Behavioral Effects of Adenosine Agonists: Evaluation by Punishment, Discrete Shuttle Avoidance and Activity Tests in Mice

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ABSTRACT—Behavioral effects of propentofylline and N\textsuperscript{6}-(L-2-phenylisopropyl)-adenosine (PIA) were evaluated by operant behavior under a punishment situation, discrete shuttle avoidance response and ambulatory activity in mice. Propentofylline (3 mg/kg, s.c.) and PIA (0.01 mg/kg, s.c.) significantly decreased the punished response without producing a significant change in the non-punished response. Propentofylline and PIA reduced the increase in the punished response induced by caffeine (30 mg/kg) and diazepam (1 mg/kg). Propentofylline and PIA also reduced the increase of ambulation induced by caffeine. Furthermore, the single administration of propentofylline and PIA decreased the response rate and/or % avoidance in the discrete shuttle avoidance situation. However, the effective doses of propentofylline and PIA to reduce the ambulation-increasing effect of caffeine and to produce a change in the avoidance behavior were much higher than those effective for eliciting a significant change in the punished response. The present results suggest that there is an intimate interaction between central adenosine and benzodiazepine systems with regards to the change of punished response.

It has been well-known that anxiolytic drugs such as benzodiazepine derivatives act to increase various types of punished responses in the operant situation (1), electrified water procedure (2), and/or hypertonic NaCl procedure (3). Furthermore, there are some reports that caffeine and theophylline, which are methylxanthine derivatives and classified into the category of central stimulant drugs, produce an increase in the punished response at certain doses (4–9). Methylxanthine derivatives possess an antagonistic action towards adenosine receptors in the central nervous system (10–12). However, the characteristics of the behavioral effects of adenosine agonists including the effect on the punished response have not been investigated sufficiently.

Hence, the present study was conducted to evaluate the effects of propentofylline, which is a xanthine derivative with an inhibitory action on the adenosine uptake process (13), and N\textsuperscript{6}-(L-2-phenylisopropyl)-adenosine (PIA), an adenosine receptor agonist (14), by the behaviors in 3 different kinds of experimental situations: punished and non-punished responses in an operant situation of food reinforcement, discrete shuttle avoidance and ambulatory activity in mice. The purposes of the punishment test were to assess whether the single administration of propentofylline and PIA produced a further suppression of the punished response and whether these two
drugs were effective for reducing the increase of the punished response induced by caffeine and diazepam. The latter two experiments were carried out to evaluate the effects of propentofylline and PIA on another type of operant behavior and unconditioned behavior and/or to confirm the specificity of the effects of propentofylline and PIA on the punished response.

MATERIALS AND METHODS

Animals
Animals used were adult male mice of the ddY strain (Japan Laboratory Animals). Groups of 10 mice were housed in standard aluminum breeding-cages (30 × 20 × 10 cm) in a controlled room (temperature: 23 ± 2°C, and light period: 6:00-18:00). These mice were freely given food (MF, Oriental Yeast) and tap water until the start of the experiment.

Drugs
The drugs used were propentofylline (Hoechst Japan), \(N^6\)-(L-2-phenylisopropyl)-adenosine (PIA, Sigma Chem.), caffeine anhydrous (Kanto Chem.) and diazepam (Cercine Inj., Takeda). Propentofylline, PIA and caffeine were dissolved in physiological saline. The commercial preparation of diazepam was diluted by 5% propylene glycol aqueous solution. All of these drug solutions were administered subcutaneously (s.c.) in a fixed volume of 0.1 ml/10 g body weight of the mice regardless of the drug doses.

Punishment test
The apparatus used in this experiment was the same as those used in our previous experiments (7). Briefly, the experimental chamber, which was made of acrilfiber and aluminum boards with dimensions of 18(W) × 9(D) × 10(H) cm (GT-8501: O’Hara & Co.), was connected to a vertically-arranged lever of stainless steel and a food dispenser for food reinforcement (20 mg pellet). The experiment was controlled and recorded by a micro-processor (GT-8805: O’Hara & Co.) and recorder (GT-7715; O’Hara & Co.).

The food-deprived mice were trained to press the lever under a multiple variable interval of 1.5 min/fixed ratio 5-punishment (MULT VI 1.5/FR 5-punishment) schedule of food reinforcement. Thus, in the safe period, which lasted for 6 min, the mouse’s lever-pressings were food reinforced at an average interval of 1.5 min without the punishment. In the alarm period, which lasted for 4 min and was indicated by an 800 Hz tone signal, every 5th lever-press was food reinforced, but it was punished by an electric shock (50–75 V, 0.2–0.3 mA, 50 Hz AC and 0.5 sec duration). The shock intensity was adjusted in each mouse so that the response rate in the alarm period was about 1/10 of that without giving the electric shock. In addition, an unavoidable shock, which was the same intensity and duration as the punishment, was delivered at the end of each alarm period according to our standard procedure (15). In each experimental inning of 10 min, the safe period was first conducted and then followed by the alarm period, and the session consisted of a repetition of 4 innings. The experiment was held every day except on Sunday. When a behavioral baseline was established for the mice, i.e., emitting a moderate to high response rate (15–20/min) in the safe period and a low response rate (around 1/min) in the alarm period, the drug-testing sessions were inserted at intervals of 3–4 days. In each drug test, the order of the doses administered were randomized. The data on the preceding days, on which saline was administered, were taken as the control and/or baseline values.

The indices of the behavior in this test were the response rates in the safe and alarm periods. The former and latter rates were used for evaluating the drug effects on general activity and motivation for food and on the punished response, respectively.

Discrete shuttle avoidance test
The experimental procedure and the apparatus were the same as those employed in our
previous experiments (16). The experimental chamber (GT-8450, O'Hara & Co.) was made of acrylfiber and aluminum boards with dimensions of 30(W) × 9(D) × 15(H) cm, with 2 photobeams at an interval of 18 cm. The behavior-controlling and data-recording apparatus (De CARES GT-M5 and TIDP-10, respectively; O'Hara & Co.) allowed us to conduct the experiment on 5 animals at the same time.

The temporal parameters of the discrete avoidance schedule were an intertrial interval of 25 sec and a warning duration of 5 sec. An electric foot shock of 100 V, 0.3 mA, 50 Hz AC was delivered through the floor grid of the chamber as the unconditioned stimulus. The maximum duration of the shock presentation was 3 sec during the training sessions, and it was constant for 0.3 sec during the drug-testing sessions. Each session consisted of a 1-hr performance in which 120 avoidance trials were carried out. The indices of the avoidance response were the overall response rate (frequency of shuttles) and % avoidance (avoidance responses/avoidance trials) in each session.

The drug administration was carried out immediately before the start of the session; and thereafter, the avoidance response of each mouse was observed for 1 hr. The drug-testing sessions were inserted at intervals of 3–4 days; and on the days before, saline was administered as the control sessions. In each drug test, the doses administered were randomized.

**Ambulatory activity test**

The apparatus for measurement of the ambulatory activity of the mice was a tilting-type ambulometer with 10 bucket-like plexiglas activity cages, 20 cm in diameter (SMA-10 O'Hara & Co.), by which the ambulatory activities of 10 mice could be observed at the same time.

Drug-naive mice were individually put into the activity cages; and after an adaptation period of 30 min, drugs were administered. Then, the ambulatory activity of each mouse was measured for 2 hr.

All experiments were held between 9:00–18:00.

**Statistical comparison**

The mean overall response rates and % avoidances during the sessions and the mean overall ambulatory activity counts for 2 hr were first analyzed by ANOVA. In a case of significant variance, comparisons between individual data were conducted by Cochran-Cox and/or paired tests. When the P value was equal to or less than 0.05, the two values were considered to be significantly different.

**RESULTS**

**Punishment test**

Figure 1 shows the dose-effect relationships for the single administration of propentofylline (1–30 mg/kg) and PIA (0.001–0.3 mg/kg) on the non-punished response (upper panel) and...
punished response (lower panel) in the MULT VI 1.5/FR 5-punishment schedule of food reinforcement. Propentofylline at 3 mg/kg and PIA at 0.01 mg/kg significantly decreased the punished response without producing a significant change in the non-punished response. PIA at 0.1 mg/kg or more almost completely inhibited both the non-punished and punished responses.

Figure 2 shows the combined effects of propentofylline with caffeine (30 mg/kg) and diazepam (1 mg/kg) on the responses in the MULT VI 1.5/FR 5-punishment schedule. Caffeine slightly but significantly increased the punished response, and the effect was reduced by propentofylline. The rates of the non-punished response after the combined administration of propentofylline (at 3 mg/kg and more) with caffeine were significantly lower than that after administration of either saline or caffeine alone. On the other hand, 1 mg/kg of diazepam markedly increased the punished response. Propentofylline reduced dose-dependently the diazepam-induced increase in the punished response without eliciting any significant change in the non-punished response.

Figure 3 shows the combined effects of PIA with caffeine and diazepam. When PIA at 0.1 or 0.3 mg/kg was administered in combination with caffeine, the rate of the punished response was significantly lower than the caffeine alone-administered value. The rate of the non-punished response was significantly lower after the combined administration of caffeine with 0.3 mg/kg of PIA than the rates after saline and caffeine alone. PIA was effective for reducing the diazepam-induced increase in the punished response at 0.01 mg/kg and more. The rate of the non-punished re-

Fig. 2. Combined effects of propentofylline (Prop.) with caffeine and diazepam on the responses on the MULT VI 1.5/FR 5-punishment schedule of food reinforcement in mice. The data are shown in the same way as in Fig. 1. Figures presented in the bottom of each column indicate the doses of drugs (mg/kg, s.c.). * and #: Significant difference as compared with the saline (SAL)- and caffeine alone-administered values, respectively (P < 0.05). N = 8 - 10.

Fig. 3. Combined effects of N^6-(L-2-phenylisopropyl)-adenosine (PIA) with caffeine and diazepam on the responses on the MULT VI 1.5/FR 5-punishment schedule of food reinforcement in mice. The data are shown in the same way as in Fig. 2. N = 8 - 10.
response after the combined administration of diazepam with 0.03 mg/kg of PIA was significantly lower than those after saline or diazepam alone.

**Discrete shuttle avoidance test**

Figure 4 shows the dose-effect relationships for propentofylline (1-100 mg/kg) and PIA (0.01-1 mg/kg) on the discrete shuttle avoidance response in terms of the response rate and the % avoidance. Propentofylline decreased the response rate at 30 mg/kg or more and decreased the % avoidance at 100 mg/kg. PIA, at 0.1 mg/kg or more, slightly but significantly decreased the response rate without eliciting a marked change in the % avoidance.

**Activity test**

Figure 5 shows the mean overall activity counts for 2 hr after the combined administration of propentofylline and PIA with caffeine. Caffeine increased the mouse's ambulatory activity with the maximum effect at around 20 min and the duration of about 2 hr after the administration. Propentofylline at 100 mg/kg and PIA at 0.1 mg/kg or more significantly reduced the caffeine-induced increase in the ambulatory activity. Such an antagonistic effect lasted for about 1 hr in the case of propentofylline and for 1-2 hr in the case of PIA, depending on the doses.

**Gross observation**

After administration of propentofylline at 100 mg/kg or PIA at 0.1 mg/kg or more, the mice became sedated.

**DISCUSSION**

It has been generally accepted that benzodiazepine anxiolytics clearly increase the punished response under various situations in animals (1). Consistent with such a general consideration as well as with the previous reports by us (7, 8), diazepam increased the punished response in the MULT VI 1.5/FR 5-punishment schedule of food reinforcement. In contrast, it has been reported that beta-carboline derivatives, which are considered to be inverse-agonists of benzodiazepine receptors that induce anxiety in humans (17), produce a further suppression of the punished response in animals (18). In these respects, it is expected that the drug-induced modification of the punished response is closely related to the drug-induced change in the anxiety-related

![Fig. 4. Effects of propentofylline and N⁸-(L-2-phenylisopropyl)-adenosine (PIA) on discrete shuttle avoidance response in mice. The data presented are the mean overall response rate and the % avoidance with S.E.M. Each figure presented in the bottom indicates the dose of drug (mg/kg, s.c.). *: Significant difference as compared with the control value after administration of saline (dose = 0) (P < 0.05). N = 10.](image)

![Fig. 5. Mean overall ambulatory activity counts with S.E.M. for 2 hr after the combined s.c. administration of caffeine (10 mg/kg) with propentofylline and N⁸-(L-2-phenylisopropyl)-adenosine (PIA) to mice. Figures presented in the bottom of each column indicate the doses of drugs (mg/kg, s.c.). * and #: Significant difference as compared with the saline (SAL)- and caffeine alone-administered control values, respectively (P < 0.05). N = 15-20 in each experiment.](image)
emotional state.

Coffin and Spealman (6) demonstrated in squirrel monkeys that adenosine receptor agonists PIA, N\textsuperscript{6}-cyclohexyladenosine and 5'-N-ethylcarboxamidoadenosine, produced an enhancement in the shock-induced suppression of the lever-pressing for food reinforcement. They also demonstrated that such an effect was reduced not only by the benzodiazepine anxiolytic chlordiazepoxide, but also by methylxanthines such as caffeine, theophylline and theobromine. Methylxanthines show an antagonistic action on the adenosine receptors (10, 11), and they sometimes attenuate conflict behaviors (4, 5-8, 19). The present experiment also demonstrated a slight but significant increase in the punished response after administration of caffeine, although the effect was much weaker than that of diazepam.

In agreement with the results reported by Coffin and Spealman (6), the present experiment also demonstrated that 3 mg/kg of propentofylline, a xanthine derivative with an inhibitory action on the adenosine uptake process (13) and 0.01 mg/kg of PIA, an adenosine receptor agonist, produced a further suppression of the punished response without eliciting a significant change in the non-punished response. Propentofylline and PIA, at these doses, produced no significant change in the discrete shuttle avoidance response and the ambulatory activity in mice (20). However, the change of the punished response by propentofylline and PIA was clearly different from that produced by central stimulants. We have reported that amphetamines decrease both the punished and non-punished response at comparatively lower doses (21), but increase the ambulatory activity and response rate in the discrete avoidance test (22). The decrease in both the non-punished and punished response after PIA, at 0.1 mg/kg and more, may reflect the sedation due to overdoses, because these doses of PIA decreased the response rate in the discrete avoidance test. Thus, these results suggest a possibility that stimulation of central adenosine systems is involved in the change in the punished response. It has been demonstrated that adenosine and/or adenosine analogs antagonize the behavioral and neurochemical changes induced by methylxanthines (23-29). However, the caffeine-induced increase in the punished response was reduced by comparatively higher doses of propentofylline and PIA. Therefore, a further study is required to make a definite conclusion about the direct relationship between central adenosine systems and the punished response and to elucidate the inverted U-shape dose-effect relations.

Interestingly, the increase of the punished response by diazepam was reduced by propentofylline and PIA in a dose-dependent manner. Particularly, the effect of PIA appeared at comparatively lower doses, suggesting that there is an intimate interaction between adenosine and benzodiazepine systems with respect to the change in the punished response, and that these two systems show an antagonistic interaction. Many researchers (6, 30-32) have also reported that adenosine agonists could reduce the benzodiazepine-induced increase in exploratory behavior, food intake, lever-pressing for food reinforcement, etc. in squirrel monkeys, rats and mice.

There is still some concern about the specificity of the combined effect of PIA and diazepam on the punished response. This is because the punished response is modified by many factors such as general activity, sensitivity to a noxious stimulus, motivation for food intake, etc. Propentofylline and PIA decreased the responses in the safe period (i.e., non-punished response), the response in the discrete shuttle avoidance test, and reduced the caffeine-induced increase in the ambulatory activity. However, 100 mg/kg of propentofylline and up to 0.1 mg/kg of PIA were required to produce significant changes in these behaviors, where as much lower doses of propentofylline and PIA had an effect on the punished response. It is therefore highly probable that the combined effect of adenosine agonists and diazepam on the punished response appeared through an interaction between adenosine and benzodiazepine systems rather than the change
in non-specific factors, although we have not conducted any experiments to evaluate the modification of the combined effect of an adenosine agonist with diazepam by benzodiazepine antagonists (such as Ro 15-1788) and adenosine antagonists (such as caffeine).

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