Pharmacology Biochemistry & Behavior, Vol. 16, pp. 851–854, 1982. Printed in the U.S.A.

Experimental Studies of the Abuse Potential of *d,l*-Glaucine·1.5-Phosphate in Rhesus Monkeys

C. R. SCHUSTER, T. AIGNER[1] AND C. E. JOHANSON

The University of Chicago, The Pritzker School of Medicine, Chicago, IL 60637

AND

T. H. GIESKE

The Dow Chemical Company, Indianapolis, IN 46268

Received 23 November 1981

SCHUSTER, C. R., T. AIGNER, C. E. JOHANSON AND T. H. GIESKE. *Experimental studies of the abuse potential of d,l-glaucine·1.5 phosphate in rhesus monkeys.* PHARMAC. BIOCHEM. BEHAV. **16**(5)851–854, 1982.—*d*-Glaucine is an alkaloid derived from *Glaucium flavum*, which is as effective as codeine as an antitussive. *d,l*-Glaucine·1.5 phosphate is a synthetic compound related to *d*-glaucine. The ability of *d,l*-glaucine·1.5 phosphate to maintain responding in rhesus monkeys was assessed in 2 procedures. In the first study responding was maintained under a fixed-ratio 10 schedule of codeine delivery during daily 3-hr sessions. When *d,l*-glaucine·1.5 phosphate (0.05–0.4 mg/kg) was substituted for codeine, responding was not maintained. In the second procedure, monkeys given 23-hr/day access to 0.5–1.0 mg/kg under a fixed-ratio schedule did not self-administer *d,l*-glaucine·1.5 phosphate above saline levels even after a 21-day period of programmed injections. Following the period of programmed injections, there were no signs of opiate withdrawal following the administration of naloxone. These results indicate that the abuse potential of *d,l*-glaucine·1.5 phosphate is low relative to codeine.

d,l-Glaucine·1.5 phosphate Self-administration Abuse potential Substitution Unlimited access Rhesus monkeys

THE *d*-isomer of glaucine is an alkaloid isolated from the *Glaucium flavum crantz* plant by Fischer in 1901. Pharmacologic testing of this isoquinoline alkaloid has shown it to be as effective an antitussive as codeine [1, 5, 6]. Preparations of *d*-glaucine hydrobromide have been marketed in Bulgaria for many years as antitussives. Synthetically produced *d,l*-glaucine·1.5 phosphate (hereafter referred to as *d,l* glaucine) and *d,l*-glaucine hydrobromide are currently undergoing clinical testing in the United States [2] and Europe [4]. Since a major contraindication to the use of codeine as an antitussive is its illegal rediversion from medical sources, the present study was designed to investigate the abuse potential of *d,l*-glaucine. Two drug self-administration procedures which have been shown to be useful in the prediction of the abuse potential of psychotropic drugs in humans were utilized in this assessment [13,14].

METHOD

Animals

Four male (6002, 6058, 7038, and 7039) and two female (2012 and 7060) rhesus monkeys weighing between 5 and 8 kg were used. Three of the animals (2012, 6058, 7039) had been used in previous experiments in which responding was maintained by drug infusions under fixed-ratio schedules. The other three animals (6002, 7038 and 7060) had no prior experimental history involving drug maintained responding. Each animal had an indwelling venous catheter implanted under sodium pentobarbital anesthesia (up to 30 mg/kg, IV) using the method of Deneau, Yanagita and Seevers [3]. Each monkey had 24-hr access to Purina monkey chow and water and received a sugar cube saturated with liquid vitamins every day. When necessary, antibiotics were administered intramuscularly to arrest a catheter tract infection.

Apparatus

Each monkey was housed in a sound-attenuated wooden cubicle (inside dimension: 70×80×70 cm) that served as the experimental space and was equipped with a fan for ventilation and masking extraneous sounds. Mounted on the door of the cubicle were two metal boxes (12.5×15 cm) located 23 cm apart. Each box contained a response lever (PRL-001 BRS/LVE, Beltsville, MD). There were two red and two white Dialco stimulus lights above the levers as well as red and white ceiling lights. Cables connected the cubicles to solid state programming and recording equipment in an adjacent room.

All animals were restrained by a stainless steel harness attached to the rear of the cubicle by a steel spring restrain-

[1]Present address: 5451 East View Park #1, Chicago, IL 60615.

ing arm [12]. Drug injections were delivered through the catheter by means of a peristaltic pump over a period of 10 seconds.

Procedure

Substitution test. Three monkeys (2012, 6058 and 7039) were trained to press the right lever 10 times (fixed ratio 10: FR 10) for a 0.1 mg/kg injection of codeine phosphate during daily 3 hour experimental sessions (baseline condition). Responses on the left lever had no programmed consequences. Drug availability was indicated by the illumination of two white stimulus lights above the lever and the illumination of a 15 W white ceiling light. Drug delivery was indicated by illumination of a 15 W red ceiling light and two red stimulus lights above the lever. After responding for codeine was stable (less than ±10% variation in the total number of infusions per session for three consecutive sessions), saline was substituted for codeine for six sessions to determine whether or not codeine was functioning as a positive reinforcer (i.e., extinction). Next, animals were returned to baseline conditions and when responding again was stable (±10% variation), a solution of *d,l*-glaucine was substituted for codeine for six sessions. Four doses of *d,l*-glaucine were tested in the following order in all three animals: 0.1, 0.05, 0.2 and 0.4 mg/kg/inj. These doses were selected on the basis of other experiments in which it was demonstrated that *d,l*-glaucine was equipotent with codeine in behavioral tests in guinea pigs (unpublished observations, T. Gieske). Monkeys were returned to baseline conditions between the testing of each dose. After stable baseline responding was reached in the codeine sessions following the last dose of *d,l*-glaucine, the monkeys finished the experiment with six sessions of saline substitution.

The data for each dose of *d,l*-glaucine were analyzed by determining the range of the number of injections during the last three sessions of each substituted dose for each monkey. This range was compared to the range of the last three sessions of the first saline substitution. A dose of *d,l*-glaucine was considered to be a positive reinforcer (i.e., to maintain responding) if the range of injections was above the range of saline injections. If the range fell below or within the range for saline (i.e., the ranges were overlapping), the dose was not considered a positive reinforcer.

23-hour access test. Three monkeys (6002, 7038 and 7060) were used in the second phase of testing. For this study the right lever was also designated as the active lever, the left lever as inactive. A single response on either lever illuminated two red stimulus lights above that lever and a red ceiling light. However, only responses on the right lever produced an injection.

Sessions were started at 12:00 noon each day and ended at 11:00 a.m. the following day. A 15 W white ceiling light indicated the session was in progress. Food intake was monitored throughout the study. A measured amount of food was placed in the food dish and the number remaining from the previous day were counted and removed. The animals were fed, watered and physically examined and cubicles were cleaned during the one hour break.

For the first seven days each response on the right lever produced a 0.2 ml/kg injection of saline. For the next 14 days each lever press on the right lever produced a 0.5 mg/kg injection of *d,l*-glaucine. Next, a period of 21 days of programmed infusions was begun. That is, if the animal did not press the active lever, a 1.0 mg/kg injection of *d,l*-glaucine was automatically delivered every three hours. A response on the active lever produced an injection of 1.0 mg/kg and also reset the programmed injection timer. After the 21 days, the programmed injections were discontinued, but the animals still had the opportunity to self-administer *d,l*-glaucine at a dose of 1.0 mg/kg/inj for an additional 7 days. On the first and fifth day after the non-contingent programmed injections of *d,l*-glaucine had been terminated, animals were tested to determine whether physical dependence of the opiate type had developed during this period. On Day 1, 0.3 mg/kg and on day 5, 1.0 mg/kg of naloxone was administered IM and the animal observed for signs of withdrawal 5, 10, 20 and 60 minutes later.

Drugs

The experimental compound was originally submitted under the Dow code number of DX-2242 and was supplied by The Dow Chemical Co. (Indianapolis, Indiana). After completion of the study the code was broken and it was revealed that the compound was *d,l*-glaucine·1.5 phosphate.

All drug doses were delivered in a 1 ml volume. Prior to each 6-session substitution test or as needed for the 23-hr test, an amount of *d,l*-glaucine was weighed and dissolved in Viaflex containers of sterile saline (Travenol Laboratories) for administration to the animal. This solution was used for the entire 6-session substitution test period. At the higher concentrations (2–3 mg/ml), there was occasionally some tendency for the compound to precipitate out of solution. Each solution was checked for precipitates prior to each experimental session. If they were observed, the solution was submerged momentarily in a warm water bath to redissolve the drug.

Codeine phosphate was obtained commercially from Merck & Company, Inc. (St. Louis, MO). Solutions were prepared in the same manner as *d,l*-glaucine.

RESULTS

Substitution

Table 1 shows the number of injections received when lever pressing was maintained by injections of saline, codeine or *d,l*-glaucine delivered under a FR 10 schedule of reinforcement. The mean number of injections of codeine self-administered was considerably greater than saline for all three monkeys. In contrast, *d,l*-glaucine across the range of doses (0.05-0.4 mg/kg/inj), failed to maintain lever pressing at rates above those generated by saline. In only one animal (6058) at one dose (0.1 mg/kg/inj) did the range of *d,l*-glaucine injections received exceed the range of saline injections over the last three sessions of access.

At the highest dose of *d,l*-glaucine tested (0.4 mg/kg/inj), vomiting was observed in two monkeys (2012 and 7039) after each had self-administered approximately five injections in rapid succession on the first day of substitution. No other untoward effects were observed at this dose. In the animal with the highest rates of responding for saline (2012), responding was reduced by *d,l*-glaucine (0.2 and 0.4 mg/kg/inj) indicating that behaviorally active doses had been achieved.

23-Hour Access

Table 2 shows the number of injections of *d,l*-glaucine self-administered before, during and after a period of programmed injections of the drug for monkeys 6002 and 7038. Monkey 7060 became severely edematous after 2 days of

TABLE 1

NUMBER OF INJECTIONS OF SALINE, CODEINE AND *d,l*-GLAUCINE PHOSPHATE SELF-ADMINISTERED BY MONKEYS 2012, 6058 and 7039

Monkey	Codeine 0.1 mg/kg/inj	Saline 1	*d,l*-Glaucine Phosphate (mg/kg/inj) 0.05	0.1	0.2	0.4	Saline 2
2012	73.3 (40–135)	32.3 (22–45)	24.7 (20–30)	20.3 (10–37)	14.0 (9–20)	3.3 (0– 7)	40.0 (19–78)
6058	104.6 (70–127)	9.3 (7–13)	12.7 (12–14)	27.3 (17–42)*	20.7 (13–32)	12.0 (4–20)	10.3 (4–16)
7039	156.2 (120–189)	5.3 (1– 9)	1.7 (1– 3)	8.7 (7–11)	3.3 (0– 7)	6.0 (3–10)	16.7 (11–22)

Values represent the mean (±range) number of injections during the last three days of the substitution(s) or for the intervening baseline codeine sessions.

*Above the range for saline.

TABLE 2

NUMBER OF INJECTIONS OF *d,l*-GLAUCINE PHOSPHATE SELF-ADMINISTERED BY MONKEYS 6002 AND 7038 DURING 23-HR ACCESS

	d,l-Glaucine Phosphate				
	Saline (0.2 ml/kg)	0.5 mg/kg/inj	1.0 mg/kg/inj (Programmed)*	1.0 mg/kg/inj	Saline (0.2 ml/kg)
Number of Days	7	14	21	7	3
Monkey 6002	7.4 (0–15)	1.5 (0– 8)	1.2 (0– 5)	0.7 (0– 4)	1.3 (0– 3)
Monkey 7038	11.1 (0–30)	4.4 (0–15)	0.9 (0–10)	22.6 (0–48)	35.7 (21–39)

Values represent the daily mean and range. Means calculated for entire period of access.

*Values represent the mean number of injections self-administered and do not include the possible 8 injections automatically delivered each 23 hr period.

programmed infusions and was withdrawn from the study. Subsequent examination of this monkey revealed massive thrombus formation around the tip of the intravenous catheter in the right atrium. It was concluded that the edema was not drug-induced but probably due to consequences of this thrombus formation.

During the initial 14 day period of 23 hour per day access to 0.5 mg/kg *d,l*-glaucine neither animal showed an increase in lever pressing over that observed in the previous seven days when saline was received after each lever press (FR 1). Further, no increased rates of responding were seen during the 21 day period during which each monkey received automatic injections of *d,l*-glaucine (1.0 mg/kg/inj) every 3 hours when it had failed to respond on the active lever. Finally, no increased rates of responding were observed during a seven day period after the non-contingent programmed injections were terminated.

Naloxone was administered IM on the first (0.3 mg/kg) and the fifth day (1.0 mg/kg) after the programmed injections had been terminated. Although both animals showed slight increases in respiration rate and vocalization, no other signs of opiate withdrawal were observed.

DISCUSSION

Glaucine has been shown in laboratory animal studies [1, 5, 6, 10] as well as during clinical trials [4,11] to be as effective an antitussive agent as codeine. Since codeine-containing cough preparations are often abused, it is of importance to find antitissuves with lower abuse potential. The results of the present study suggest the *d,l*-glaucine should have significantly less abuse potential in humans than codeine. Codeine has been demonstrated to serve as a reinforcer in the rhesus monkey under a wide variety of experimental conditions including substitution and unlimited access procedures [3, 7, 15]. In the present study *d,l*-glaucine did not generally serve as a reinforcer under either experimental condition. In only one animal, at one dose, did infusions of *d,l*-glaucine maintain responding at higher rates than those maintained by saline infusions. Although there may be conditions under which *d,l*-glaucine would be a more robust reinforcer, most drugs of abuse which have been tested under the conditions of the present experiments maintain responding in most, if not all, animals [3, 8, 9]. It also appears that *d,l*-glaucine at a dose of a least 8 mg/kg/day intravenously does not produce physical dependence of the opiate type after 21 days of exposure, since naloxone administration failed to produce any significant signs of withdrawal. This observation is in accord with a previously published report on the failure of glaucine to produce morphine-like physical dependence in rats [10,11]. These data showing that *d,l*-glaucine neither serves as a reinforcer nor produces physical dependence would lead to the prediction that the abuse potential of glaucine in humans will be significantly lower than that of codeine.

ADDED NOTE

Since this article was prepared it has been brought to our attention that a similar study has been conducted in Japan. Yanagita, T., T. Wakasa and H. Nakanishi. Drug dependence potential of DL-832 tested in rhesus monkeys and rats. *Cent. Inst. exp. Anim. preclin. Rep.* **8:** 121–130, 1982.

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