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Etorphines: μ-opioid receptor-selective antinociception and low physical dependence capacity

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Abstract

Comparative analgesic studies revealed that dihydroetorphine was more potent than etorphine in the tail-flick and hot-plate tests, respectively and nearly equipotent in the phenylquinone assay. Both compounds were short acting. Studies with selective opioid receptor antagonists β-funaltrexamine, nor-binaltorphimine and naltrindole revealed that both etorphines were μ-selective agonists. Presumptive evidence for competitive antagonism of these compounds with naloxone was provided by Schild regressions with slopes of near unity. In a suppression test in rhesus monkeys maximally dependent on morphine, dihydroetorphine and etorphine dose-dependently replaced morphine. Drug-naive simians chronically exposed to frequent, intermittent and escalating doses of dihydroetorphine for 42 days showed few withdrawal signs when challenged with large doses of naloxone or were abruptly withdrawn from this drug. The results suggest that these atypical opioids may be useful in the clinical treatment of pain and opiate drug abuse. © 1997 Elsevier Science B.V.

Keywords: Dihydroetorphine; Etorphine; Antinociception; Physical dependence; (Rhesus monkey); (Mouse)

1. Introduction

Bentley and Hardy (1967) reported that dihydroetorphine an 6,14-endothenotetrahydro-oripavine derivative, was the most powerful analgesic ever tested in animals. Its potency was calculated at about 12 000 times that of the standard morphine sulfate! Etorphine to which dihydroetorphine was structurally closely related was less active but still impressively potent at 3200 times the standard. Because etorphine substituted for morphine in a single-dose suppression test in rhesus monkeys physically dependent on morphine and because, at that time, all drugs which suppressed withdrawal produced physical dependence, Deneau and Seevers (1963) concluded that etorphine was likely to produce physical dependence. In 1966, the World Health Organization ruled that etorphine was liable to abuse by humans. Later, etorphine was reported to be clinically effective at 1 μg/kg in cancer patients with chronic pain (Blane and Robbie, 1970). Other studies involving opiate-dependent and former human addicts led Jasinski et al. (1975) to conclude that etorphine had a high abuse potential. Moreover, etorphines's remarkable potency was perceived by many as a liability; it was considered too dangerous and unsuitable for general use in humans. However, etorphine was used by veterinarians to treat pain and to immobilize large animals (cited in Blane et al., 1967).

After a long hiatus, other preclinical and clinical reports surfaced. They reconfirmed that dihydroetorphine was a potent analgesic. Unexpectedly, some of these reports also claimed that dihydroetorphine had a low dependence liability and was effective in treating human addicts (Wang et al., 1992a,b; Qin, 1993; Tokuyama et al., 1993). Electrophysiological studies on sensory dorsal-root ganglion neurons in culture, Crain and Shen (1992a,b, 1995a,b) and Shen and Crain (1992, 1994, 1995) suggested that most opioid agonists including morphine have bimodal excitatory and inhibitory activating properties. The excitatory effects are apparently mediated by opioid receptors coupled via a cholera toxin sensitive-stimulatory G proteins (G_s) to adenylate cyclase/cyclic AMP/protein kinase A-dependent ionic conductances that extended the action potential duration of a dorsal root ganglion preparation. In turn, the inhibitory effects are mediated by pertussis toxin-sensitive (inhibitory) G_i/G_o-coupled opioid receptors that shortened the action potential. These investigators hypothesized that sustained activation of G_s coupled opioid

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receptors was associated with the development of physical dependence and supersensitivity to naloxone. Etorphine and dihydroetorphine elicited only inhibitory agonist effects. In addition, these etorphines also displayed stimulatory antagonist properties that blocked the development of tolerance development in chronic opioid-treated dorsal-root ganglion neurons (Shen and Crain, 1994). The combination of inhibitory opioid agonist and stimulatory antagonist properties was considered an especially effectual combination. From these studies Crain and Shen (1995a) posited that a drug with selective opioid inhibitory agonist properties: (1) would have analgesic properties; (2) would have low physical dependence liability; and (3) would be useful in the pharmacotherapy of opioid dependence.

Other investigations regarding etorphine-induced analgesia have been published. Etorphine was reported to bind with similar affinities to μ-, κ- and δ-opioid receptor sites in vitro (Kosterlitz and Paterson, 1980; Chang et al., 1981; Pfeiffer and Herz, 1981) however, in receptor binding assays in vivo, it apparently manifested μ-opioid receptor selectivity (Perry et al., 1982; Richards and Sadee, 1985). In a comparative study (Niwa et al., 1995) the same affinities for μ-, κ- and δ-opioid receptors were reported for etorphine and dihydroetorphine. In antinociceptive assays both etorphines exhibited μ-opioid receptor selectivity (Zimmerman et al., 1987; Xu et al., 1992; Wang et al., 1995; Kamei et al., 1995).

Needless to say, interest in dihydroetorphine and etorphine was renewed. Our primary objective was to determine whether or not dihydroetorphine produced physical dependence in the rhesus monkey. In addition, etorphine and dihydroetorphine would be tested and compared using the single-dose substitution assay. The use of the rhesus monkeys as subjects was considered important because the development and expression of physical dependence on opioids most nearly mimics the withdrawal syndrome observed in humans. We also decided to reconfirm and characterize further the pharmacological properties of these interesting compounds in the mouse.

2. Materials and methods

All animals received care according to ''Guide for the Care and Use of Laboratory Animals'', U.S. Department of Health and Human Services, 1985. The facilities are certified by the American Association for the Accreditation of Laboratory Animal Care. These studies were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University.

2.1. Mouse opioid agonist / antagonist evaluation

2.1.1. General methods

ICR male mice (Harlan–Sprague–Dawley, Indianapolis, IN, USA) weighing 20–30 g, were used. Each animal was tested once only. Both etorphines were given by the subcutaneous (s.c.) route. β-Funaltrexamine was administered by the intracerebroventricular (i.c.v.) route. At least 3 doses were tested, and 6–10 animals per dose were used.

2.1.2. Tail-flick agonist, antagonist or apparent pA_2 tests

This procedure was first reported by D'Amour and Smith (1941) and modified by us (Dewey et al., 1970; Dewey and Harris, 1971). 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 or noxious stimulus was turned on, heat was focused on the tail, and the mouse responded by flicking its tail out of the groove. As a result, light passed through the slit and activated a photocell which, in turn, stopped the recording timer. The heat source was adjusted to produce tail-flick latencies of 2–4 s under control conditions. Only mice meeting this criterion were used. Mice were injected with test drug or vehicle, and except as indicated below in the time-course studies, were tested 20 min later. Antinociception was calculated as % MPE (per cent maximum possible effect) = (test latency − control latency/(10 s − control latency) × 100 for each dose tested. Cut-off time was 10 s. In the naloxone antagonism test, naloxone was given 10 min before the dihydroretorphine or etorphine ED_{80} (dose producing 80% increase in MPE) was injected and latencies were measured 20 min later. For each point of the dose–response curve, percent antagonism was calculated as [1-(Naloxone + dihydroetorphine or etorphine MPE)/(dihydroetorphine or etorphine MPE ED_{80})] × 100).

Each apparent pA_2 for naloxone (negative logarithm of the molar concentration of antagonist required to produce a two-fold shift of the agonist dose–response curve to the right) was calculated using Schild and constrained plots as described by Tallarida and Murray (1987). Dose–response lines for antinociception were plotted using at least 3 doses, 10 mice per dose, of test substance in combination with vehicle or naloxone. Dose ratio (x) was calculated by dividing the ED_{50} (dose producing a 50% increase in the MPE) of dihydroetorphine or etorphine in the presence of a given dose of antagonist by that of the agonist alone. $\log(x-1)$ was plotted against the negative logarithm of the molar dose of antagonist. At least 4 $\log(x-1)$ points were plotted. Each apparent pA_2 was calculated from the point of intersection of the regression line with the abscissa.

2.1.3. Phenylquinone abdominal-stretching assay

The procedure described by Pearl and Harris (1966) with modifications as indicated below was used. Six mice were injected per dose of test drug or vehicle and 10 min later received 2 mg/kg intraperitoneally (i.p.) of a freshly prepared paraphenylquinone solution. They were then placed in 3 cages in groups of 2 each. Then, the total number of stretches observed per group during each 1 min period was counted at 10 and 15 min. The total number of

stretches for the 3 groups was determined. A stretch was characterized by an elongation of the mouse's body, development of tension in the abdominal muscles and extension of the hindlimbs. The antinociceptive response was expressed as % inhibition of the paraphenylquinone-induced stretching response and was calculated as [1-(total number of stretches in the medicated mice)/(total number of stretches in the control mice)] × 100.

2.1.4. Hot-plate test

The method originally described by Eddy and Leimbach (1953) was used. Modifications are indicated below. A modified 1000 ml pyrex beaker (bottom removed) was placed on the hot plate maintained at 56°C. The test was initiated by placing a mouse in the specially designed beaker. This arrangement served to confine a mouse to a specific area of the hot plate. Each mouse was exposed to the hot plate for 2 trials spaced 5 min apart. Only mice that gave a control response latency in the range of 6 to 10 s on both trials served as subjects. Each subject received a dose of test drug and 30 min later was again tested on the hot plate. Activity was scored as positive if the mouse jumped, licked or shook its paws at least 5 s beyond its average control latency. Cut-off time was 15 s. Per cent activity for each dose tested was calculated as (total number of mice scored as positive)/(total number tested) × 100.

2.2. Rhesus monkey studies

2.2.1. Single-dose substitution test

Male and female rhesus monkeys (*M. mulata*) weighing 2.5 to 7.5 kg were used. They were housed in pens in socially compatible groups of 4 or 5 subjects per pen and received 3 mg/kg s.c. of morphine sulfate every 6 h. All the animals had received morphine for at least 3 months and were maximally dependent on morphine (Deneau and Seevers, 1963). A minimal 2-week recuperation period was allowed between tests. At least 3 subjects were used per dose. The assay was initiated by a s.c. injection of the test drug or control substances, morphine, or 25% aqueous solution of hydroxypropyl-β-cyclodextrin (vehicle) into animals in a group that had not received morphine for 14–15 h 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; or (c) vehicle control, 1 ml/kg. The withdrawal signs designated slowing, drowsiness, fighting (aggressor), vocalizes, rigid abdominal muscles, vocalizes on palpation of abdomen, restlessness (pacing), tremors, coughing, retching, vomiting, wet-dog shakes and masturbation were scored once, when observed, during each of five 30 min observation periods. The trained observer was 'blind' regarding treatment assignments. At the end of the study, the data were grouped according to dose and drug and analyzed as indicated below. In addition, the mean cumulative score ± S.E.M. was calculated for each 30 min time point and illustrated in figure form. Details of this method were published (Aceto et al., 1977, 1978).

2.2.2. Primary physical dependence study

One female and 4 male drug-naive monkeys in the weight range of 3.2–3.9 kg were housed in a pen and medicated with dihydroetorphine. They were observed daily by a trained observer after dihydroetorphine administration, when challenged with naloxone or when placed in abrupt withdrawal. Additional details are provided in Table 4.

2.3. Statistical analyses

MPE ED_{50} values, ED_{80} values or AD_{50} values and apparent pA_2 values were calculated according to the methods described by Litchfield and Wilcoxin (1949) and Tallarida and Murray (1987), respectively. The data from the monkey studies were analyzed nonparametrically using Kruskal–Wallis ANOVA (Analysis of Variance). Post hoc comparisons were made using the Mann–Whitney test. The StatView 512 + statistical package (Brainpower, Agoura Hills, CA, USA) was used for these analyses.

2.4. Pharmacological agents

Dihydroetorphine · HCl, etorphine · HCl, nor-binaltorphimine · HCl and naloxone · HCl were furnished by the National Institute on Drug Abuse. Morphine sulfate was purchased from Mallinckrodt (St. Louis, MO, USA). Naltrindole · HCl and β-funaltrexamine · HCl were obtained commercially from Research Biochemicals International (Natick, MA, USA). Dihydroetorphine was dissolved in hydroxypropyl-β-cyclodextrin (American Maize-Products, Hammond, IN, USA), in sterile distilled water (vehicle).

3. Results

3.1. Mouse

3.1.1. Opioid agonist / antagonist evaluation

As can be seen in Table 1, dihydroetorphine and etorphine displayed the same profile of activity, i.e., they were potently active antinociceptively. The ED_{50} doses expressed as ng/kg of dihydroetorhine in the tail-flick, phenylquinone and hot-plate tests were 150, 200 and 100 respectively, whereas etorphine's were lower namely, 2000, 400 and 1000 for each one. The apparent pA_2 values (see Figs. 1 and 2) are in the range reported by us for naloxone/morphine and naloxone/sufentanil (Aceto et al., 1996). The Schild regressions with slopes of near unity

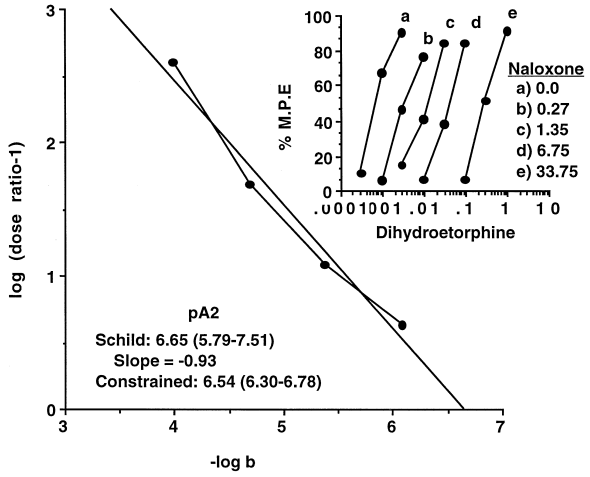

Fig. 1. Competitive antagonism of and Schild regression to dihydroetorphine-induced antinociception by naloxone in the tail-flick test.

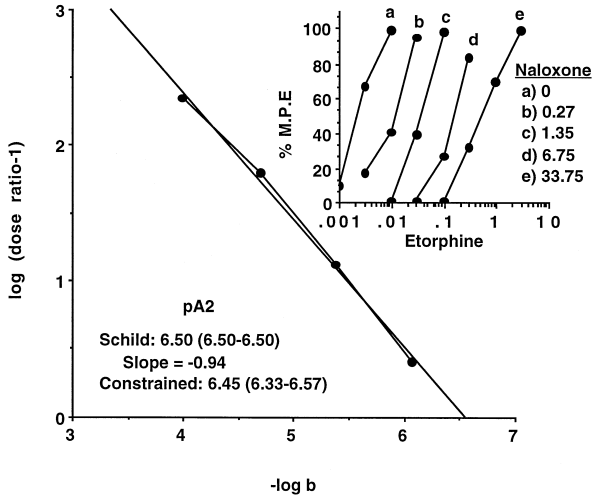

Fig. 2. Competitative antagonism of and Schild regression to etorphine-induced antinociception by naloxone in the tail-flick test.

suggested that the interaction at the opioid receptor was competitive. Quantitatively, dihydroetorphine appeared to be about 13 and 10 times more potent than etorphine in the tail-flick and hot-plate assays, respectively. Curiously, regarding potency, the dihydroetorphine and etorphine ED_{50}s were not remarkably different in the paraphenylquinone test.

3.1.2. Dihydroetorphine, etorphine and morphine time-course studies

In the tail-flick test, both drugs displayed similar time courses (Table 2). Onset was rapid, within 20 min, and activity was waning at 40 min and no longer detectable at 90 min. As can be seen morphine's onset was also rapid and activity had waned considerably by 180 min.

3.1.3. Interactions with opioid receptor antagonist subtypes

The antinociceptive actions of both dihydroetorphine and etorphine were antagonized by the selective opioid receptor antagonists, β-funaltrexamine (μ). Nor-binaltorphimine (κ) and naltrindole (δ) were ineffective in this regard. The results indicate that dihydroetorphine and etorphine are highly selective μ-opioid receptor agonists devoid of κ- and δ-opioid properties (Table 3).

3.2. Rhesus monkey

3.2.1. Single-dose substitution in abruptly withdrawn morphine-dependent monkeys

The results of this study are illustrated in Fig. 3 for dihydroetorphine. The data for etorphine are not illustrated; however, the statistical evaluation is presented below. Both drugs dose-dependently substituted completely for morphine; however, dihydroetorphine was active in the potency range of 20,000 to 100,000 times morphine whereas the etorphine potency range was 1500 to 6000 times morphine. These oripavines acted promptly. At the high doses they had durations of action of at least 2.5 h at the high doses. Kruskal–Wallis one-way analysis of variance of the data at 2.5 h generated values of $H = 9.462$; χ^2 $0.05(3) = 7.82$ and $H = 6.66$; χ^2 $0.1(3) = 6.25$ for dihydroxyetorphine and etorphine, respectively. The critical value for dihydroetorphine was statistically significant and that for etorphine approached significance. Post hoc comparisons between the high dose and vehicle controls or low dose and vehicle controls in the dihydroetorphine study also showed statistically significant differences ($P < 0.05$) as did similar comparisons of the data in the etorphine study.

Table 1
Effects of dihydroetorphine and etorphine in the tail flick, paraphenylquinone and hot plate tests in the mouse

Assay	Results: ED_{50} or AD_{50} (95% C.L.) or ng/kg	
	dihydroetorphine	etorphine
(1) Tailflick	150 (60–400)	2000 (1000–4000)
(2) Tailflick-naloxone AD_{50} versus dihydroetorphine or etorphine ED_{80}	40 000 (10 000–80 000)	90 000 (40 000–250 000)
(3) Phenylquinone test	200 (60–400)	400 (200–900)
(4) Hot plate	100 (50–300)	1000 (400–3000)

Table 2
Time-course studies for dihydroetorphine, etorphine and morphine of the tail-flick ED_{80} values in the mouse tail flick assay

% Inhibition of Nociception ± S.E.M.			Pretreatment time (min)
dihydroetorphine	etorphine	morphine	
77 ± 13	82 ± 12	89 ± 11	20
41 ± 10	66 ± 18	87 ± 13	40
4 ± 2	18 ± 8	83 ± 11	60
1 ± 0.8	4 ± 4	92 ± 8	90
NT[a]	NT[a]	41 ± 13	120
NT[a]	NT[a]	9 ± 4	180

[a] Not tested.

3.2.2. Primary physical dependence study

A synopsis of the results is presented in Table 4. The starting dose of 30 ng/kg was based on the results of the tail-flick test. This dose would probably elicit potent analgesic effects in monkeys because it effectively suppressed the sign designated vocalization when abdomen palpated which is believed to be a reaction to pain. Initially, dihydroetorphine produced the usual agonist behavioral signs associated with the administration of opiates to non-tolerant subjects such as body sag, ataxia, slowing, ptosis and scratching. Because dihydroetorphine had a relatively short duration of action, the frequency of injections was increased from every 6 h to 6 times a day on weekdays (at 6, 10 and 12 a.m. and 2, 6 and 12 p.m.). On day 8, when the dose had been raised to 1200 ng/kg, one monkey lost consciousness for a brief period. The following day, 2 monkeys lost consciousness briefly. As a result, the dose was reduced to 600 ng/kg at the noon injection. Fewer agonist signs were noted at the lowered dose indicating some tolerance had developed.

On day 16, approximately 2 h after the 6 a.m. injection of dihydroxyetorphine, the monkeys were challenged with naloxone (0.05 mg/kg/s.c.). This dose would normally precipitate a severe withdrawal syndrome in morphine-treated monkeys receiving 3 mg/kg every 6 h for at least 90 days (Aceto et al., 1977). However, naloxone was ineffective. One-half h later, the dose of naloxone was raised by a factor of 10 and the monkeys were challenged again. As described in Table 4, a very mild withdrawal syndrome developed. Nevertheless, two critically important withdrawal signs were not seen; namely, rigid abdominal muscles and vocalization associated with palpation of the abdomen. We concluded that only a very mild degree of physical dependence had developed. After the precipitated withdrawal test was conducted, dihydroetorphine was given and the dose was raised to 900 ng/kg. The usual agonist signs were recorded. The following day the dose of dihydroxyetorphine was raised to 1200 ng/kg and maintained at that level until day 21 when loss of consciousness was again observed in 2 monkeys. The dose was reduced to 900 ng/kg for the remainder of the study. Agonist signs were noted throughout this period. On day 31, the monkeys were again challenged with a very high dose (0.55 mg/kg s.c.) of naloxone. Only a very mild withdrawal syndrome was elicited. Dihydroxyetorphine was abruptly withdrawn (Abrupt Withdrawal) on day 41 and the animals were evaluated for signs of withdrawal. Again, very mild withdrawal behavior was noted. Sixteen h after abrupt withdrawal (day 42), dihydroetorphine (900 ng/kg) was again administered and 0.5 h later, the monkeys were challenged for the third time with naloxone (0.55 mg/kg s.c.). Wet-dog shakes were elicited in only 1 of 5 subjects. Interestingly, during the 0.5 h-observation period, some dihydroxyetorphine agonist signs were still evident. Throughout the study no remarkable body weight changes were observed (data not shown).

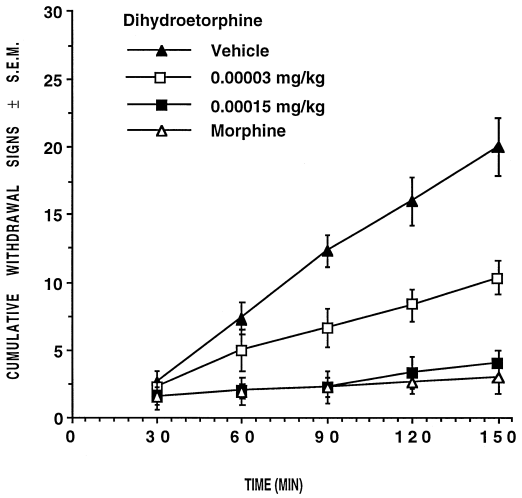

Fig. 3. The effect of dihydroetorphine on the attenuation of withdrawal signs in withdrawn, morphine-dependent monkeys. Doses of morphine and vehicle were 3.0 mg/kg and 1.0 ml/kg, respectively.

Table 3
Interactions of dihydroetorphine and etorphine with opioid antagonist subtypes in the tail-flick assay

Opioid subtype	Results: AD_{50}/(95% C.L.)[a]	
	dihydroethorphine (s.c.)	etorphine (s.c.)
μ: β-Funaltreximine-4 h[b] (i.c.v.)	9.3 (3.4–25.0) μg/brain	4.7 (2.5–8.6) μg/brain
κ: Nor-binaltorphimine-2 h[b] (mg/kg)	inactive at 1, 10 and 30	inactive at 1, 10 and 30
δ: Naltrindole-20 m[b] (mg/kg)	inactive at 1, 10 and 30	inactive at 1, 10 and 30

[a] mg/kg or μg/brain as indicated.
[b] Pretreatment time.

Table 4
Synopsis of dihydroetorphine primary physical dependence study: Abrupt and precipitated withdrawal in rhesus monkeys

Day(s)	Dose (ng/kg, s.c.)	Observations
1–6	30–300	Body sag, ataxia, slowing, ptosis, drowsy, scratching.
7–8	600–1200	Same as above. Dosing frequency increased to 6 times/day on weekdays because of short duration of action. One monkey on day 8 and 2 on day 9 briefly lost consciousness.
9–16	600	Dose reduced because one monkey lost consciousness. Same behavioral signs as above except no loss of consciousness.
16		**1st PRECIPITATED WITHDRAWAL** On day 16, 2 h after dihydroetorphine, a challenge dose of naloxone (0.05 mg/kg) which would produce severe withdrawal in morphine-dependent monkeys was ineffective. Naloxone dose increase by a factor of 10. Very mild withdrawal syndrome noted (restlessness, 1/5; wet-dog shakes, 3/5; retching, 1/5; fighting, 1/5; coughing,1/5; and masturbation, 1/5). All monkeys had relaxed abdominal muscles and failed to vocalize when palpated.
17–20	1200	Dose raised to 1200 ng/kg after precipitated withdrawal test completed. Only the signs designated as body sag, slowing and scratching were noted.
21–30	900	Same as above. However, dose reduced to 900 ng/kg because of brief loss of consciousness in two monkeys.
31	900	**2nd PRECIPITATED WITHDRAWAL** A very high dose of naloxone (0.55 mg/kg s.c.) elicited very mild withdrawal (lying down, 1/5; pacing, 1/5; wet-dogs, 3/5; and coughing 1/5).The usual mu-agonist signs designated body sag, jaw sag, slowing and scratching were noted.
31–41	900	Same signs noted as above.
41		**ABRUPT WITHDRAWAL** 6 h after the last dose of dihydroetorphine, 2/5 avoid contact, 1/5 vocalized and 1/5 pacing. 12 h after last dose of dihydroetorphine 2/5 pacing and 1/5 had tremors. At both time intervals all had relaxed abdominal muscles and none vocalized when palpated.
42	900	**3rd PRECIPITATED WITHDRAWAL** Monkeys were given dihydroetorphine, 900 ng/kg, and 0.5 h later challenged with naloxone (0.55 mg kg). Wet-dog shakes observed in 1/5. However, abdomens were relaxed. Some dihydroetorphine-agonist signs still evident.

4. Discussion

In our laboratory, dihydroetorphine and etorphine displayed the same profile of activity in mice. The results of time-course studies in mice, suggested that they are short-acting antinociceptive agents compared to morphine. Interactions with selective opioid-receptor antagonists established that etorphine and dihydroetorphine-induced antinociception was mediated by μ-opioid receptors. Schild regressions with slopes of near unity provided evidence that the etorphines acted competitively with naloxone at the opioid receptor. Interestingly, Matthes et al. (1996) showed that genetically altered mice lacking μ receptors were devoid of morphine-induced analgesia, reward effects and physical dependence liability. In addition, they demonstrated that δ-and κ-opioid receptors did not mediate any of morphine's main effects. Other investigators (Tokuyama et al., 1993; Kamei et al., 1995; Wang et al., 1995) also concluded that dihydroxyetorphine-induced antinociception was solely μ-opioid receptor mediated. Apparent differences between etorphine and dihydroetorphine have also been recorded. For example, Wang et al. (1995) found that etorphine, but not dihydroetorphine, nonselectively interacted with μ-, δ- and κ-opioid receptors. Other workers (Xu et al., 1992) demonstrated that etorphine-induced antinociception was associated wholly with μ-opioid receptors when given i.c.v. and nonselective when administered intrathecally. Furthermore, the inhibitory effects of etorphine and dihydroetorphine on dorsal-root ganglion neurons also appeared to act nonselectively on all opioid receptor subtypes (Shen and Crain, 1994; Crain and Shen, 1996).

There are also reports indicating high binding affinities for these oripavine derivatives for μ-, κ- and δ-opioid receptors (Magnan et al., 1982; Xu et al., 1992; Wang et al., 1995; Niwa et al., 1995). In fact, differential effects were demonstrated for [^{3}H] etorphine binding in vitro and in vivo (Kurowski et al., 1982). Nevertheless, differences between in vitro and in vivo studies are not uncommon; the possible causes are many. For example, one cannot be certain that the concentration of agonist is sufficient to achieve pharmacological function in vivo as opposed to in vitro. Also, cloned δ-opioid receptors have been reported to change their coupling from G_i to G_s when transfected (Wu et al., 1995). Too, binding alone is insufficient to effect a pharmacological response if coupling is required. Lack of correspondence between binding and efficacy may also be related to disturbances of the dynamic interactions with G-coupled components or transduction mechanisms of the receptor or its environment. Perhaps too, the etorphines may bind to more than one site on the opioid receptor(s) or on the same site that is coupled to both G_s and G_i/G_o regulatory proteins as was suggested by Fan and Crain (1995).

According to the American Psychiatric Association (1994), physical dependence is not a necessary criterion for the diagnosis of psychoactive substance abuse disorder. Nevertheless, for opiate addicts, physical withdrawal is so distressful that addicts use opiates to stop the pain and other withdrawal effects (Kleber cited in Altman et al., 1996).

In our laboratory, in single-dose substitution studies in morphine-dependent monkeys in withdrawal, dihydroetorphine and etorphine behaved similarly, i.e., they both substituted completely for morphine. Again, dihydroetorphine was more potent. These results are in accord with those reported in monkeys for etorphine (Deneau and Seevers, 1963) and for dihydroetorphine (Wang et al., 1992a; Huang et al., 1994). Dihydroetorphine (Wang et al., 1992b; Ge et al., 1994) and etorphine (Jasinski et al., 1975), also suppressed withdrawal in opioid-dependent human subjects. Our primary physical dependence study suggested that dihydroetorphine had little, if any, potential in this respect. The present results are in good agreement with previous studies on chronic dihydroetorphine-treated monkeys in China (Wang et al., 1992a) and chronic etorphine-treated dorsal-root ganglion neurons in culture (Shen and Crain, 1994; Crain and Shen, 1995a). Finally, it should be noted that etorphine was said to produce physical dependence in mice and rats in the absence of tolerance (Roerig et al., 1985).

Some anomalies were noticed in our studies. The duration of action of antinociception in mice and of the overt behavioral signs observed in monkeys not dependent on morphine was short. Yet, when dihydroetorphine was given to monkeys in a preexistent state of morphine-induced physical dependence, withdrawal was suppressed for at least 2.5 h. Perhaps, morphine-induced physical dependence is associated with a conformational change or dissociation of the μ-opioid receptor or with altered interactions among different opioid receptors.

Many inherent physicochemical and pharmacokinetic properties including pH, lipophillicity, hydrosolubility, protein binding, absorption, distribution, clearance and metabolism, can influence the development and expression of physical dependence. Continuous exposure to a drug usually overcomes these problems and greatly facilitates its development. Patrick and Harris (1997) conducted such an experiment in rats and reported atypical results. For example, although some opiate-like behavioral abstinence signs were observed when dihydroetorphine was abruptly withdrawn, pronounced body weight loss was not observed. In this laboratory, body weight loss is critical for assessing the physical dependence capacity of a typical opiate in the rat.

Although physical dependence is a significant factor contributing to the abuse of opioids, other important properties such as tolerance, reward, compulsive abuse and relapse potential must also be addressed. Recently, Beardsley and Harris (1997) using an FR10 schedule (fixed-ratio 10), demonstrated that dihydroetorphine was self-administered by rhesus monkeys. Most, but not all drugs that are

self-administered in humans are reinforcers in animals exposed to operant paradigms (Schuster and Johanson, 1981) and are often associated with high dependence liability (e.g. Gardner, 1992). In this regard, etorphine and dihydroetorphine are unique opioid alkaloids. Importantly, dihydroetorphines's atypical effects may only be manifested after chronic administration.

Finally, although we have focused our discussion on physical dependence, we should like to emphasize that opioids with limited ability to produce tolerance and physical dependence may be particularly useful in the clinical treatment of chronic pain.

Although it may be premature to conclude that these etorphines are the long sought 'ideal opioids', there are sufficient intriguing data to justify fully exploring their mechanism(s) of action and therapeutic potential.

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