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Dependence in Rats After One Injection of Heroin-, LAAM- or Hydromorphone-Zinc Tannate[1]

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BRANDS, B., J. C. BASKERVILLE, M. HIRST AND C. W. GOWDEY. *Dependence in rats after one injection of heroin-, LAAM- or hydromorphone-zinc tannate.* PHARMAC. BIOCHEM. BEHAV. 11(3) 279–282, 1979.—Complex zinc tannate salts of heroin, hydromorphone and *l*-α-acetylmethadol were synthesized and injected in a slow-release vehicle, into rats. One, 3, 7, 10 and 14 days after the drug was administered rats were injected with naloxone hydrochloride (10 mg/kg) and during the following 4 hours body weights, core temperature and behavioral signs such as diarrhea, writhing, teeth chattering and wet dog shakes were recorded. On every naloxone testing day the narcotic-treated groups presented behavioral signs of abstinence, but weight loss and temperature changes were much less consistent. Reduction of core temperature following naloxone administration seems to be an earlier indicator of physical dependence than weight loss. According to the parameters tested a level of physical dependence can persist for at least two weeks after a single injection of these narcotic salts.

Narcotic zinc tannate salts Heroin Hydromorphone *l*-α-acetylmethadol Phsyical dependence
Naloxone Weight loss Core temperature Abstinence scores

WE have demonstrated in mice that sparingly soluble pamoate and 3,5-di-*tert*-butyl-2,6-dihydroxybenzoate salts of heroin significantly increase the duration of analgesia when compared to that after the hydrochloride salt [4]. When naloxone was administered 24 hr after single injections of these salts, the degree of physical dependence as demonstrated by jumping activity correlated well with the durations of analgesia. Gray and Robinson [9,10] had previously synthesized sparingly soluble salts of several narcotic antagonists which produced pharmacological effects of long duration when injected as suspensions. The complex zinc tannate salts were particularly effective, antagonism to morphine being evident after several weeks. We have prepared complex zinc tannates of the narcotic agonists heroin, hydromorphone and *l*-α-acetylmethadol and preliminary studies show them to produce long-lasting analgesia in rats [5]. The present study was undertaken to evaluate their potential to cause physical dependence when they were suspended in a slow-release vehicle and given as a single injection.

METHOD

Preparation and Analyses of the Complex Zinc Tannate Salts

The zinc tannate salts of heroin (HZT), hydromorphone (Dilaudid=DZT; Pentagone Laboratories, Ltd.) and *l*-α-acetylmethadol (LAAM=LZT) were prepared by the method described by Gray and Robinson [9,10]. Digestion by dilute hydrochloric acid permitted analyses for the content of narcotic analgesic and zinc. Quantitative analyses for the heroin, hydromorphone and LAAM were done by the acid-dye technique of Beckstead and French [1] and zinc assays were performed by atomic absorption spectrophotometry.

Treatments

The complex salts were finely ground in a mortar and suspended in the slow-release vehicle of Collier [7], consisting of saline, mineral oil and mannide mono-oleate, using an ultrasonic probe (Sonifier Cell Disruptor Model W185D, Heat Systems—Ultrasonics Inc., Plainview, NY). (Photomicrographs revealed that the particle sizes in the suspension were less than 10 microns.) The resulting viscous preparations were injected subcutaneously into rats, weighing 250–350 g, in doses equivalent to 41.0 and 82.9 mg/kg of heroin base, 40.0 and 79.9 mg/kg hydromorphone base and 41.0 and 81.9 mg/kg of LAAM base. The volumes of injections were 5 ml/kg for the heroin preparations and 10 ml/kg for the LAAM and hydromorphone preparations. Following injection, the rats were placed in holding cages in a temperature-controlled room on a fixed lighting schedule. Food and water were supplied ad lib.

Induction and Withdrawal

On various days (1, 3, 7, 10, 14) after the drug salt complexes or vehicle had been administered, four rats from each

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TABLE 1

RANKING SYSTEM FOR QUANTIFYING PRECIPITATED WITHDRAWAL OVER THE 4 HR PERIOD*

Abstinence Behavior	Score
No change in behavior	0
Abnormal posturing, writhing irritability, diarrhea (any 3 our of these 4 abstinence signs)	1
Teeth chattering or salivation	2
Two more escape attempts or 3 or more wet shakes	3

*modified from Wei, 1973 [17].

group were selected randomly and placed in Plexiglas observation cages at 0800 hr. Thirty min later they were injected subcutaneously with naloxone hydrochloride (10 mg/kg) in saline. The volume of this injection was 3.3 ml/kg. During the following 4 hours body weights were taken and core temperatures measured using a rectal probe (YSI 423) every 15 min for the first hour and at subsequent 30 min periods. Behavioral signs were monitored and those characteristic of abstinence were recorded and scored by a system shown in Table 1.

Statistical Design and Analyses

The experiment was designed to assess the effects on the body weights and core temperatures of rats of six drug treatments and a vehicle control (drugs=7) at several time periods following the day on which the rats received their treatment (days=5) and at 10 intervals after the naloxone injection (times=10); thus there is a $7\times5\times10$ factorial set of treatment combinations with repeated measures. The temperature and weights were analysed by the appropriate ANOVA for this design using BMD and SPSS computer programs. Multiple comparisons of drug means with the SRV control were made using Dunnett's procedure with the nominal α-level adjusted for the number of sets of such comparisons made [8]. Thus the α-level stated is an error rate for the entire set of comparisons made (i.e., 350).

After the ANOVA was completed, the maximal temperature response of each of the drug-treated groups was compared to that of the control group (SRV). Although only 6 comparisons were actually made for each day, these were not pre-selected and thus it was necessary to use an experimental error rate to control the probability of making one or more faulty inferences [15]. An experimental error rate of approximately 0.05 was achieved by employing a method of adjustment known as an application of the Bonferroni inequalities (i.e., P [at least one type I error in K comparisons] $\leq$ KP [a type one error in any one comparison]=Kα). Because of the complex nature of the behavioral signs accompanying withdrawal and the difficulties in quantifying them, they were not subjected to statistical analyses.

RESULTS

Twenty-four hours after the injection of the vehicle (SRV), the rats were normally active in contrast to the drug-treated rats which appeared sedated. Within 15 min of the naloxone challenge, all drug-treated groups became hyperirritable and most rats were assigned an abstinence score of 2 (by the criteria in Table 1). Rats with this score invariably had diarrhea and most of the other signs included in the abstinence score of 1. These signs became apparent within 15–30 min after the naloxone challenge and continued throughout the 4 hr testing period. At no time during the two week duration of the study did the SRV drug treated groups show any signs of abstinence (Table 2). No significant differences in weight loss were found between the drug-treated groups and the SRV group on Day 1. The mean core temperature decreased after naloxone in all groups except the SRV and low-dose heroin. The maximum change in core-temperature during the 4 hr post-naloxone period was significantly greater (α=0.05) in the four groups which had received DZT and LZT than in the SRV group.

On Day 3 most drug-treated rats appeared quiet and docile. After the naloxone challenge, all drug-treated groups lost significantly more weight than the SRV controls, but significant hypothermia was found only in the LZT HIGH, the DZT LOW, and DZT HIGH groups. The drug-treated rats were hyperirritable, difficult to handle and some LZT-treated rats mutilated themselves. In general, the rats treated with either DZT or LZT had higher abstinence scores than the HZT-treated groups.

Seven days after the various treatments were administered, the HZT-injected rats appeared to be less irritable than the other drug-treated groups. After the naloxone challenge all drug-treated groups lost weight, but only the DZT-treated groups were significantly different from control and only these groups had significant hypothermia. The HZT groups showed milder abstinence signs than the other drug-treated groups.

On Day 10, rats which had been treated with the high doses of either DZT or LZT were hyperirritable and difficult to handle. Following the naloxone challenge, these groups presented more severe abstinence signs. All groups, with the exception of HZT LOW, lost weight but only the HZT HIGH and DZT LOW groups achieved significance, although the LZT HIGH and DZT HIGH groups closely approached statistical significance. Marked hypothermia was found in those rats treated with LZT HIGH, DZT HIGH and DZT LOW.

Two weeks after the single injection of HZT, DZT or LZT most groups did not behave differently than the SRV controls when they were handled. LZT and DZT rats had varying degrees of alopecia. All groups lost weight, but the loss was significant only in the LZT HIGH, DZT LOW and DZT HIGH groups. Of these groups, only the DZT HIGH also had significant hypothermia.

DISCUSSION

Wei *et al.* [18] maintain that the quantitative assessment of physical dependence, if it is measured by behavior after precipitated withdrawal, is difficult "because the withdrawal syndrome is represented by a constellation of signs with different onset, duration and intensity." It is obvious from the data presented here that in order to assess physical dependence adequately both behavioral signs and objective parameters such as loss of body weight and fall in body temperature must be monitored. From Table 2 it may be seen that on *every* testing day the narcotic-treated groups pre-

TABLE 2

CHANGES IN BODY WEIGHT, TEMPERATURE AND BEHAVIOR IN 4 HR AFTER NALOXONE CHALLENGE

Day	Treatment Group	Wt. Loss at 4 HR (%) (Mean ± SEM)	Maximal Temperature Response (°C) (Mean ± SEM)	Abstinence Score (Mean ± SEM)
1	SRV	6.02 ± 0.47	1.08 ± 0.41	0
	HZT LOW	7.24 ± 0.51	0.43 ± 0.28	1.50 ± 0.29
	HZT HIGH	7.02 ± 0.92	−0.65 ± 0.13	2.00 ± 0.00
	LZT LOW	5.68 ± 0.45	−1.25 ± 0.31*	2.00 ± 0.00
	LZT HIGH	6.44 ± 1.07	−1.15 ± 0.47*	2.50 ± 0.29
	DZT LOW	6.56 ± 0.99	−2.23 ± 0.26*	2.00 ± 0.00
	DZT HIGH	6.88 ± 0.46	−2.30 ± 0.24*	2.00 ± 0.00
3	SRV	3.32 ± 0.46	0.83 ± 0.18	0
	HZT LOW	8.17 ± 0.47*	1.10 ± 0.15	2.00 ± 0.00
	HZT HIGH	8.46 ± 0.97*	0.60 ± 0.31	2.00 ± 0.00
	LZT LOW	8.28 ± 1.44*	0.53 ± 0.26	3.00 ± 0.00
	LZT HIGH	8.77 ± 0.98*	−1.55 ± 0.48*	3.00 ± 0.00
	DZT LOW	11.17 ± 0.98*	−2.20 ± 0.15*	3.00 ± 0.00
	DZT HIGH	9.26 ± 1.00*	−2.23 ± 0.63*	3.00 ± 0.00
7	SRV	4.75 ± 1.13	−0.38 ± 0.19	0
	HZT LOW	8.60 ± 0.89	−0.33 ± 0.05	2.00 ± 0.00
	HZT HIGH	8.35 ± 0.11	−1.05 ± 0.16	2.00 ± 0.00
	LZT LOW	7.91 ± 1.15	−1.20 ± 0.34	3.00 ± 0.00
	LZT HIGH	6.76 ± 0.36	−1.18 ± 0.49	2.75 ± 0.25
	DZT LOW	9.81 ± 1.13*	−3.28 ± 0.56*	2.75 ± 0.25
	DZT HIGH	10.46 ± 1.14*	−3.08 ± 0.37*	3.00 ± 0.00
10	SRV	4.82 ± 0.41	0.58 ± 0.29	0
	HZT LOW	4.73 ± 0.49	−0.60 ± 0.11	2.00 ± 0.00
	HZT HIGH	8.87 ± 1.02*	−0.90 ± 0.15	2.00 ± 0.00
	LZT LOW	7.20 ± 0.54	−0.83 ± 0.30	2.25 ± 0.25
	LZT HIGH	8.56 ± 1.17	−2.05 ± 0.38*	3.00 ± 0.00
	DZT LOW	9.63 ± 0.28*	−3.05 ± 0.26*	2.75 ± 0.25
	DZT HIGH	8.59 ± 0.91	−3.00 ± 0.40*	3.00 ± 0.00
14	SRV	3.15 ± 0.64	−0.80 ± 0.17	0
	HZT LOW	3.81 ± 0.65	−0.90 ± 0.18	1.25 ± 0.25
	HZT HIGH	6.45 ± 0.67	−1.13 ± 0.25	2.00 ± 0.00
	LZT LOW	6.76 ± 0.95	−0.80 ± 0.64	2.00 ± 0.00
	LZT HIGH	9.68 ± 0.16*	−1.82 ± 0.19	2.50 ± 0.29
	DZT LOW	7.53 ± 0.99*	−1.30 ± 0.18	2.50 ± 0.29
	DZT HIGH	10.21 ± 1.20*	−4.03 ± 0.23*	2.67 ± 1.54

*$\alpha = 0.05$
SRV=slow release vehicle
HZT=heroin zinc tannate
LZT=l-α-acetylmethdol zinc tannate
DZT=hydromorphine zinc tannate

sented behavioral signs of abstinence, whereas weight loss and temperature changes were much less consistent. On Day 1 the fall in core temperature of the LAAM- and hydromorphone-treated rats was significantly greater than that of the control rats at a time when the naloxone-induced withdrawal signs were unmistakable, but no statistical difference in body weights was found. The present results, in agreement with the conclusions of Bhargava [2], suggest that it is possible that early in the development of physical dependence a decrease in body temperature may be a more sensitive indicator than weight loss. When naloxone was given on the third day, all drug-treated groups lost weight and had high abstinence scores, but by the seventh day the withdrawal responses of only the hydromorphone groups met the strict statistical criteria of dependence. Even 14 days after a single injection of the narcotic zinc tannate salts, signs of physical dependence were evident.

Taken together, the results underline the recurrent problem of the quantitative assessment of the physical dependence syndrome. Weight loss, changes in core temperature, and behavioral signs are obviously important, but because of the different time courses of these variables it is impossible

to determine what magnitude each of these components should be assigned in the total expression of withdrawal.

Notwithstanding these difficulties, the results suggest that under the conditions of this experiment the severity of dependence resulting from these treatments could be ranked as: DZT>LZT>>HZT. These differences are probably attributable to the fact that virtually identical amounts of the drugs rather than equipotent narcotic doses were administered.

Laska and Fennessy [13] reported that naloxone induced no significant temperature changes in their vehicle-treated control rats and that in their morphine-dependent rats naloxone produced a consistent reduction in body temperature not exceeding, 1.1°C. In the vehicle-treated rats of the present experiments, naloxone produced temperature changes ranging from −0.38°C to 1.08°C and these were not statistically different from the pre-naloxone values. The rats treated with the narcotic agonists generally showed hypothermic responses with a maximum change in temperature of −4.03 ± 0.23°C.

Of special interest was the observation that after the naloxone challenge even the SRV group lost weight. This phenomenon resembles that reported in rats after removal of implanted placebo pellets and injection of naloxone [2]; their weight loss was comparable to that found in the present experiment. The vehicle treated rats did not show the behavioral signs characteristic of withdrawal and, like the rest, had ready access to food. In other experiments we have shown [6] that naloxone injected either in the morning or evening reduced the food intake of both fasted rats and those feeding ad lib.

Bläsig and his colleagues [3] state that one criticism of cellulose morphine pellets is that encapsulation occurred and drug release ceased after 3 days. In the present experiments some encapsulation was evident from Day 3 on, but enough of the narcotic agonist was still being released so that even two weeks after the injection naloxone could induce withdrawal effects within a short period. Isom and his group [11], using a silicone slow-release morphine tablet which they claimed did not undergo encapsulation, stated that the maximum development of tolerance and physical dependence occurred 72 hr after implantation. Encapsulation or not, three days was the time reported for the onset of maximal dependence with cellulose morphine tablets [14,16], with silicone morphine tablets [11,14], and with the heroin, LAAM and hydromorphone zinc tannate salts reported here. Moreover, Table 2 indicates that a high level of dependence can persist in some groups for at least two weeks after a single injection of these narcotic complexes.

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