.) - Naltrexone and NIH 11037 vs morphine.

Naltrexone	NIH 11037
AD50 = 1.92 (0.69 - 5.31)	2.69 (0.99 - 7.30)

Subtype testing as a kappa antagonist:

NIH 11037 AD50 vs ED80 of enadoline, a kappa agonist = 0.196 (0.045-0.0.849).

MONKEY DATA
(PPT-W)

NIH 11037 precipitated withdrawal in morphine-dependent monkeys at doses of 0.03 and 0.15 mg/kg. As shown in the accompanying figure, this drug appeared to be more potent than naloxone, the reference standard. Onset of action was rapid and offset seemed longer than that of naloxone.

NIH 11037 (Continued)

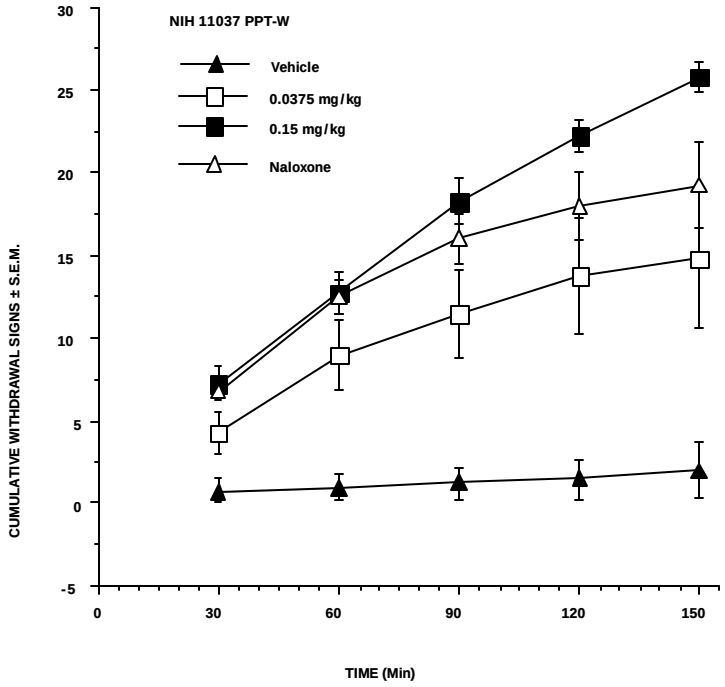


Fig. NIH 11037. Results of study in which NIH 11037 was administered to morphine-dependent monkeys (PPT-W).

Comment: Based on the results of studies in mice and morphine-dependent monkeys, we conclude that NIH 11037 is a potent mu- and kappa-opioid receptor antagonist. Whether or not this drug also has delta-opioid receptor antagonist activity remains to be determined.

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