Pharmacological Actions of the Racemic and the Enantiomeric 1,4-Dimethyl-10-Hydroxy-2,3,4,5,6,7-Hexahydro-1,6-Methano-1H-4-Benzazonines (C-Homobenzomorphans)

Mario D. ACETO* and Louis S. HARRIS
Department of Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298, U.S.A.

James H. WOODS, Jonathan L. KATZ, Charles B. SMITH and Fedor MEDZIHRADSKY
Department of Pharmacology, The University of Michigan Medical School, Ann Arbor, Michigan 48109-0010, U.S.A.

Arthur E. JACOBSON
Medical Chemistry Section, Laboratory of Chemistry, National Institutes of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205, U.S.A.

Shunsaku SHIOTANI
College of Liberal Arts, University of Toyama, Gofuku 3190, Toyama 930, Japan

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Abstract—The racemate and optical isomers of the C-homobenzomorphans, 1,4-dimethyl-10-hydroxy-2,3,4,5,6,7-hexahydro-1,6-methano-1H-4-benzazonine, were evaluated in a number of assays sensitive to narcotics of different types. All three C-homobenzomorphans were active in vitro in guinea pig ileum, mouse vas deferens, and rat brain membrane binding assays, but were of low potency. These C-homobenzomorphans showed different profiles of in vivo activity. The (+)-isomer and racemate were active as agonists in the tail-flick assay, whereas the (-)-isomer was inactive. At higher doses, the (-)-isomer and the racemate behaved as antagonists of morphine in the tail-flick assay. All three compounds were active in the phenylquinone test, but naloxone did not block this effect. In addition, all three were potent in the hot-plate test. Neither of the isomers substituted for morphine in dependent rats or monkeys. However, the (+)-isomer precipitated withdrawal in these monkeys. The (-)-isomer produced opioid-like physical dependence in both rats and monkeys. Some of the implications regarding the results with these remarkable homobenzomorphans are discussed.

C-Homobenzomorphans (1,4 mono or dialkyl-10 hydroxy-2,3,4,5,6,7-hexahydro-1,6-methano-1H-4-benzazonines) have a clear molecular resemblance (Fig. 1) to a well-characterized class of analgesic agonist-antagonists which were originally designated as 6,7-benzomorphans (6,11 mono or dialkyl-8-hydroxy-1,2,3,4,5,6, hexahydro-2,6-methano-3-benzazocines). Extensive studies of the optical isomers in the N-methyl benzomorphan series revealed a remarkable separation of morphine-like properties among several species. It was found that the (-)-isomers had potent antinociceptive activity in the mouse hot-plate assay (1), precipitated abstinence signs in morphine-dependent
monkeys (2) and were naltorphine-like in the guinea pig ileum (3). Further, the 6,11-diethyl compound did not produce physical dependence in monkeys after chronic administration, yet, it and the 6,11-dimethyl homolog were morphine-like in man (4, 5). On the other hand, 4 of the 5 corresponding (+)-isomers studied also had antinociceptive properties in mice. Indeed, although the (+)-isomers were always less potent than their respective enantiomers, the potency differences were as little as 6-fold for the 6,11-dimethyl compounds. Interestingly, the (+)-isomers were inactive as antagonists. Instead, they substituted for morphine in dependent monkeys (6).

Fig. 1. Chemical structure of 8-hydroxy-3,6,11-alpha-trimethyl-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocine (a: 6,7-benzomorphan) and 1,4-dimethyl-10-hydroxy-2,3,4,5,6,7-hexahydro-1,6-methano-1H-4-benzazoline (b: C-homobenzomorphan).

The initial finding that racemic 1,4-dimethyl-10-hydroxyl-2,3,4,5,6,7-hexahydro-1,6-methano-1H-4-benzazocine (b, Fig. 1) was active in the tail flick test and did not bind with high affinity to the opiate receptor spurred us to evaluate further this compound and its optical isomers.

Materials and Methods

Rat membrane receptor binding

The procedures used for isolation of rat brain membranes and assessing opioid receptor binding have been described previously (7, 8). Male Sprague-Dawley rats weighing 200 g were used. Following decapitation, the brain tissue was washed briefly in 50 mM Tris HCl, pH 7.4. The tissue was disrupted with 100 parts of the cold buffer using a homogenizer. The homogenate was centrifuged at 20,000 × g for 15 min in the cold, and the obtained pellet was resuspended with the original amount of buffer. Aliquots of this suspension, sufficient for a set of experiments on one given day, were frozen at -70°C. Prior to use, the suspension of membranes was quickly thawed and dispersed. The protein concentration of the homogenate was determined by the method of Lowry et al. (9).

Assays were conducted in a total volume of 500 μl, consisting of 400 μl of membrane suspension, 50 μl of H2O or 1.57 M NaCl, 25 μl of a solution of dextrorphan, levorphanol or the drug being investigated, and 25 μl of a 6 × 10⁻⁸ M solution of ³H-etorphine. Final concentrations in the medium were 1.5 × 10⁻¹ M NaCl and 3 × 10⁻⁹ M ³H-etorphine. In assays containing dextrorphan or levorphanol, final concentration in the medium was 6 × 10⁻⁷ M.

The binding of ³H-etorphine in the presence of a given drug was related to the maximum stereospecific etorphine binding, obtained as the difference between binding in the presence of an initially established appropriate excess of dextrorphan and excess of levorphanol. The IC₅₀ values were obtained graphically from log-probit plots of the binding data. Each drug was investigated at 5 concentrations, run in duplicates. The sodium response ratio (10) for a given drug was expressed as the ratio of IC₅₀ values obtained in the presence and in the absence of 150 mM NaCl in the medium.

Mouse vas deferens and guinea pig ileum

Male, albino Swiss-Webster mice, weighing between 25 and 30 g, were sacrificed by decapitation. The vasa deferentia were removed, and 1.5 cm segments were suspended in organ baths which contained a modified Krebs' physiological buffer. The buffer contained the following: 118 mM NaCl, 4.75 mM KCl, 2.54 mM CaCl₂, 1.19 mM MgSO₄, 1.19 mM KH₂PO₄, 11 mM glucose, 25 mM NaHCO₃, 0.07 mM hexamethonium bromide, 0.3 mM pargyline, 0.2 mM tyrosine, 0.1 mM ascorbic acid, and 0.03 mM disodium edetate. The buffer was saturated with 95% O₂-5% CO₂ and kept at 37°C. The segments were attached to a strain-gauge transducer and suspended between two platinum electrodes. After a 15 min equilibration period, the segments
were stimulated once every ten seconds with pairs of pulses of 1 msec duration, 1 msec apart, and at supramaximal voltage. The segments were stimulated for 30 min or until a stable twitch height was achieved. Cumulative concentration-response curves were determined for the various drugs by increasing the concentration of the drug by three-fold increments until a maximum response was obtained. EC50 values were calculated by probit analysis.

The isolated guinea-pig ileum was prepared as described (11). Segments of ileum were suspended in a Krebs physiological buffer at 37°C, saturated with 95% O2-5% CO2. The composition of the buffer was the same as described for the vas deferens preparation, except that it contained 0.0125 mM pyrilamine maleate and did not contain the pargyline, tyrosine, ascorbic acid, or disodium edetate. Tissues were equilibrated for 30-40 min, with washes every ten min. After the equilibration period, cumulative concentration-response relationships were determined for the various drugs. EC50 values were calculated by probit analysis.

**Mouse antinociceptive assays**

Male mice, weighing 20-30 g, were used. All drugs were dissolved in distilled water and administered s.c. in a volume of 0.1 ml/10 g of body weight. All doses are expressed as the respective salt. At least three doses per curve were tested, and 6-10 animals per dose were used. ED50's were calculated using computerized probit analysis.

**Tail-flick:** The procedure (12) and modifications (13) have been described in the literature. Briefly, the mouse's tail was placed in a groove which contained a slit under which was located a photo-electric cell. When the heat source or noxious stimulus was turned on, it focused on the tail, and the animal responded by flicking its tail out of the groove. Thus, light passed through the slit and activated the photocell which, in turn, stopped the recording timer. The heat source was adjusted to produce tail-flick latencies of 2-4 sec under control conditions. Mice were injected s.c. with drug or vehicle and tested 20 min later. In the assays for antagoni

**Hot-plate:** The method has been reported previously (15-17). The hot plate was held at a constant 55°C via a refluxing 1:1 mixture of ethyl formate and acetone. Mice were placed on the HP and activity was noted as a delay of five sec or more, but no more than 30 sec beyond the control time for the movement of the hind leg of the mouse, over at least two consecutive time periods. The mice were tested at 5, 10, 20, 30, 60 min and longer, if necessary, until responses returned to control levels.

**Dependence studies**

**Rat continuous infusion tests:** The method of Teiger (18) was used with some modification. Rats were anesthetized, and 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 in 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 Sprague-Dawley rats received a continuous infusion of 7 to 10 ml of solution every 24 hr for six days.

The animals received morphine, 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 4-6. A test drug or H2O was then substituted for 2 days. The animals were
observed for changes in body weight and for behavioral withdrawal signs for 1/2 hr at 6, 24, 48, 72 and/or 96 hr after stopping the infusion of morphine.

For primary physical dependence evaluation, the animals received a dose schedule of a compound for 6 days and then were placed in abrupt withdrawal and observed as above.

Substitution for morphine in rhesus monkey: Male and female rhesus monkeys (M. mulatta) in the weight range of 2.5 to 7.5 kg were used, and they received 3 mg/kg, s.c., of morphine every six hr. All the animals had received morphine for at least three months. Each animal was tested with a new compound with a minimum of two weeks between tests.

The assay was initiated by the subcutaneous injection of the test drug or control substances (morphine and vehicle H2O) into animals in a group that had not received morphine for 14–15 hr and showed definite signs of withdrawal. Each animal was randomly allocated to 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 (19–21) for suppression of withdrawal signs during a 2 1/2 hr observation period. The observer was “blind” regarding the allocation of treatments. At the end of the study, the data were grouped according to dose and drug, and the results were analyzed using the Mann-Whitney-U-test (22).

Precipitated withdrawal in rhesus monkeys: This evaluation was done under the same conditions as described above, except that the animals were administered a compound 2 or 3 hr after the last dose of morphine. Naloxone (0.05 mg/kg, s.c.) served as the positive control.

Primary physical dependence in rhesus monkeys: In this test, five animals in a group were given a substance 4–6 times a day for 45 days. The starting dose was usually based on the results of previous studies in monkeys described above. In this study, the observer knew the dose. The animals selected for this study were either experimentally naive or had not received another drug for at least two months. The dose was increased as an attempt to obtain effects after each injection. These animals were observed 1/2 hr after injection for 15 min. As indicated below, the animals were placed in abrupt withdrawal or challenged with a dose of naloxone.

Drugs

The racemate and isomer were prepared as described in the literature (23–25). The method of preparation and optical rotation and melting points of the enantiomers leave no doubt about their optical purity. They were administered as the hydrobromide salt. All other drugs were obtained commercially.

Table 1. Displacement of stereospecific 3H-etorphine binding in rat membrane by the racemate and isomers of homobenzomorphan·HBr and propoxyphene and dextrorphan

| Homobenzomorphan     | EC50 (nM) |
|-----------------------|-----------|
| (±)                  |           |
| (+)-                  |           |
| (-)-                  |           |
| +Na                   | 5410      |
| -Na                   | 3580      |
| +Na/−Na               | 1.51      |
| Propoxyphene          |           |
| Dextrophan            |           |
| 1040                  |
| 14,000                |

In vitro assays

As shown in Table 1, the racemate and its optical isomers had low potencies in displacing tritiated etorphine in rat brain membrane binding. They were less potent than propoxyphene and more potent than the non-narcotic dextrorphan. In Table 2, both the racemate and (+)-isomer caused less than a full inhibition of the twitch of the mouse vas deferens. The racemate is about one-twentieth as potent as morphine and two-thirds as efficacious, whereas the (+)-isomer is one-tenth as potent as morphine and one-third as efficacious in this preparation. Over the concentration range studied
Table 2. Depression of twitch in electrically driven mouse vas deferens by the isomers and racemate of homobenzomorphan-HBr

| Drug | (±) EC50 (M) | (+) EC50 (M) | (−) EC50 (M) |
|------|--------------|--------------|--------------|
| Drug alone* | 6.05×10^{-7} ±2.80 | 2.68×10^{-7} ±0.78 | >3×10^{-4} ± |
| Maximum depression | 68.7%±4.5 | 34.4%±2.3 | None |
| After naltrexone, 10^{-8} M | 6.75×10^{-9} ±1.80 | 2.98×10^{-6} ±2.87 | >3×10^{-4} ± |

*EC50's were determined assuming percent depression as the maximum response.

Table 3. Comparison of the profile of analgesic activity of the racemate and isomers of homobenzomorphan-HBr, morphine SO₄ and nalorphine HCl

| Mouse test          | (±) ED50 or AD50 mg/kg, s.c. | (+) ED50 or AD50 mg/kg, s.c. | (−) ED50 or AD50 mg/kg, s.c. | Morphine ED50 or AD50 mg/kg, s.c. | Nalorphine ED50 or AD50 mg/kg, s.c. |
|---------------------|-------------------------------|-----------------------------|-----------------------------|-----------------------------------|-----------------------------------|
| Tail-flick          | 6.4 (3.9–10.5)ᵃ              | 8.2 (3.1–21.8)ᵇ             | Inactive at 3.0, 6.0, 10.0 and 30.0 | 5.8 (5.7–5.9)ᶜ                   | Inactive at 10.0                  |
| Tail-flick vs. Morphine | 19.8 (10.5–37.2)            | Inactive at 3.0, 6.0, 10.0 and 30.0 | 5.5 (1.2–25.9)             | Inactive at 30.0                  | 2.6 (0.7–10.0)                    |
| PPQ                 | 0.5 (0.2–1.3)ᵈ               | 0.9 (0.4–2.2)ᵉ              | 0.4 (0.1–1.3)ᵈ              | 0.23 (0.20–0.25)ᶠ                 | 0.6 (0.30–1.4)ᵍ                   |
| Hot plate           | 0.4 (0.25–0.6)              | 2.6 (2.1–3.4)               | 1.8 (1.3–2.4)               | 1.0 (0.8–1.1)                    | 9.9 (5.7–17.2)                    |

ᵃ Antagonized by naloxone, AD50=0.8 (0.6–1.0). ᵇ Antagonized by naloxone, AD50=1.7 (0.2–15.5). ᶜ Antagonized by naloxone, AD50=0.03 (0.01–0.1). ᵈ Naloxone inactive as an antagonist up to 20.0 mg/kg. ᵉ 55% antagonism by naloxone at 20.0 mg/kg. ᶠ Antagonized by naloxone, AD50=0.5 (0.1–1.2). ᵍ Antagonized by naloxone, AD50=0.4 (0.08–2.4).
(10⁻⁹ M–10⁻⁴ M), the (-)-isomer was only weakly active in inhibiting the twitch. Concentrations of the racemate above 3x10⁻⁵ M caused increases in baseline tension. In the presence of naltrexone, the inhibitory effects of the racemate were potentiated. Very high concentrations, 10⁻⁴ M and higher, of the (+)-isomer caused marked increases in magnitude of the twitch as well as increases in the baseline tension. The slight inhibitory response of this isomer was antagonized by naltrexone.

In the guinea pig ileum, the racemate and the (+)-isomer were inactive in the concentration range of 10⁻⁹ M to 3x10⁻⁴ M. At concentrations of 3x10⁻⁹ M and higher, both enhanced the magnitude of the electrically elicited twitch and, at concentrations of 10⁻⁵ M and higher, they caused increases in baseline tension. Naltrexone had little effect upon the responses. On the other hand, an EC50 for the 82% inhibition of the twitch for the (-)-isomer was calculated as 2.51x10⁻⁶ M±0.24.

In vivo assays

Analgesia: An examination of the data in Table 3 reveals distinct antinociceptive profiles of activity for the racemate, (+)-isomer, and morphine in the tail-flick assay. The activity of the racemate and (+)-isomer in the tail-flick test was antagonized by naloxone; a larger amount of naloxone was required to antagonize the effects of the homobenzomorphan when compared to morphine. In turn, the racemate and the (-)-isomer antagonized morphine in the tail-flick test. In the PPQ assay, agonist activity of each of the homobenzomorphans was obtained; the effects were not antagonized by naloxone. Morphine had agonist activity in both antinociceptive tests which was antagonized by naloxone, and it was devoid of antagonist properties. Nalorphine was inactive in the tail-flick test, but showed antagonist properties vs. morphine. In the PPQ test, it showed agonist activity which was reversed by naloxone. All the compounds including the isomers of the homobenzomorphans were active in the hot plate assay.

Dependent studies: Rodents: In the rat-
infusion studies, neither of the isomers substituted for morphine at either the 100 mg/kg dose (data not shown) or the 200 mg/kg dose (Fig. 2). The weight losses were not statistically different from those calculated at 24, 48 or 72 hr for the morphine-dependent rats in which H2O was substituted. When compared with H2O controls, significant differences (P<0.05) were calculated for morphine and the isomers. Weight loss is considered to be the most reliable withdrawal sign in this test. A withdrawal syndrome characterized by signs of hypersensitivity, squealing, aggression, wet-dog shakes, rubbing and chewing, developed when either of the isomers or H2O was substituted for

![Table 4. Substitution for morphine in the rat: Effects of the isomers of homobenzomorphan-HBr](image)

| Substitution | Hours after termination of morphine infusion |
|--------------|---------------------------------------------|
| H2O Control, N=6 | 24  48  72 |
| Morphine infusion: (+)-isomer, N=4 | 9.3  15  13.8 |
| Substitution (200 mg/kg) | (P=0.005) (P=0.005) (P=0.005) |
| Morphine infusion: (-)-isomer, N=4 | 10.3  12.3  13.3 |
| Substitution (200 mg/kg) | (P=0.005) (P=0.005) (P=0.005) |
| Morphine infusion: H2O | 15.2  18.8  12.6 |
| Substitution, (N=5) | (P=0.063) (P=0.002) (P=0.041) |

Mean number of withdrawal signs\(^1\) noted during 1/2 hr observation period at specified intervals and calculated probability values\(^2\) for comparisons between H2O only group and (-)-isomer, and (+)-isomer and morphine control. \(^1\) Hypersensitivity, squealing, aggression, wet-dog shakes, rubbing and chewing. \(^2\) One-tailed test (Mann-Whitney U-test)

![Fig. 3. Body weight changes in primary physical dependence studies in rats.](image)
morphine (Table 4); thus the isomers failed to prevent the morphine withdrawal syndrome. When the (-)-isomer was infused in the rat for 6 days in the primary dependence study at two dose schedules, statistically significant (P<0.05 by the Mann-Whitney U-test), dose-related weight loss at 24, 48, 72 and 96 hr (Fig. 3) and behavioral withdrawal signs (Table 5) developed when the drug was discontinued; however, the signs were not as large in magnitude as those associated with morphine.

**Dependence studies: Rhesus monkeys:** Neither of the isomers of the homobenzo-

| Table 5. Primary physical dependence rat infusion study |
|---|---|---|---|---|
| Hours in withdrawal | 6 | 24 | 48 | 72 | 96 |
| H₂O Control (N=4) | 0 | 1.8 | 1.3 | 0.5 | 1.5 |
| Morphine infusion: | | | | | |
| H₂O Substitution (N=4) | 4.3 (P=0.01) | 14.8 (P=0.01) | 18.5 (P=0.01) | 21.0 (P=0.02) | 13.3 (P=0.06) |
| (-)-isomer infusion 50, 100, 200×4 mg/kg; H₂O Substitution (N=5) | 3.2 (P=0.05) | 6.8 (P=0.45) | 9.2 (P=0.05) | 12.0 (P=0.05) | 10.0 (P=0.14) |
| (-)-isomer 25, 50, 100×4 mg/kg; H₂O Substitution (N=5) | 4.2 (P=0.05) | 0.8 (P=0.36) | 1.4 (P=0.45) | 3.0 (P=0.05) | 2.2 (P=0.36) |

Mean number of withdrawal signs\(^1\) noted during 1/2 hr observation period at specified intervals and calculated probability values \(^2\) for comparisons between H₂O only group and the (-)-isomer and morphine control. \(^1\) Hypersensitivity, squealing, aggression, wet dog shakes, rubbing and chewing. \(^2\) One-tailed test (Mann-Whitney U-test)

| Table 6. Comparison of individual signs noted after (+)-homobenzomorphan·HBr in the single dose substitution test in morphine-dependent rhesus monkeys\(^a\) |
|---|---|---|---|
| Treatment | H₂O | Morphine | (-)-Isomer |
| Dose mg/kg, s.c. | 3.0 | 6.0 | 3.0 | 1.5 |
| No. of animals | 3 | 3 | 3 | 3 |
| Lying on side or abdomen | 6 | 1 | 13 | 10 | 9 |
| Fighting | 3 | 1 | 2 | 1 |
| Avoids contact | 1 | 3 | 4 | 4 |
| Vocalizes | 1 | 2 | 2 | 2 |
| Crawling and rolling | 4 | — | 6 | 3 | — |
| Restless | 12 | — | 8 | 12 | 9 |
| Drowsy | 1 | 1 | — | — | 2 |
| Tremors | 2 | — | 11 | 9 | 6 |
| Wet dogs | 4 | 1 | 2 | 3 | 3 |
| Retching | 9 | — | 13 | 7 | 9 |
| Vomiting | 1 | 1 | 2 | 1 |
| Coughing | — | — | 1 | 1 |
| Salivation | — | — | — | 2 |
| Vocalizes when abdomen palpated | 15 | 2 | 15 | 15 | 15 |
| Abdominal muscles (rigid) | 15 | — | 15 | 15 | 15 |
| Masturbation | 3 | — | 2 | — | — |
| Ataxia\(^b\) | — | — | — | 1 | 1 |
| Total of signs | 77 | 7\(^c\) | 91 | 86 | 80 |

\(^a\) Animals observed for 2 1/2 hr. \(^b\) Ataxia is not considered to be a withdrawal sign. \(^c\) Significantly different from H₂O controls (P<0.05, Mann-Whitney U-test).
moran substituted for morphine to prevent the occurrence of morphine withdrawal (Tables 6 and 7). Rather, it seemed that the (+)-isomer exacerbated withdrawal since the total number of withdrawal signs at each dose was greater than that of the H2O controls (Table 6). These effects of the (+)-isomer were also dose-related. Regarding the (-)-isomer (Table 7), it should be noted that the total number of withdrawal signs at the 3.0 mg/kg dose was smaller, but so was the number of animals tested at this dose. At the highest dose, ataxia was observed, which is not considered to be a withdrawal sign.

The (+)-isomer precipitated withdrawal in a dose-related manner in non-withdrawn morphine-dependent rhesus monkeys (Table 8). On the other hand, the (-)-isomer did not produce the full withdrawal syndrome. More than 50% of each total score can be accounted for by the signs of drowsiness and ataxia (Table 9).

Primary physical dependence: Rhesus monkeys: As shown in Table 10, the (-)-isomer produced some of the signs of opiate-like physical dependence upon abrupt discontinuation of administration or following the administration of naloxone. Tolerance was noted as reduced effects of the compound with continued administration over 1.5 months. The physical dependence capacity may be judged intermediate relative to a standard of 3 mg/kg/6 hr of morphine.

Discussion

The C-homobenzomorphans have a clear molecular resemblance to the 6,7-benzomorphans (Fig. 1). The aromatic ring and nitrogen atom of the former can be shown by molecular models and x-ray crystallography to nearly overlap these moieties in the 6,7-benzomorphans (24). The fact that the racemate and its enantiomers were relatively impotent in the mouse vas deferens, guinea pig ileum, and rat brain membrane binding assays was somewhat surprising to us. Thus, for example, neither the homobenzomorphane racemate nor its enantiomers appeared to interact with high potency with opioid receptors from rat brain membranes.

Table 7. Comparison of individual signs noted after (-)-homobenzomorphan-HBr in the single dose substitution test in morphine-dependent rhesus monkeys

| Treatment                              | H2O | Morphine | (-)-Isomer |
|----------------------------------------|-----|----------|------------|
| Dose mg/kg, s.c.                       |     | 3.0      | 6.0        | 3.0        | 1.5        |
| No. of animals                         | 3   | 3        | 3          | 2          | 3          |
| Lying on side or abdomen               | 2   | 4        | 6          |            |            |
| Fighting                               | 2   |          | 1          |            |            |
| Avoids contact                         | 2   | 8        | 9          |            |            |
| Vocalizes                              |    | 1        |            |            |            |
| Crawling and rolling                   |    |          |            |            |            |
| Restless                               | 12  | 4        | 8          | 13         |            |
| Drowsy                                 |    |          |            | 1          | 2          |
| Tremors                                |    | 7        | 1          | 3          |            |
| Wet dogs                               | 6   | 2        | 6          | 3          |            |
| Retching                               | 13  | 4        | 1          | 4          |            |
| Vomiting                               |    |          |            | 3          |            |
| Coughing                               |    |          |            |            |            |
| Salivation                             |    |          |            |            |            |
| Vocalizes when abdomen palpated        | 10  | 15       | 10         | 15         |            |
| Abdominal muscles (rigid)              | 10  | 15       | 10         | 15         |            |
| Masturbation                           |    |          |            |            |            |
| Ataxia<sup>b</sup>                     |    |          |            |            |            |
| Total of signs                         | 57  | 3<sup>c</sup> | 72         | 43         | 62         |

<sup>a</sup> Animals observed for 2 1/2 hr.  <sup>b</sup> Ataxia is not considered a withdrawal sign.  <sup>c</sup> Significantly different from H2O controls (P<0.05, Mann-Whitney U-Test).
Table 8. Comparison of individual signs noted after (+)-homobenzomorphan-HBr in the precipitated withdrawal test in rhesus monkeys

| Treatment                  | H₂O | 0.05 | 10.0 | 3.0 | 2.5 |
|----------------------------|-----|------|------|-----|-----|
| Dose mg/kg, s.c.           |     |      |      |     |     |
| No. of animals             | 3   | 3    | 3    | 3   | 3   |
| Lying on side or abdomen   |     | 5    | 3    | —   | 2   |
| Fighting                   |     | 5    | —    | —   | —   |
| Avoids contact             | 5   | 3    | 2    | 1   | 2   |
| Vocalizes                  |     | —    | —    | —   | —   |
| Crawling and rolling       |     | —    | —    | —   | —   |
| Restless                   |     | —    | —    | —   | —   |
| Drowsy                     | 3   | 9    | 11   | 11  | 7   |
| Tremors                    |     | 2    | 15   | —   | 7   |
| Wet dogs                   |     | 3    | 4    | —   | 1   |
| Retching                   |     | 5    | 5    | —   | 1   |
| Vomiting                   |     | 2    | —    | —   | —   |
| Coughing                   |     | 1    | 2    | —   | —   |
| Salivation                 |     | —    | 3    | 2   | —   |
| Vocalizes when abdomen palpated |     | 9    | 8    | 8   | 9   |
| Abdominal muscles (rigid)  |     | 6    | 9    | 9   | 6   |
| Sagging and ptosis         |     | —    | —    | 5   | —   |
| Masturbation               |     | 4    | —    | —   | —   |
| Ataxia b                   |     | —    | 10   | 3   | —   |
| Total of signs             | 8   | 59   | 73   | 40  | 38  |

a Animals observed for 2 1/2 hr.  b Ataxia is not considered a withdrawal sign.  c Significantly different from H₂O controls (P<0.05, Mann-Whitney U-Test).

Table 9. Comparison of individual signs noted after (-)-homobenzomorphan-HBr in the precipitated withdrawal test in rhesus monkeys

| Treatment                  | H₂O | 0.05 | 12.0 | 6.0 | 3.0 |
|----------------------------|-----|------|------|-----|-----|
| Dose mg/kg, s.c.           |     |      |      |     |     |
| No. of animals             | 3   | 3    | 3    | 3   | 3   |
| Lying on side or abdomen   |     | 6    | 6    | —   | —   |
| Fighting                   |     | 1    | —    | —   | —   |
| Avoids contact             | 4   | 1    | —    | —   | 2   |
| Vocalizes                  |     | —    | —    | —   | —   |
| Crawling and rolling       |     | —    | —    | —   | —   |
| Restless                   |     | 7    | 1    | 3   | 4   |
| Drowsy                     | 1   | 6    | 9    | 6   | 6   |
| Tremors                    |     | 3    | 3    | 1   | —   |
| Wet dogs                   |     | 6    | —    | —   | —   |
| Retching                   |     | 8    | 1    | —   | —   |
| Vomiting                   |     | 2    | —    | —   | —   |
| Coughing                   |     | 2    | —    | —   | —   |
| Salivation                 |     | 2    | —    | —   | —   |
| Vocalizes when abdomen palpated |     | 9    | —    | 1   | 1   |
| Abdominal muscles (rigid)  |     | 6    | —    | —   | —   |
| Masturbation               |     | 3    | 2    | —   | —   |
| Ataxia b                   |     | —    | 9    | 8   | 3   |
| Total of signs             | 5   | 62   | 31   | 19  | 17  |

a Animals observed for 2 1/2 hr.  b Ataxia is not considered a withdrawal sign.  c Significantly different from H₂O controls (P<0.05, Mann-Whitney U-Test).
With regard to the results of the antinociceptive studies, a number of conclusions can be made. Neither the racemate nor its optical isomers have profiles of activity similar to those of morphine or nalorphine. Although all these homobenzomorphans were active in the PPO assay, an important distinction is that both morphine's and nalorphine's actions were reversed by naloxone, whereas naloxone was inactive when tested against either the homobenzomorphan racemate or its antipodes. Interestingly, naloxone will antagonize cyclazocine and other benzomorphan agonist-antagonist compounds in this test. These results suggest that the homobenzomorphans are exerting analgesic activity at non-opioid sites in these assays. However, the (+)-isomer showed opioid agonist activity in the tail-flick test which was reversed by large doses of naloxone and the (-)-isomer showed antagonist activity against morphine in the same test. The racemate seemed to incorporate the features common to both isomers in the tail-flick test, i.e., it is active as an agonist at lower doses and, at higher doses, showed antagonist properties. Clearly, the homobenzomorphan and its isomers had unusual profiles of activity in these assays.

Neither of the isomers substituted for morphine in the morphine-dependent rat or monkey. The (+)-isomer precipitated abstinence in the dependent monkey. These results are in sharp contrast with those noted in the mouse tail-flick tests where the (-)isomer antagonized morphine's antinociceptive effects. On the other hand, the (-)-isomer produced opioid-like primary physical dependence in both the rat and the monkey. This isomer was selected for primary physical dependence studies because it was considered the least likely to produce dependence.

Species and optical isomer differences are also common for the N-substituted benzomorphans. It is probably not a mere coincidence that the structurally similar N-methyl homobenzomorphans and benzomorphans...
both exhibit species differences. Equally intriguing is the activity associated with the (+)-isomer. Among the opioids, it is a common rule that most or all of the activity is associated with one isomer, usually the (-) isomer. Further studies with these stereoisomers may shed more light on the properties of the opioid receptor system and may lead to the development of an "ideal" analgesic. At a more pragmatic level, it is evident that not too much emphasis must be placed on any one test system when evaluating potential analgesic drugs. It is also evident that studies with racemates may be misleading and that, whenever possible, that enantiomers should be studied.

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