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Potent reinforcing effects of dihydroetorphine in rats

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Abstract

Dihydroetorphine is a novel opioid that is an extremely potent analgesic in rodents. The reinforcing potency was determined in rats trained to self-administer heroin and compared to those of fentanyl, heroin, 6-acetylmorphine and morphine for assessment of the abuse potential of dihydroetorphine using a procedure that determines the dose–effect curve in individual sessions. Dihydroetorphine produced a bimodal dose–effect curve similar to that of other opioids. Potency ratios were determined with morphine for the ascending and descending limbs of the dose–effect curve, as well as the dose that yielded maximal response rate. Fentanyl, heroin and 6-acetylmorphine were approximately 100, 8 and 2 times more potent than morphine in maintaining self-administration, respectively. Dihydroetorphine was roughly 1500 to 3000 times more potent than morphine, however, depending upon the limb of the dose–effect curve used for comparison. These potency ratios of dihydroetorphine to morphine were somewhat less than has been reported for analgesia assays, and therefore this compound may have some clinical advantages over other opioids. However, these studies indicate significant abuse liability for dihydroetorphine given its potency in maintaining self-administration in these animals.

Keywords: Fentanyl; Heroin; Morphine; 6-Acetylmorphine; Reinforcement

1. Introduction

Drug self-administration procedures were developed in the late 1960s as a preclinical model of human drug abuse and have provided a wealth of information on the abuse liability of novel compounds and on mechanisms of drug reinforcement. Lever-pressing can be readily engendered in rats by reinforcement with infusions of a variety of potent opioids (Koob, 1992). This model provides a direct assessment of the reinforcing efficacy of compounds. Coupled with preclinical models of opioid analgesia, self-administration studies can be useful in assessing the abuse potential of a novel compound for the purpose of developing efficacious analgesics with lower abuse liability.

Dihydroetorphine is an opioid analgesic with extraordinary potency. Potency ratios of dihydroetorphine to morphine in mouse tail flick are on the order of several thousand (Huang and Qin, 1982). Furthermore, this compound produces a physical withdrawal syndrome that is much less severe than morphine in mice (Wang et al., 1995), rats (Patrick et al., 1996) and monkeys (Aceto et al., 1996). Therefore, dihydroetorphine shows promise as a clinically useful opioid analgesic. However, information regarding the abuse liability and reinforcing potency of this drug is lacking. The potency of dihydroetorphine in maintaining self-administration in rats trained to self-administer heroin was determined and compared with those of fentanyl, 6-acetylmorphine and morphine. These data will be useful for the preclinical assessment of the abuse liability of dihydroetorphine and for comparison with analgesic potency.

2. Materials and methods

2.1. Subjects

Male, Fischer 344 rats (250–300 g) were used for these experiments (SASCO, Omaha, NE, USA). Animals were housed individually on a reversed light-dark cycle (dark 15:00–03:00) and given ad libitum access to food and water prior to surgery.

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2.2. Surgical procedures

All animals were anesthetized with pentobarbital (50 mg/kg, i.p.) and implanted with intravenous jugular catheters according to previously published methods (Martin et al., 1996). The catheter was inserted into the right exterior facial vein, terminated just above the right auricle and extended subcutaneously to the back of the animal where it exited between the scapulae. The catheter was connected to an infusion pump (Razel, Stamford, CT, USA) through a fluid swivel and a spring leash. The spring leash was attached to the animal by a polypropylene plate that was encased in Teflon mesh and implanted subcutaneously. Penicillin G procaine (75 000 units) was administered i.m. and exterior surgical wounds were dressed with antibiotic powder (Polysporin, Wellcome-Glaxo, Research Triangle Park, NC, USA) following surgery. All animals were housed individually in acrylic cages ($24 \times 26 \times 21$ cm). Catheter patency was maintained by hourly infusions of 0.2 ml of 0.9% saline (w/v) with heparin (pH 7.4, 1.7 units/ml) and checked periodically by observing the length of time necessary for loss of consciousness following administration of methohexital (10 mg/kg, i.v.).

2.3. Apparatus

All experimental sessions were conducted in sound-attenuated chambers and were controlled by an IBM-compatible computer through an interface (MED Associates, St. Albans, VT, USA). The operant chamber (21 cm $\times$ 21 cm $\times$ 28 cm) contained a response lever 6.8 cm above the floor and 1.1 cm from the rear wall and a light located 4.0 cm above the response lever. Each sound attenuated chamber contained a house light, tone generator and a ventilator fan. The fluid swivel and catheter were connected to an infusion pump located outside of the sound-attenuated enclosure through a 20 gauge Luer hub and a 22 gauge male connector.

2.4. Training of heroin self-administration

Following 5–7 days of recovery from surgery, responding on a lever was established using a multiple-dosing procedure with a range of four doses of heroin under a fixed-ratio 1 schedule of reinforcement according to a previously published method (Martin et al., 1996). The dose of heroin was varied by altering the duration that the infusion pump was activated such that operation of the pump for 1.7, 2.8, 5.6 or 9.3 s delivered infusions of 5.4, 9, 18 or 30 μg of heroin (18, 30, 60 or 100 μg/kg for a 300 g rat). The concentration of heroin delivered was 90 μg/ml. During self-administration sessions, the animals were placed in operant chambers and, following a 10 min acclimation period, an infusion of the dose of heroin available for the first hour was delivered, and the lever light was illuminated to indicate drug availability. Upon completion of the ratio requirement, the lever light was darkened and an infusion of heroin was delivered. A 30 s time-out period followed that was signaled by the operation of the house light and tone. Each successive session hour was initiated by delivery of an infusion of the dose of heroin available for that hour. The order of dose presentation was randomized using a random number generator in the Med-PC programming language (MED Associates) at the beginning of each session for each animal. The four hourly segments of the session were separated by 20 min time-out periods during which all lights were extinguished and lever presses had no programmed consequences. Responding was considered stable when the number of infusions at each dose of heroin for each of five successive days did not vary by more than 10% of the mean. When stable responding was established, the ratio requirement was increased from 1 to 10 across experimental sessions. Saline was substituted for all doses of heroin within a session following 5 days of stable responding at FR10.

2.5. Determination of dose–effect curves

Both the ascending and descending portions of the dose–effect curves were determined for heroin, morphine, fentanyl, 6-acetylmorphine and dihydroetorphine by substituting these drugs for the training doses of heroin on Tuesdays or Thursdays, provided that the number of heroin infusions administered did not vary by more than 10% of the mean in the previous session for each dose. The infusion times were the same as for heroin, and the range of available doses was varied by changing the concentration of each drug in the syringe during substitution sessions.

2.6. Drugs and chemicals

Heroin hydrochloride, morphine sulfate and fentanyl hydrochloride were obtained from Research Triangle Institute (Research Triangle Park, NC, USA) and dissolved in 0.9% saline (w/v) pH 7.4 with 1.7 U/ml of heparin. Dihydroetorphine was provided by the National Institute on Drug Abuse and was dissolved in 0.0014% 2-hydroxypropyl-β-cyclodextrin (w/v) in 0.9% saline(w/v) pH 7.4 with 1.7 U/ml of heparin. All drugs for self-administration were sterilized by filtration through a polyethersulfone filter (Supor Acrodisc, Gelman Sciences, Ann Arbor, MI, USA). Pentobarbital (Nembutal) was purchased from Abbott Laboratories (North Chicago, IL, USA) in a vehicle of 10:40:50 (v/v) ethanol-propylene glycol-water. Atropine sulfate and 2-hydroxypropyl-β-cyclodextrin were purchased from Sigma (St. Louis, MO, USA) and dissolved in 0.9% (w/v) sodium chloride at a pH of 7.4. Methohexital (Brevital) was purchased from Eli Lilly (Indianapolis, IN, USA) and dissolved in sterile water. Antibiotic powder (Polysporin) was purchased from Wellcome-Glaxo and heparin sodium was purchased from Elkins-Sinn (Cherry

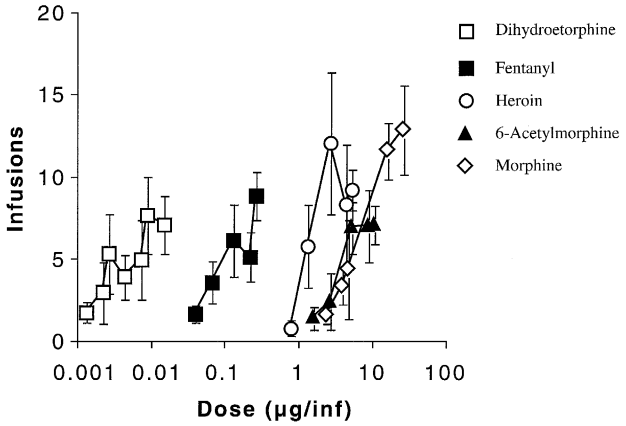

Fig. 1. Ascending dose–effect curves for self-administration. Shown are the mean (S.D.) for the number of infusions administered during the hourly component for each dose of the indicated drug. $n = 8$.

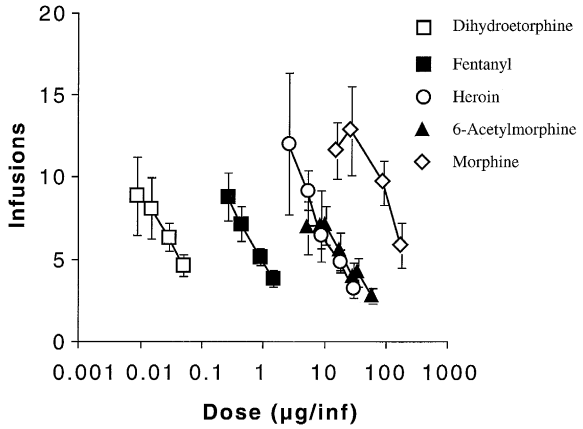

Fig. 2. Descending dose–effect curves for self-administration. Shown are the mean (S.D.) for the number of infusions administered during the hourly component for each dose of the indicated drug. $n = 8$.

Hill, NJ, USA). Penicillin G procaine was purchased from Butler (Columbus, OH, USA) as an aqueous suspension of 300 000 U/ml. All doses are reported in terms of the free base of the drug.

2.7. Data analysis

The dose–effect curves were fit to the general logistic form of the dose–effect equation using commercially available curve-fitting software (Prism2, GraphPad, San Diego, CA, USA). Estimates of the log ED_{50}'s, standard errors and 95% confidence intervals were generated from the fitted curves. The maximum number of infusions obtained for each drug was compared using analysis of variance (ANOVA).

3. Results

All of the drugs tested maintained responding in a biphasic manner with respect to dose, and the ascending and descending limbs of the dose–effect curves are presented separately in Figs. 1 and 2, respectively. Substitution of saline or 0.0014% β-cyclodextrin for heroin significantly decreased responding, with the mean (S.E.M.) number of infusions administered in the successive hourly components being 1.1 (0.5), 1.5 (0.8), 0.5 (0.3) and 0.4 (0.4) for saline and 0.8 (0.3), 0.2 (0.1), 0.8 (0.4) and 0.8 (0.4) for 2-hydroxypropyl-β-cyclodextrin. Dihydroetorphine displayed tremendous reinforcing potency in these animals, with ascending and descending ED_{50} values of 5.0 and 38.0 ng/infusion, respectively (Table 1). As can be seen from the 95% confidence intervals, the ED_{50} values were significantly different between all of these compounds with the exception of heroin and 6-acetylmorphine (Table 1). The maxima of the dose–effect curves were not significantly different between any of the drugs. The maximum number of infusions of dihydroetorphine administered was 8.8 (2.4) at a dose of 9 ng/infusion. The maximum number of infusions of fentanyl administered was 8.8 (1.5) at a dose of 0.27 µg/infusion. The maximum number of infusions of heroin administered was 12.0 (4.4) at a dose of 2.7 µg/infusion. The maxima of the 6-acetylmorphine and morphine dose–effect curves were 7.0 (1.1) at a dose of 10.8 µg/infusion and 12.8 (2.7) at a dose of 27 µg/infusion, respectively. The slopes were not significantly different for either the ascending or descending limbs of the fitted dose–effect curves between any of these compounds (data not shown).

The potency ratios of these compounds differ depending upon the whether the ascending or descending limb of the dose–effect curve was used for calculation. Using the

Table 1
Potency comparisons between dihydroetorphine and standard opioids

	Ascending			Descending		
	log ED_{50}	ED_{50}	95% C.L.	log ED_{50}	ED_{50}	95% C.L.
Dihydroetorphine	−2.30 (0.10)	0.005	0.003–0.009	−1.42 (0.05)	0.038	0.024–0.060
Fentanyl	−0.93 (0.10)	0.12	0.06–0.25	−0.20 (0.04)	0.64	0.45–0.89
Heroin	0.11 (0.05)	1.29	0.92–1.81	1.11 (0.06)	12.76	8.31–19.56
6-Acetylmorphine	0.49 (0.06)	3.08	1.72–5.52	1.10 (0.05)	12.54	9.24–17.02
Morphine	0.85 (0.01)	7.15	6.23–8.20	2.02 (0.09)	104.70	44.53–246.40

Shown are the ED_{50} estimates (µg/infusion) from the nonlinear curve fit of dose–effect data.

ED_{50} values of the ascending limb (Table 1), the potency ratios of dihydroetorphine, fentanyl, heroin and 6-acetylmorphine to morphine are 1430, 60, 5.5 and 2.3, respectively. Using the ED_{50} values of the descending limb (Table 1) the respective potency ratios to morphine are 2755, 164, 8.2 and 8.3. Using the dose that yields the maximum in the dose–effect curve (given above), the respective potency ratios for these compounds are 3000, 100, 10 and 2.5 for dihydroetorphine, fentanyl, heroin and 6-acetylmorphine.

4. Discussion

Dihydroetorphine substituted for heroin with tremendous potency, being approximately 300 times more potent than heroin and 1500 to 3000 times more potent than morphine. These studies therfore predict significant abuse potential for dihydroetorphine in humans. The potency ratios of the other compounds compared to morphine are consistent with literature reports. Fentanyl and heroin are more potent than morphine in maintaining self-administration in rats with reported potency ratios of 260 and 13 compared to morphine, respectively (Van Ree et al., 1978), which are similar to those reported in the present study (see Results). Heroin and 6-acetylmorphine are equipotent in decreasing the reward threshold for intracranial self-stimulation and are 40 times more potent than morphine (Hubner and Kornetsky, 1992). The potency ratios of heroin and 6-acetylmorphine to morphine are approximately 6.5 and 1.7, respectively, in increasing the escape threshold for intracranial stimulation in rats (Hubner and Kornetsky, 1992). These data are consistent with the present findings of potency ratios of 5.5–10 for heroin and 2.5–8.3 for 6-acetylmorphine compared to morphine.

The potency ratios for each compound compared to morphine were dependent upon the region of the dose–effect curve that was used for the calculation. This is undoubtedly due to the fact that the inverted U-shaped dose–effect curves are the result of multiple drug effects, and that the potency ratios of these compounds with morphine for producing these effects do not necessarily have to be similar. It has been suggested that the ascending limb of the dose–effect curve generated in self-administration experiments is more indicative of the reinforcing potency of a drug, and that the descending limb is due to both drug satiety and response rate-decreasing effects of the drug (Katz, 1989). Therefore, the potency ratios generated using the data from the ascending limb may be more indicative of the relative potencies of these compounds with respect to morphine for producing reinforcing effects. The potency ratios generated using the data from the descending limb may indicate relative potencies for producing locomotor effects or other response rate-decreasing effects. Differences in the descending limb may also be the result of pharmacokinetics, since fewer infusions of a drug with a comparably longer half-life will be taken once a maximally reinforcing dose has been reached. The apex of the dose–effect curve is a result of the combination of response rate-increasing and response rate-decreasing effects, and depends upon the potency and efficacy of each compound for producing these various effects. Therefore, the complex nature of the data generated from drug self-administration experiments has led to the calculation of multiple potency ratios for these compounds with respect to morphine.

The potency ratios of these compounds to morphine are comparable to those obtained using analgesia as the pharmacological measure. Heroin is 3–4 times more potent than morphine in producing analgesia in mice, with 6-acetylmorphine being slightly less potent than heroin (Umans and Inturrisi, 1981). Fentanyl is approximately 80 times more potent than morphine in producing analgesia in rats (Paronis and Holtzman, 1991). Dihydroetorphine is several thousand times more potent in analgesic assays than morphine (Huang and Qin, 1982). Therefore, dihydroetorphine would have less abuse potential than morphine at equianalgesic doses. These findings together with the purported limited ability of dihydroetorphine to produce physical dependence in laboratory animals, suggest that this compound has pharmacological advantages over morphine that may prove useful clinically. The present data clearly demonstrate that dihydroetorphine posesses significant abuse potential, however.

Dihydroetorphine may also be a valuable research tool. It would be important to determine if this compound displays a higher efficacy as well as potency compared to other opioids using irreversible antagonists. Given the diminished ability of this compound to produce a physical withdrawal syndrome, it would be informative to determine if this difference is due to its ability to bind to the opioid receptor or its ability to stimulate second messenger systems. Such studies may ultimately prove useful for investigating the mechanisms of opioid tolerance and withdrawal.

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