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A Simple, Reliable Method for Predicting the Physical Dependence Liability of Narcotic Antagonist Analgesics in the Rat

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HOWES, J. F. *A simple, reliable method for predicting the physical dependence liability of narcotic antagonist analgesics in the rat.* PHARMAC. BIOCHEM. BEHAV. **14**(5) 689–692, 1981.—The rat intraperitoneal infusion procedure was used to chronically administer drugs for evaluation of the physical dependence liability of narcotic antagonist analgesics. Three methods were used to assess dependence liability: presence of withdrawal signs upon abrupt cessation of chronic infusion (primary dependence), attenuation of the withdrawal signs produced by cessation of chronic morphine infusion (morphine substitution), and production of withdrawal signs when chronically morphine-infused rats were administered the drugs (precipitated withdrawal). Butorphanol, nalbuphine and pentazocine all caused a mild withdrawal in the rat primary dependence model which agrees with the conclusions from experiments with monkey and man. None of these agents substituted for morphine in the rat and all three appeared to precipitate withdrawal. Two experimental drugs, Codorphone and TR5400, did not induce primary dependence in the rat, and in chroni-morphinized rats, they precipitated a withdrawal syndrome comparable to naloxone. Another experimental drug, TR5257, substituted for morphine. The correlation between these observations in the rat and previously published data from the monkey are excellent. It is proposed that the rat could be used as a reliable indicator of potential physical dependence liability for the narcotic antagonist analgesics.

Rats Narcotic antagonist analgesic Physical dependence Intraperitoneal infusion Morphine substitution

WHILE narcotic antagonist analgesics do not cause the same degree of physical dependence as morphine and other narcotic analgesics, pentazocine [6], nalorphine [6], nalbuphine [6] and butorphanol [7] all produce a mild abstinence syndrome in man following abrupt withdrawal. In the chronically morphinized monkey they all precipitate an abstinence syndrome [4, 9, 12]. Primary dependence studies in the monkey have demonstrated an abstinence syndrome following abrupt withdrawal of either chronic nalorphine [3] pentazocine [3] or nalbuphine [11]. Data for butorphanol are not available. While this mild physical dependence liability does not pose a major drawback to clinical use, it would be desirable to have an agent which was completely free of this complication. The monkey has traditionally been used to determine the physical dependence liability of new drugs. Large amounts of material are required (100–200 g), which may not be available during the early stages of drug development. A simple, reliable method in rodents for predicting physical dependence liability for narcotic antagonist analgesics would, therefore, be of great value. The rat intraperitoneal infusion procedure of Teiger [10] is an economical and rapid method for studying the physical dependence properties of narcotic analgesics and can serve as an early screen for detecting compounds with significant physical dependence liability. The purpose of this study is to evaluate the physical dependence liability of standard and novel structures of the narcotic antagonist analgesic class by this procedure. Where possible these data were compared with data previously generated in monkeys and humans.

METHOD

Male, Charles River CD Rats (Charles River Laboratories, Wilmington, Massachusetts), 180–200 g were used for this study. The method described by Teiger [10] with the following modifications was used: animals were anesthetized with methoxyfluorane by exposure to vapor in a closed chamber. A small incision was made in the abdomen to expose the wall of the peritoneum in which a small hole was made. A 10 cm length of surgical silk was tied to a 15 cm length of PE50 polyethylene cannula, about 2 cm from one end. This cannula was inserted into the peritoneal cavity, to the level of the surgical silk. The surgical silk was stitched to the peritoneal wall and tied to hold the cannula in place. A flexible probe was pushed subcutaneously from the abdominal incision to a position between the ears of the animal. The cannula was looped through the eye of the probe and drawn through. The protruding cannula was tied in place using surgical silk. The abdominal incision was closed using a wound clip, and the animal was allowed to recover for four days

before being attached via a harness and swivel arrangement to a Harvard Model 975 Compact Infusion Pump adapted to control six 20 cc syringes. Drugs were infused for periods up to six days. Three types of experiments were performed using this technique. All drugs were dissolved in normal saline. The drug solutions were prepared daily. The animal's weight was recorded and the concentration of drug required to deliver the stated 24 hour dose was prepared based on an infusion rate of 0.0052 ml/min. The following infusion schedules were used for the study: morphine (50 mg/kg/day for 1 day, 100 mg/kg/day for 2 days and 200 mg/kg/day for 4 days), pentazocine (200 mg/kg/day), butorphanol (40 mg/kg/day), nalbuphine (100 mg/kg/day), codorphone (200 mg/kg/day), TR5400 (60 mg/kg/day and TR5257 (40 mg/kg/day in Experiment 1 and 80 mg/kg/day in Experiment 2). Doses were selected on the basis of antinociceptive potency relative to morphine. The morphine schedule was determined in a series of pilot experiments.

Primary Dependence Studies

The drug was infused for six days and then abruptly terminated. The animals were weighed at this time and at intervals of 24 hours up to 96 hours. The following behavioral effects were recorded at each 24 hour observation period: Vocalization, aggression, piloerection, diarrhea, "wet dog shakes," tremors and compulsive behavior [8]. These parameters were recorded as either present or absent.

Substitution Studies

In these studies, the rats were infused with morphine according to the following schedule: Day 1—50 mg/kg/day, Day 2—100 mg/kg/day and Day 3 through Day 6—200 mg/kg/day. On the 7th day, the animals were infused with the test drug for 24 hours. The animals were weighed 6 hours into the test drug infusion, at 24 hours when the infusion stopped, at 30 hours, and then at every 24 hour period up to 96 hours. The animals were observed for the behavioral effects described above.

Precipitation Studies

The animals were infused with morphine as described above. On Day 6, the infusion was terminated and the animals were transferred immediately to individual clear-sided cages and given a subcutaneous dose of the test compound or naloxone (for doses see Results). The one hour period following this injection is divided into twenty 3-min periods and the animals were observed continuously. The number of animals exhibiting chewing, teeth chatter or tremors during each of these periods was recorded. At the end of the hour the mean number of animals showing these effects per period was recorded. The total number of wet dog shakes during this 60 min period was recorded. At one hour the animals were examined to see if they showed signs of vocalization, aggression or irritability, as described by Meyer and Sparber [8]. The animals were weighed at 6 hr, 24 hr, 30 hr and every 24 hour period up to 96 hours. The presence of diarrhea was noted.

Statistics

Means and standard errors were calculated and recorded in Tables 1, 2 and 3. Analyses of variance (one way classification, many sample comparison) were calculated for each time interval using a Hewlett Packard HP-67 programmable calculator. Statistical significance for individual treatments was determined using Students t test.

TABLE 1

PERCENT CHANGE IN WEIGHT FOLLOWING SIX DAY INFUSIONS

		Days Post Infusion			
Drug	N	Day 1	Day 2	Day 3	Day 4
Morphine	8	−16.8*	−12.7*	− 7.8*	− 1.6
		± 1.3	± 1.9	± 2.3	± 4.6
Pentazocine	4	− 7.7*	+ 3.6	+ 3.7	+ 3.8
		± 3.9	± 2.7	± 5.0	± 6.5
Butorphanol	4	− 6.6*	− 3.1	+ 0.9	+ 2.2
		± 2.1	± 1.5	± 1.9	± 2.3
Nalbuphine	6	− 8.5*	− 6.9*	− 1.1	+ 2.8
		± 2.7	± 1.4	± 0.7	± 2.1
Codorphone	8	+ 5.1	+ 4.9	+11.9	+13.6
		± 2.5	± 4.8	± 4.3	± 7.3
TR5400	6	+ 6.6	+ 8.3	+ 9.7	+10.9
		±1.2	± 1.9	± 1.8	± 2.2
TR5257	6	+ 1.2*	+ 2.0	+ 6.1	+ 9.6
(Experiment 1)		± 1.1	± 2.9	± 3.2	± 2.9
TR5257	4	+ 1.7*	+ 3.1	+ 5.3	+ 9.5
(Experiment 2)		± 1.9	± 2.0	± 2.3	± 2.7
Control	6	+ 6.8	+10.2	+14.5	+15.7
		± 1.0	± 1.5	± 2.7	± 4.2

*Significantly different from corresponding control values, $p<0.05$.

Materials

Materials for this study were supplied by the following manufacturers: morphine sulphate (Merck and Co., Rahway, NJ), nalbuphine hydrochlonde and naloxone hydrochloride (Endo Laboratories, Garden City, NY), pentazocine (Sterling Winthrop, Rensselaer, NY), butorphanol (Bristol-Myers, Syracuse, NY), codorphone [8β-ethyl-N-cyclopropylmethyl-dihydro-norcodeinone], TR5400 [14-methoxy-N-cyclopropylmethyl, 6-oxo-3-methylmorphinan] and TR5257 [8β-methyl-N-cyclobutyl-methyl-6-oxo-morphinane] (Miles Laboratories, Elkhart, IN).

RESULTS

Table 1 lists the effects of the test compounds on the percent change in body weight of rats following a six day infusion. Analysis of variance indicated that the regimens differed from each other on Day 1 and Day 2 only. Students t test indicated which regimens differed from controls. Following a saline infusion, rats gained weight steadily. Morphine caused a marked fall in body weight during the first 24 hours post infusion. Weight changes were still significantly different from controls at 3 days. At the 24 hour period, all rats showed signs of opiate withdrawal including tremor, chewing, teeth chatter and "wet dog shakes," Increased urination and defecation were also observed. Pentazocine, butorphanol and nalbuphine all caused a fall in weight during the first 24 hours. This fall was accompanied by piloerection and vocalization in all rats. Nalbuphine caused "wet dog shakes" in 4 out of 6 animals. TR5257 did not, but these

TABLE 2

EFFECTS OF TEST DRUG INFUSIONS ON PERCENT CHANGE IN WEIGHT IN THE CHRONICALLY MORPHINIZED RAT

			Time After Beginning Test Drug Infusion					
Test Drug	Dose	N	+6 hrs	+1 day	+30 hrs	+2 days	+3 days	4 days
Pentazocine	100 mg/kg	6	−9.2	−15.1	−14.3	−11.9	−4.9	−3.1
			±2.3	± 2.7	± 2.6	± 2.1	±3.7	±2.6
Butorphanol	40 mg/kg	6	−7.2	−16.2	−10.4	− 8.00	−5.8	−2.9
			±2.1	± 2.1	± 3.2	± 3.5	±4.1	±3.8
Nalbuphine	100 mg/kg	6	−8.6	−17.9	−17.1	−11.6	−7.0	−4.9
			±1.3	± 0.9	± 0.6	± 0.5	±0.8	±0.9
TR5257	125 mg/kg	9	−6.1	− 7.5*	− 2.6	− 1.4*	−0.1	−2.7
			±2.9	± 2.1	± 1.5	± 2.4	±3.1	±3.9
Saline	—	5	−3.5	−14.8	−16.7	−14.6	−9.9	−3.8
			±1.4	± 2.0	± 2.5	± 4.01	±3.8	±2.9

*Significantly different from corresponding control value, $p<0.05$.

TABLE 3

INDUCTION OF WITHDRAWAL SIGNS BY CODORPHONE, TR5400, AND NALOXONE IN MORPHINE DEPENDENT RATS

Symptom	Codorphone (10.0 mg/kg IP)	Naloxone (2.0 mg/kg IP)	Saline	TR5400 (10.0 mg/kg IP)
Teeth Chattering*	1.17 ± 0.17	3.20 ± 1.01	0.00	2.40 ± 0.40
Tremors*	8.67 ± 0.99	7.20 ± 1.61	0.00	2.60 ± 0.68
Chewing*	8.17 ± 0.31	3.40 ± 1.12	0.00	3.20 ± 0.79
Wet Dog Shakes†	15.50 ± 3.87	8.20 ± 2.67	0.00	7.60 ± 3.20
Irritability‡	6/6	5/5	1/5	5/5
Aggression‡	3/6	1/5	0/5	2/5
Vocalization‡	4/6	1/5	1/5	5/5
Diarrhea‡	2/6	3/5	1/5	3/5
4 hr weight loss (%)§	7.67¶ ± 1.38	6.22¶ ± 1.01	2.14 ± 0.71	6.03¶ ± 0.51

*Mean number of 3 min periods/hr that animals showed response.
†Mean number of wet dog shakes per hr.
‡Ratio of animals showing response at 1 hr.
§Expressed as mean % loss at 4 hrs.
¶Significantly different from corresponding control value, $p<0.05$.

animals did gain significantly less weight than control animals. The animals were vocal and irritable but no other signs were observed. Codorphone and TR5400 caused neither a loss in weight, nor opiate withdrawal signs following the six day infusion.

Pentazocine, butorphanol and nalbuphine all failed to substitute for morphine in the chronically morphinized rat (Table 2). The early appearance of withdrawal signs in these studies indicated that these agents were precipitating withdrawal. TR5257 at a dose of 125 mg/kg/day did suppress some of the signs of withdrawal when substituted in the chronically morphinized rat.

In the chronically morphinized rat, single injections of codorphone, naloxone or TR5400, precipitated withdrawal signs (Table 3).

DISCUSSION

The withdrawal signs produced in the rat by abrupt cessation of opiate exposure are tremor, "wet dog shakes," vocalization, aggression, increased urination and defecation, and a marked loss of weight during the first 24 hours following discontinuation of the drug [8,10]. Our results using the rat infusion procedure clearly demonstrated these signs. In this procedure, compared to morphine, narcotic antagonist analgesic drugs produced a much milder syndrome. Pentazocine and butorphanol caused some weight loss in the first 24 hour period and this was accompanied by some vocalization. In addition, in the monkey chronic pentazocine causes a mild withdrawal syndrome [3] characterized by yawning, stretching and scratching. Similarly, pentazocine [6] and butorphanol [7], cause a mild withdrawal syndrome in man following abrupt withdrawal. Data for the effects of chronic butorphanol in the monkey are not available.

Nalbuphine caused a 24 hour weight loss similar in degree to that of pentazocine. The rats given nalbuphine, however, showed more symptoms than those given pentazocine. This agrees with the data generated by Villarreal and Seevers [11] who demonstrated that abrupt termination of nalbuphine in

monkeys receiving 32.0 mg/kg every 6 hours for 31 days, led to a morphine-like abstinence syndrome. In a similar experiment, these authors were able to demonstrate that naloxone precipitated a withdrawal in nalbuphine-tested animal. They also demonstrated that nalbuphine could precipitate abstinence in chronically morphine treated monkeys. In man, chronic nalbuphine treatment results in a mild abstinence syndrome on abrupt withdrawal [6]. Thus, for these standard compounds, the correlation from the rat to the monkey and man is excellent. Codorphone, TR5400 and TR5257 are novel compounds which are currently being developed as analgesics. In the rat model, TR5400 and codorphone did not cause primary dependence but did precipitate a withdrawal syndrome in the morphinized rat. In the monkey, neither compound supported morphine dependence [1,2]. Both compounds precipitated a withdrawal syndrome in the chronically morphine treated monkey [1,2]. Chronic TR5257 caused a mild withdrawal syndrome in rats and partially suppressed morphine withdrawal in the substitution procedure. In the monkey, a single dose of TR5257 suppressed partially the withdrawal signs following abrupt withdrawal of chronic morphine [1]. For these three experimental compounds, the correlation between the rat and the monkey is excellent. The implications for the human use of these agents is that both codorphone and TR5400 would have less physical dependence liability than pentazocine, butorphanol or nalbuphine, whereas TR5257 might have some limited dependence liability.

Jasinski [6,7], studying chronic doses of narcotic antagonist analgesics in man, demonstrated that pentazocine, cyclazocine, nalorphine, nalbuphine and butorphanol are all able to induce a state of dependence and a characteristic withdrawal. When the withdrawal signs were ranked with respect to their contribution to the total observed response, a qualitative difference between morphine and this group was found to exist. The withdrawal in each case was noticeable but not severe.

Substitution for morphine, may not be relevant as an index of abuse liability since butorphanol, pentazocine and nalbuphine all fail to substitute for morphine in both the monkey and the rat. All of these agents produce primary dependence in the rat, the monkey and man. For studying substitution, it may be preferable to chronically treat rats with a narcotic antagonist which gives a measurable withdrawal. Pentazocine or nalorphine would qualify as candidates. We are presently studying the use of nalorphine for substitution studies in the rat.

The primary dependence procedure in the rat appears to give reliable data. The results agree with those generated in the monkey. The rat is a less expensive and more readily available animal. Furthermore, the quantity of material required to carry out a primary dependence study in rats is much less than that required for the monkey. Physical dependence liability can be estimated earlier in the development of a new compound.

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