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Dihydroetorphine: physical dependence and stereotypy after continuous infusion in the rat

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Abstract

In a previous study in this laboratory, exposure of rhesus monkeys to intermittent, high doses of dihydroetorphine for 42 days did not evoke behavioral signs of physical dependence on this opioid either after it was abruptly withdrawn or after challenge with a high dose of naloxone. To investigate further the physical dependence capacity of this opioid, it was given by infusion to rats thereby exposing receptors chronically and continuously to this opioid. Abstinence expressed as body weight loss, irritability, and wet-dog shakes was observed after abrupt withdrawal of the low-dose regimen (5,10, 40 and 40 μg/kg per day for 4 days, respectively). The high-dose regimen (10, 20 and 80 μg/kg per day for 3 days, respectively) produced stereotypy and physical dependence. Although many reported molecular events and dependence studies suggest otherwise, dihydroetorphine's propensity to produce physical dependence, an important determinant of opioid abuse, is real.

Keywords: Dihydroetorphine; Morphine; Physical dependence; Stereotypy; Continuous infusion; (Rat)

1. Introduction

Dihydroetorphine, a 6,14-endoethenotetrahydro-oripavine derivative is one of the most potent opioids ever synthesized. Its antinociceptive potency estimate was reported to be in the range of 10 000–20 000 times morphine (Bentley and Hardy, 1967; Aceto et al., 1997). Although high binding affinities for μ-, κ-, and δ-opioid receptors were reported (Magnan et al., 1982; Xu et al., 1992; Niwa et al., 1995; Wang et al., 1995), in vivo testing suggested that dihydroetorphine-induced antinociception was μ-opioid receptor mediated (Tokuyama et al., 1993; Kamei et al., 1995; Wang et al., 1995; Aceto et al., 1997). Unexpectedly, physical dependence on dihydroetorphine was not observed in mice, rats and monkeys receiving intermittent doses (Wang et al., 1992a; Qin, 1993; Tokuyama et al., 1993; Huang et al., 1994). In this laboratory, even after subjecting rhesus monkeys to four to six injections per day for 42 days at doses which included loss of consciousness, we could not demonstrate physical dependence on dihydroetorphine either after its abrupt withdrawal or after periodic challenges with high doses of naloxone (Aceto et al., 1997). Interestingly, dihydroetorphine was also reported effective in detoxifying human opioid addicts (Wang et al., 1992b; Qin, 1993).

Nevertheless, dihydroetrophine evoked heroin-like discriminative stimulus effects in rats and was self-administered by rats and rhesus monkeys (Beardsley and Harris, 1997; Martin et al., 1997) and after a decade of use as an analgesic in China, widespread abuse was cited (B.-Y. Qin, personal communication cited in Beardsley and Harris, 1997).

We decided to evaluate dihydroetorphine's physical dependence liability under conditions in which the rats were exposed chronically and continuously to this μ-opioid receptor agonist.

2. Methods

2.1. Subjects

All rats received care according to ''Guide for the Care and Use of Laboratory Animals'', DHHS Publication, Revised, 1996. The facilities are certified by the American

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Association for the Accreditation of Laboratory Care. These studies were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University.

2.2. Continuous-infusion protocol

The experimental procedure was described earlier (Aceto et al., 1998) and is based on the method reported by Teiger (1974). Briefly, adult male Sprague Dawley rats were purchased from Harlan Sprague, Incorporated (Indianapolis, IN). Upon arrival the rats were examined and placed in quarantine. They were in the weight range of 260–280 g when assigned to a study. Once selected they were housed individually in stainless steel cages. The vivarium was temperature and humidity controlled with alternating light–dark cycles (lights on at 06:00 h and off at 24:00 h).

The rats were acclimated to their new surroundings for at least 3 days before intraperitoneal (i.p.) cannulas were implanted. After they were anesthetized with pentobarbital (45 mg/kg administered i.p.), the lateral side of the lower left abdomen and back of the neck were shaved and the exposed skin cleansed with Pavidone–Iodine Solution (Redi-Product, WV). Then, each rat was fitted with a cannula (PE90 tubing, Clay Adams, NJ). The cannula passed subcutaneously from the nape of the neck to the lateral side of the lower abdomen. The peritoneal end of the cannula was enclosed in silastic tubing to prevent foreign body reaction. It was introduced into the peritoneal cavity through a stab-wound entry site. The cannula was secured with sutures at both sites. Then, each rat was fitted with a harness. The harness consisted of a flat stainless steel plate fitted with a shoulder collar, a narrow strip of Velcro and a spring coil. Its collar was passed over the head of the rat and the harness was secured by means of the strip of Velcro which girdled the chest. The cannula passed through the harness and spring coil and was then attached to a flow-through swivel (Instech Lab., Horsham, PA). The swivel allowed the rat to move about in its cage and eat and drink normally. An infusion pump (Harvard Apparatus, S. Natick, MA, Model-945) delivered the solutions to the swivel in a volume of 8 ml every 24 h. During the administration of drugs or vehicle, fresh solutions were prepared daily after the rats were weighed.

2.3. Primary physical dependence study

A synopsis of the experiments is in Table 1. Each rat was randomly allocated one of the treatment regimens designated: (1) vehicle controls; (2) morphine controls; (3) dihydroetorphine-low; and (4) dihydroetorphine-high. They were then randomly assigned to a home 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. Finally, the dose regimens selected for dihydroetorphine are related to the antinociceptive ED_{50}.

Table 1
Synopsis of the dose regimens and combined number of subjects employed in the evaluation of dihydroetrophine

Treatment regimen	Day				Number of subjects
	1	2	3	4	
High Dose Regimen Dihydroetorphine μg/kg per 24 h[a]	10	20	80	–	5
Low Dose Regimen Dihydroetorphine μg/kg per 24 h	5	10	40	40	5
Morphine Dose Regimen mg/kg per 24 h	50	100	200	200	10
Vehicle Regimen 8 ml/24 h	–	–	–	–	10

[a]This treatment regimen was stopped on day 3 because stereotyped behavior developed. Data not included in the statistical analysis.

2.4. Behavioral ratings

During the infusion of vehicle, dihydroetorphine or morphine, the rats were weighed and observed daily for 1 h for overt behavioral signs. In addition, they were watched for withdrawal signs at 24 and 48 h following the abrupt withdrawal of vehicle, dihydroetorphine or morphine. Body weight was recorded at 24 h intervals through day 8. During abrupt withdrawal, the following signs were seen: irritability, front-paw shakes; wet-dog shakes; facial rubbing with front paws; eyelid ptosis; and immobility. The sign of wet-dog shakes was quantified. Irritability was scored as proposed by Teiger (1974). Scoring was as follows: 0 (remained tame when touched and on being grasped and lifted); 1 (remained tame when touched and on being grasped and lifted made only a feeble attempt to wiggle free); 2 (remained tame when touched but when grasped and lifted clawed, bit and or vocalized); and, 3 (reacted to initial touch by vocalizing and biting and to attempts to grasp it by rolling over on its back and clawing). Stereotypy was evaluated using a modified scoring system (Johansson et al., 1991) that was originally proposed by Magos (1969). If a rat showed no stereotyped behavior, it received a score of 0. The rat was scored 1 for continuous sniffing; 2 for continuous licking of cage parts or grid or poking nose through grid floor or mock biting; 3 for either stereotyped forceful biting of cage parts or grid or continuous gnawing on paws; and 4 for autophagia or gnawing and biting which was accompanied by tissue damage. All other signs were simply noted. A trained observer was blind regarding treatment assignments.

2.5. Statistical analysis

The data from two experiments were combined and then grouped by treatment regimen and analyzed. Quantified data were assessed using repeated measures analysis of variance. If overall significance was found, the conservative Scheffé test (pair-wise comparisons) was used for post-hoc analysis. Scored data were analyzed using the nonparametric Kruskal–Wallis one-way analysis of variance. Post hoc comparisons were made using the Mann–Whitney *U*-test. In all cases significance was set at $P = 0.05$ or less. The StatView statistical package (Brainpower, Agoura Hills, CA) was utilized for these analyses.

2.6. Chemical supplies

Dihydroetorphine was obtained from the National Institute on Drug Abuse. Morphine sulfate was purchased from Mallinckrodt, St. Louis, MO. Sterile water (USP), was acquired from Baxter Healthcare, Deerfield, IL. Other necessary supplies were bought from sources indicated above. All drugs were dissolved in sterile water.

3. Results

Because pronounced stereotypy developed in the high-dose-treated rats on day 3 and to avoid the imminent development of autophagia, the infusion of dihydroetorphine was stopped and vehicle was substituted. The abstinence data of this 3-day dose regimen was excluded from the statistical analysis of the 4-day infusion results. However, 24 h later the rats receiving this dose regimen lost approximately 10% of their body weight, were irritable, and developed wet-dog shakes. And, withdrawal was of the same magnitude as that of the low dose dihydroetorphine-treated rats (data not shown).

3.1. Withdrawal associated body-weight changes

The low-dose dihydroetorphine group data as well as that of the vehicle and morphine controls are illustrated in Fig. 1. Two-factor repeated measures analysis of variance indicated significant differences among the treatment groups ($F = 11.199$, $P = 0.0004$) and for days ($F = 106.176$, $P = 0.0001$). One factor analysis of variance of the body weights at the start of the experiment indicated no significant differences ($F = 0.146$, $P = 0.8655$). Analysis of variance for day 1($F = 10.044$, $P = 0.008$) day 2 ($F = 7.487$, $P = 0.05$), day 3 ($F = 8.219$, $P = 0.0022$), day 5 ($F = 22.395$, $P = 0.0001$), day 6 ($F = 122.578$, $P = 0.0001$), day 7 ($F = 108.412$, $P = 0.0001$), and day 8 ($F = 69.965$, $P = 0.0001$) but not day 4 ($F = 2.31$, $P = 0.1228$) indicated significant differences in body weight among treatments. Post hoc comparisons showed that the morphine controls initially gained weight during the first 2 days ($P = 0.05$); but, by day 4 body, weights had returned to pre-infusion levels. The rats that received dihydroetorpine also gained significantly more weight than the vehicle-treated rats. Body weight on day 3 were also significantly greater than that of the morphine controls. Then, after dihydroetorphine was abruptly withdrawn, body weights fell precipitously compared to vehicle controls. The difference was greatest on day 5 (24 h after abrupt withdrawal). For the rest of the experiment, body weights began increasing although they were still depressed significantly compared to vehicle. Body weight changes of the morphine-treated rats followed the same course except that the peak loss occurred on day 6. The vehicle controls gained weight slowly as expected. Finally, post hoc comparisons indicated statistically significant differences between the morphine group and dihydroetorphine group on days 6 (48 h), 7 (72 h), and 8 (96 h).

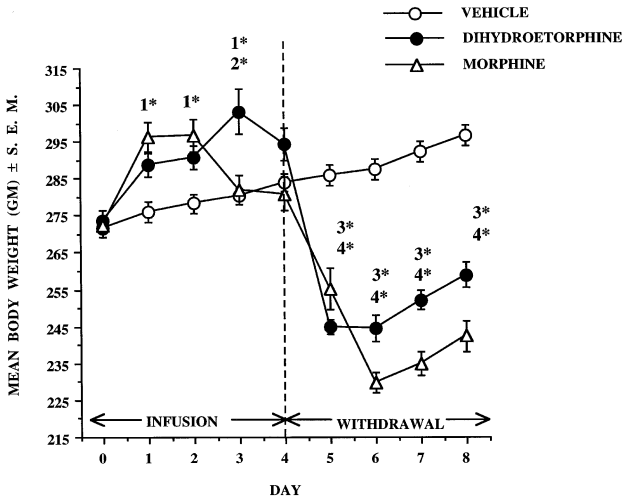

Fig. 1. Effects on body weights of continuous infusions of solutions of a low dose of dihydroetorphine, morphine or vehicle prior to, and after abrupt withdrawal. Each treatment schedule was given i.p. for 4 days. Then, drug regimens were abruptly stopped and vehicle was substituted. (1) During the infusion, body weights of both opioids were significantly greater than that of the vehicle control. (2) During the infusion, body weights in the dihydroetorphine group were significantly greater than those in the morphine-treated rats. (3) After withdrawal, the body weights of both opioid-treated rats were significantly less than those of the vehicle-treated rats. (4) After withdrawal, the body weight of the dihydroetorphine-treated rats were significantly less than those of the morphine-treated rats. Data were analyzed using repeated measures analysis of variance. The conservative Scheffé test (pair-wise comparisons) was used for post-hoc analysis. *P* was set at 0.05 or less. The data in the figure are expressed as mean ± S.E.M.

3.2. Withdrawal associated wet-dog shakes

The results are depicted in Fig. 2. Repeated measures ANOVA indicated significant *F* values for days ($F = 24.99$, $P = 0.0001$), and for wet-dog shakes ($F = 6.373$, $P = 0.0066$). One factor analysis of variance of the data on day 4 (pre-withdrawal) indicated no significant differences among treatments. Significant differences among treat-

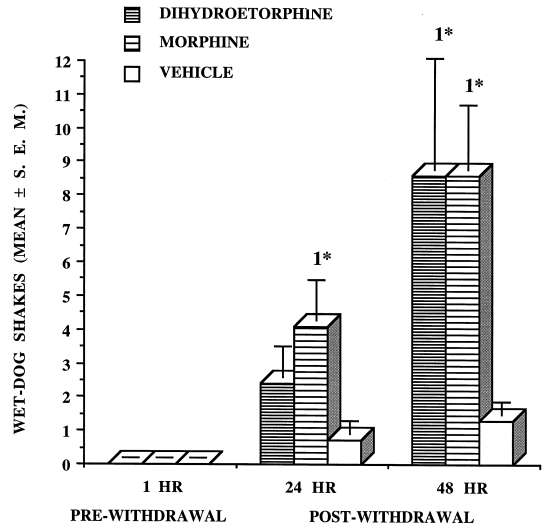

Fig. 2. Incidence of wet-dog shakes 1 h prior to, and at 24 and 48 h after abrupt withdrawal of the low-dose dihydroetorphine group, and the morphine sulfate or vehicle groups. Data were assessed using repeated measures analysis of variance. The conservative Scheffé test (pair-wise comparisons) was used for post-hoc analysis. * Significant when compared to corresponding vehicle controls. $P = 0.05$ or less. The data in the figure are expressed as mean ± S.E.M.

ments were evident at 24 h ($F = 4.019$, $P = 0.0325$) and 48 hr ($F = 6.284$, $P = 0.0069$) after withdrawal. Post hoc testing showed that the morphine controls displayed, significantly more wet-dog shakes compared to the vehicle controls at 24 and 48 h after it was abruptly withdrawn.

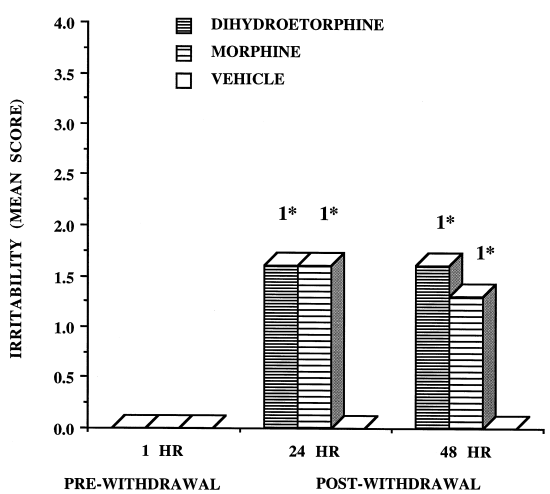

Fig. 3. Irritability score 1 h prior to and 24 and 48 h after abrupt withdrawal of the low-dose dihydroetorphine group, and the morphine and vehicle groups. Irritability was scored: 0 (remained tame when touched and on being grasped and lifted); 1 (remained tame when touched and on being grasped and lifted made only a feeble attempt to wiggle free); 2 (remained tame when touched but when grasped and lifted clawed, bit and or vocalized); and, 3 (reacted to initial touch by vocalizing and biting and to attempts to grasp it by rolling over on its back and clawing). The data were analyzed using the nonparametric Kruskal–Wallis one-way analysis of variance. Post-hoc comparisons were made using the one-tailed Mann–Whitney *U*-test. Significance was set at $P = 0.05$ or less. * Significant when compared to the corresponding vehicle score.

The dihydroetorphine-treated rats showed a substantially ($P = 0.05$) increased number of wet-dog shakes compared to vehicle at 48 h. Importantly, no statistically significant differences were found between dihydroetorphine and morphine.

3.3. Withdrawal-induced irritability

Analysis of variance using the Kruskal–Wallis nonparametric test indicated no significant differences among irritability scores on the day before abrupt withdrawal (day 4, $H = 0$). Twenty-four and 48 h following abrupt withdrawal, the calculated H values indicated significant differences among treatments $F = 14.931$, ($\chi^2_{0.005} = 10.60$) and $F = 15.047$ respectively. Post hoc comparisons using the Mann–Whitney *U*-test revealed that there were no significant differences between the dihydroetorphine and morphine groups at 24 and 48 h after withdrawal. Furthermore, the scores in the morphine or dihyroetorphine groups were significantly higher than those in the vehicle group ($P = 0.05$). The data are graphically portrayed in Fig. 3.

3.4. Stereotypy

As depicted in Fig. 4, stereotypy was evident on day 3 only. Kruskal–Wallis analysis of variance of this data

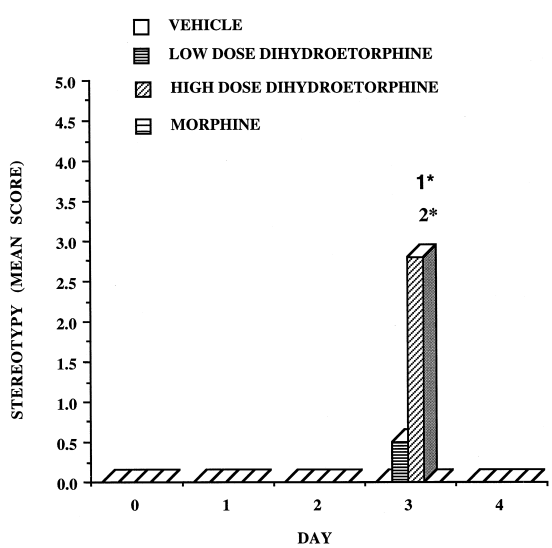

Fig. 4. Development of stereotypy during the chronic administration of the low- and high-dose dihydroetorphine regimens, and the morphine and vehicle schedules. If a rat showed no stereotypy, it received a score of 0. The rat was scored 1 for continuous sniffing; 2 for continuous licking of cage parts or grid or poking nose through grid floor or mock biting; 3 for either stereotyped forceful biting of cage parts or grid or continuous gnawing on paws; and 4 for autophagia or gnawing and biting which was accompanied by tissue damage. The data were analyzed using the nonparametric Kruskal–Wallis one-way analysis of variance. Post-hoc comparisons were made using the Mann–Whitney *U*-test. Significant at $P = 0.05$ or less. * Significant when compared with corresponding vehicle score. 2* Significant when compared with the dihydroetorphine group. Finally, the dihydroetorphine low-dose scores were not statistically greater than those of the vehicle group.

revealed a significant H value of 9.395 (c^2 0.02 = 9.35). Post hoc comparisons indicated that the difference between the dihydroetorphine high-dose regimen scores and vehicle scores was significant ($U = 2.5$, $P \leq 0.015$). In addition, the difference between scores for the groups receiving vehicle and low-dose regimens was not significant ($U = 12.5$, $P = 0.331$) whereas the difference between scores for the low and high doses were significantly different from one another ($H = 5$, $P = 0.041$). Dihydroetorphine was abruptly withdrawn in the high-dose regimen group to avoid further tissue damage. Stereotypy was no longer evident the next day. Instead, the rats were found in withdrawal.

4. Discussion

The literature on dihydroetorphine is replete with studies that suggest fundamental differences between it and other opioids. For example, opioid peptides and etorphine reportedly produced activation and rapid internalization of μ-opioid receptors. In sharp contrast morphine-activated m opioid receptors eluded desensitization and internalization (Keith et al., 1996, 1998; Whistler and Von Zastrow, 1998). Consequently, activated G proteins and disturbance of the β arrestin-mediated regulatory cycle were proposed to play a critical role in the development of tolerance and dependence. It is also reported that morphine has stimulatory and inhibitory effects (Smart and Lambert, 1996). In a series of studies on dorsal–root ganglion neurons in culture Crain and Shen (1992a,b, 1995a,b, 1996) and Shen and Crain (1992, 1994, 1995) found that most opioid agonists including morphine had bimodal excitatory and inhibitory properties. The excitatory effects are apparently mediated by opioid receptors coupled via a cholera toxin sensitive–stimulatory G protein (G_s) to adenylate/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 receptor that shortened the action potential. These investigators hypothesized that sustained activation of G_s-coupled opioid was associated with the development of physical dependence. Etorphine and dihydroetorphine elicited only inhibitory effects and therefore were not expected to produce physical dependence. In addition, the etorphines also displayed stimulatory antagonist effects that blocked tolerance development in chronic opioid-treated dorsal root ganglion. Others (Smart and Lambert, 1996) proposed that the stimulatory effects of opioids on the activation of inositol (1,4,5)-triphosphate, protein kinase C, calcium ion, and cyclic adenosine monophosphate (cAMP) and the subsequent stimulatory signal transduction and cross-talk played a role in the development of tolerance.

Indeed, multiple signaling roles for seven transmembrane domain receptors were proposed by Lefkowitz (cited in Schwartz and Ijzerman, 1998). For example, protein kinase A-mediated phosphorylation could switch a receptor from G_s to G_i coupling. Results reported in preclinical studies (Wang et al., 1992a; Tokuyama et al., 1993; Aceto et al., 1997) and in human opioid addicts (Wang et al., 1992b; Qin, 1993) are consistent with these reports. Remarkably, Wang et al. (1992a) also noted that substitution of dihydroetorphine for morphine in physically dependent rats and monkeys for 9 days apparently reverted the state of physical dependence to normal as evidenced by the fact that naloxone challenge did not precipitate abstinence signs.

Regarding our results, physical dependence on dihydroetorphine was evident and was at least of the same magnitude as that of morphine. In this study, the starting doses for the low- and high- dose regimens were 0.21 and 0.42 mg/kg per hour or approximately 1.4 and 2.8 times, respectively, of its antinociceptive ED_{50} per hour. The starting dose regimen for morphine was approximately 2.1 mg/kg per hour which was equivalent to its antinociceptive ED_{50} per hour. The results of this study were unlike those we observed in rhesus monkeys receiving intermittent injections (Aceto et al., 1997). Species issues aside, intermittent versus continuous stimulation of μ-opioid receptors could have different outcomes. With the intermittent protocol, wide fluctuations in the concentration of dihydroetorphine at the μ-opioid receptor site probably occurred. It is noteworthy that intermittent dose schedules were used in all the other published pre-clinical studies in which physical dependence on dihydroetorphine was not demonstrated (Wang et al., 1992a; Qin, 1993; Tokuyama et al., 1993). Studies involving human opioid addicts (Wang et al., 1992b; Qin, 1993) also utilized intermittent protocols. On the other hand, the continuous-infusion model and escalating-dose regimens we used presumably insured a steady and increasing concentration of dihydroetorphine on μ-opioid receptors. In a preliminary study, using a model similar to ours, Patrick and Harris (1997) suggested that physical dependence on dihydroetorphine was atypical because body weight loss, the most reliable index of opioid physical dependence (Akera and Brody, 1968; Aceto, 1990), was less than that of the morphine controls. In sharp contrast, in our study weight loss after abrupt withdrawal of dihydroetorphine was of the same magnitude as that of the morphine controls. Also, stereotypy was not observed in their study.

In other laboratories, dihydroetrophine evoked heroin-like discriminative stimulus effects in rats and was self-administered by rats and rhesus monkeys (Beardsley and Harris, 1997; Martin et al., 1997). Positive reinforcing effects of opioids were linked to μ-opioid receptors on neurons in the mesolimbic dopaminergic tract and the release of dopamine (Di Chiara and North, 1992). Most but not all drugs that evoke discriminative stimulus properties and are reinforces in animals are associated with high dependence liability in humans (Schuster and Johanson, 1981).

It is known that dependence on opioids involves different brain areas and neurotransmitter systems which have been linked to human opioid drug abuse behaviors. The pontine locus coeruleus and the periaqueductal gray area were implicated in the development of tolerance to and physical dependence on opioids (Aghajanian, 1978; Maldonado et al., 1992; Nestler et al., 1993) and that increased cyclic AMP-dependent protein kinase activity in the locus coeruleus correlated with the development of tolerance to and physical dependence on morphine (Nestler and Tallman, 1988). Stereotypy was reported associated with activation of the ventral tegmentum area dopaminergic neurons. Its proposed role in opioid abuse is in the acquisition and relapse stages (Altman et al., 1996).

In summary, dihydroetrophine, produced overt signs of physical dependence and stereotypy in the rat when given continuously. These manifestations involved separable neural systems and may reflect important aspects of human opioid drug abuse. The results of this study indicate that dihydroetorphine's potential abuse liability is real. Nevertheless, the judicious use of intermittent dose schedules of this high efficacy opioid could be used to advantage in the management of pain and opioid drug abuse.

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