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Cocaine-like discriminative stimulus effects of phendimetrazine and phenmetrazine in rats

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Abstract

Phendimetrazine is a clinically available anorectic and candidate medication for the treatment of cocaine addiction. Phendimetrazine can be metabolized to the amphetamine-like monoamine releaser phenmetrazine, but it is unclear if phendimetrazine functions as an inactive pro-drug or might have activity on its own. As one method to address this issue, the present study compared the potency and time course of phendimetrazine and phenmetrazine to produce cocaine-like discriminative stimulus effects in adult, male rats (N=5) trained to discriminate cocaine (5.6 mg/kg i.p.) from saline in a two-key food-reinforced discrimination procedure. We hypothesized that, if metabolism to phenmetrazine was required for phendimetrazine effects, then phendimetrazine would be less potent and have a slower onset and offset of effects than phenmetrazine. Both phendimetrazine and phenmetrazine produced dose-dependent cocaine-like discriminative stimulus effects, and phendimetrazine was 7.8-fold less potent than phenmetrazine. However, the time courses of discriminative stimulus effects produced by phendimetrazine and phenmetrazine were similar, with peak effects at 10 min and offset by 100 min. These results show the effectiveness of phendimetrazine to rapidly produce cocaine-like behavioral effects in rats and support other nonhuman primate evidence to suggest that metabolism to phenmetrazine may not be required for phendimetrazine effects.

Keywords

phendimetrazine; phenmetrazine; rat; cocaine; and discrimination

Introduction

Phendimetrazine is a clinically available anorectic and candidate medication for cocaine addiction (Banks et al., 2013a). Structurally, phendimetrazine is an *N*-methyl analog of the

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monoamine releaser phenmetrazine, and phendimetrazine can be metabolized to phenmetrazine in vivo (Rothman et al., 2002; Banks et al., 2013b). Moreover, it has been suggested that phendimetrazine itself is inactive, but that it functions as a pro-drug for phenmetrazine as the active metabolite (Rothman et al., 2002). Consistent with this hypothesis, only phenmetrazine promoted in vitro dopamine release from rat brain synaptosomes and increased in vivo microdialysis measures in the nucleus accumbens in rats (Rothman et al., 2002). However, in disagreement with this hypothesis, we recently found that phendimetrazine produced cocaine-like discriminative stimulus effects in rhesus monkeys within 10 min of its administration, a time when plasma phenmetrazine plasma levels were too low to account for behavioral effects (Banks et al., 2013b). These results were interpreted to suggest that phendimetrazine might have pharmacological activity in rhesus monkeys independent of its metabolism to phenmetrazine. They also suggested potential species difference in phendimetrazine effects. To address these issues, the present study compared the potency and time course of phendimetrazine and phenmetrazine to produce cocaine-like discriminative stimulus effects in rats. We hypothesized that, if metabolism to phenmetrazine is required for phendimetrazine to produce behavioral effects in rats, then phendimetrazine should have lower potency and produce a slower onset and offset of effects than phenmetrazine.

Methods

Subjects

Studies were conducted in a total of 12 adult male Sprague Dawley rats (Harlan, Frederick, MD) that were fed standard laboratory chow after daily behavioral sessions to maintain a 300 – 370 g body weight. Experiments were conducted during the light cycle. Animal maintenance and research were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), and Institutional Animal Care and Use Committee-approved protocols.

Training Procedure

Rats were trained to discriminate 5.6 mg/kg i.p. cocaine from saline in a two-lever, food-reinforced discrimination procedure as previously described (Banks et al., 2014; Caine et al., 2000). Discrimination training was conducted during daily sessions consisting of a 10-min response period and rats could earn up to 25 food pellets (F0165 45 mg pellets, BioServ, Flemington, NJ) by responding under a fixed-ratio 10 schedule of pellet presentation. Saline or 5.6 mg/kg cocaine was administered i.p. 10 min prior to the start of the response period, in a double alternating pattern across days. The criterion for accurate discrimination was 75% injection-appropriate responding before delivery of the first reinforcer, 90% injection-appropriate responding for the entire component, and responses rates 0.02 responses/sec (sufficient to earn at least one pellet) for 5 out of 6 consecutive sessions.

Testing Procedure

Test sessions were identical to training sessions except that response requirement completion on either lever produced food, and drugs were administered as described below. Test sessions were conducted on Tuesdays and Fridays and only if criteria for accurate

discrimination had been met during the preceding training session. The pretreatment time for (–)-cocaine, (+)-phendimetrazine, and (+)-phenmetrazine dose-effect determinations was 20 min. For time course studies, different pretreatment times (10–300 min) were tested across different test session days. Dose-effect studies were conducted before time course studies, and both dose and pretreatment time were examined in ascending order.

Drugs

(–)-Cocaine HCl was provided by the National Institute on Drug Abuse Drug Supply Program (Bethesda, MD). (+)-phenmetrazine fumarate and (+)-phendimetrazine hemifumarate were synthesized by Bruce Blough. All drugs were dissolved in sterile saline to yield an injection volume of 1 mL/kg. All drug doses were expressed as the salt forms listed above.

Data Analysis

The primary dependent measures were (1) percent cocaine-appropriate responding (% CAR) (defined as [number of responses on the cocaine-associated key divided by the total number of responses on both the cocaine-and saline-associated keys]*100), and (2) rates of responding in responses per second. % CAR was included in the analysis only if at least one ratio requirement was completed. These dependent measures were then plotted as a function of drug dose or time after test drug administration. Dose effects were analyzed using one-way ANOVA (Prism 6.0f for Mac, GraphPad, La Jolla, CA), and in the presence of a significant dose effect, a Dunnett post-hoc test was conducted to determine differences compared to saline. In addition, the effective drug dose to produce 50% CAR (ED₅₀) in dose-effect studies, and time for % CAR to decline to 50% (T₅₀) in time-course studies, were determined in each rat by either linear regression when at least three data points were available or interpolation when only two data points were available (one below and one above 50% CAR). Individual cocaine, phendimetrazine, and phenmetrazine ED₅₀ and T₅₀ values were then averaged to yield mean values and 95% confidence limits. Mean cocaine, phendimetrazine, and phenmetrazine ED₅₀ and T₅₀ values were compared by one-way ANOVA.

Results

Cocaine-like discriminative stimulus effects of phendimetrazine and phenmetrazine

On cocaine and saline training days preceding all test days, mean $\pm$ SEM percentages of injection-appropriate responding were 96.8 ± 2.3 and $97.1 \pm 4.4\%$, respectively. Rates of responding during cocaine and saline training days were 0.5 ± 0.1 and 0.8 ± 0.1 responses/s, respectively. Figure 1A shows that cocaine, phendimetrazine, and phenmetrazine produced dose-dependent % CAR. Saline administration during a test session produced <10% CAR in all rats. For cocaine, both 1.8 and 5.6 mg/kg produced 90% CAR in all 5 rats. For phendimetrazine, 10 mg/kg produced 90% CAR in all 5 rats. For phenmetrazine, 1.0 mg/kg produced 90% CAR in 3 out of 5 rats and 3.2 mg/kg produced 90% CAR in all 5 rats. These descriptive results were supported by one-way ANOVA ($F_{10,49} = 26.1, p < 0.001$). In addition, the ED₅₀ value (95% confidence limits) was significantly ($F_{2,14} = 68.79, p < 0.001$) larger for phendimetrazine [5.0 mg/kg (3.97–6.28)] than for phenmetrazine [0.64 mg/kg

(0.37–1.11)] or cocaine [0.66 mg/kg (0.45–0.99)], demonstrating that phendimetrazine was 7.8-fold less potent than phenmetrazine. Figure 1B shows that no cocaine, phendimetrazine, or phenmetrazine dose significantly altered rates of responding.

Figure 2A shows the time course for 5.6 mg/kg cocaine, 10 mg/kg phendimetrazine, and 3.2 mg/kg phenmetrazine. Both phendimetrazine and phenmetrazine produced cocaine-like effects at 10 min (first time point assessed) which returned to saline-like levels by 100 min. The T_{50} values (95% confidence limits) for phendimetrazine [54.7 min (36.3–82.4)] and phenmetrazine [46.1 min (33.8–62.9)] and were not different. The T_{50} value for cocaine was [108.3 min (71.3–164.5)]. Figure 2B that shows cocaine, phendimetrazine, or phenmetrazine did not significantly alter rates of responding.

Discussion

This study compared the potency and time course of phendimetrazine and phenmetrazine in a rat cocaine discrimination procedure. There were two main findings. First, both phendimetrazine and phenmetrazine produced dose-dependent and complete cocaine substitution, but phendimetrazine was 7.8-fold less potent. Second, phendimetrazine and phenmetrazine had similar time courses. In particular, the substitution profile within 10 min parallel results in rhesus monkeys and suggest that phendimetrazine may not require metabolism to phenmetrazine to produce its behavioral effects in rats. Overall, these results provide further evidence that ‘agonist-like’ cocaine medication effects of phendimetrazine maybe mediated via both phendimetrazine and its metabolite phenmetrazine.

The present results agree with a previous finding that phenmetrazine substituted for cocaine in rats (Wood and Emmett-Oglesby, 1988); however, this is the first study in rats to examine cocaine-like effects of phendimetrazine and compare phendimetrazine and phenmetrazine potencies and time courses. The full effectiveness and 7.8-fold lower potency of phendimetrazine vs. phenmetrazine to substitute for cocaine in rats is similar to the full effectiveness and 3.6-fold lower potency of phendimetrazine vs. phenmetrazine to substitute for cocaine in rhesus monkeys (Banks et al., 2013b). Phendimetrazine was also fully effective, but approximately 3- to 5-fold less potent than phenmetrazine to substitute for the discriminative stimulus effects of amphetamine in pigeons (Evans and Johanson, 1987) and rhesus monkeys (de la Garza and Johanson, 1987). Overall, the present rat results are generally consistent with previous drug discrimination results in other species.

The lower potency of phendimetrazine vs. phenmetrazine to substitute for cocaine could reflect a metabolism requirement to convert phendimetrazine to phenmetrazine; however, the time course data do not support this hypothesis. In addition to having lower potency than their active metabolites, inactive pro-drugs also usually have a slower onset and offset rate. For example, lisdexamfetamine is an inactive prodrug for amphetamine, and lisdexamfetamine had both lower potency and a slower onset and offset rate than the active metabolite amphetamine to produce cocaine-like discriminative stimulus effects in rhesus monkeys (Banks et al., 2015) and amphetamine-like discriminative stimulus effects in rats (Heal et al., 2013). In contrast, phendimetrazine and phenmetrazine have now been shown to produce cocaine-like discriminative stimulus effects within 10 min in both rats (present

study) and rhesus monkeys (Banks et al., 2013b). Although rapid metabolism of phendimetrazine to phenmetrazine might account for these behavioral effects, pharmacokinetic studies found that metabolism to phenmetrazine could not account for phendimetrazine effects at 10 min in rhesus monkeys (Banks et al., 2013b). Overall, these results indicate that phendimetrazine may not require metabolism to phenmetrazine to produce behavioral effects.

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Figure 1.

Potency of cocaine-like discriminative stimulus effects of (–)-cocaine (0.56 – 5.6 mg/kg), (+)-phenmetrazine (0.32 – 3.2 mg/kg), and (+)-phendimetrazine (1.0 – 10 mg/kg) in rats trained to discriminate 5.6 mg/kg, IP cocaine from saline. The upper panel (A) shows percent cocaine-appropriate responding (% CAR) as a function of unit drug dose, and the lower panel (B) shows the corresponding rates of responding in responses per second. Symbols above “S” represent data collected after saline administration during a test session. Points represent mean $\pm$ SEM of 5 rats. Filled symbols denote statistical significance ($p < 0.05$) compared to saline.

Figure 2.

Time course of cocaine-like discriminative stimulus effects of (–)-cocaine (5.6 mg/kg), (+)-phenmetrazine (3.2 mg/kg), and (+)-phendimetrazine (10 mg/kg) in rats trained to discriminate 5.6 mg/kg, IP cocaine from saline. The upper panel (A) shows percent cocaine-appropriate responding (% CAR) as a function of time post administration and the lower panel (B) shows the corresponding rates of responding in responses per second. Symbols above “S” and “C” represent the group averages $\pm$ SEM for all training sessions preceding test session when the saline- and cocaine-appropriate keys were correct, respectively. All points represent the mean $\pm$ SEM of 5 rats.