Rolipram as a Discriminative Stimuli: Transfer to Phosphodiesterase Inhibitors

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Abstract—Rats were trained to discriminate ($\pm$)-rolipram (0.32 mg/kg, i.p.) from saline in a two-lever food-reinforced drug discrimination procedure (FR 10). ($\pm$)-Rolipram served as a drug discriminative stimulus, and its discrimination was readily established with a mean of 42 training sessions to achieve criterion performance (at least 80% correct response in the consecutive generalization tests with both saline and ($\pm$)-rolipram). Thereafter, this stimulus was stably maintained. The (–)-isomer of rolipram was generalized at about one-third of the training dose of the ($\pm$)-compound, but the (+)-isomer was generalized only at 10 times the dose of the ($\pm$)-compound. This finding suggests that the (–)-compound is more extensively involved as a stimulus than either ($\pm$)-rolipram or the (+)-isomer. Ro20–1724, caffeine and theophylline, which are phosphodiesterase inhibitors, were generalized to ($\pm$)-rolipram. This result strongly suggests that phosphodiesterase inhibition may be an important factor involved in ($\pm$)-rolipram discrimination.

Drug discrimination learning using drugs as discriminative stimuli is considered to reflect the substitution effect of drugs. Recently, drug discrimination has been frequently utilized as a unique behavioral method for the classification of drugs (1) and for the preclinical evaluation of subjective effects of drugs in man (2, 3). Psychoactive drugs generally have discriminative properties (4), but drug discriminative learning is said to be comparatively difficult with antidepressants as they produce less effective discriminative stimuli (5).

($\pm$)-Rolipram [4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidone] is a dialkoxypheyl pyrrolidone derivative which a) antagonizes reserpine-induced hypothermia and hypoactivity (6), b) significantly decreases the immobility time in a forced swimming test (7), and c) potentiates yohimbine toxicity (6). In view of these actions, this compound has been regarded as a new antidepressant (8). Biochemical studies have shown that, like atypical antidepressants, rolipram has no anti-cholinergic

effect and inhibits neither monoamine oxidase nor monoamine re-uptake (6, 9). On the other hand, it significantly inhibits cerebral phosphodiesterase (10, 11).

The present study was designed to ascertain whether ($\pm$)-rolipram would serve as a discriminative stimulus in drug discrimination learning and, provided that drug discrimination learning could be established, to clarify the discriminative characteristics of ($\pm$)-rolipram.

Materials and Methods

Animals: Ten male rats of the Slc: Wistar-KY strain (Shizuoka Laboratory Animals Center) were used. The rats were housed in groups of 4 per cage in a vivarium controlled at $21 \pm 2^\circ\text{C}$ and maintained at approximately 80% of free-feeding weight throughout the experimental period. Each rat was given 10 g laboratory food pellets after completion of each daily session. Water was available ad lib.

Drugs: The following drugs were used: ($\pm$)-rolipram and its (+)-isomer and (–)-

isomer (Schering AG., Berlin), Ro20-1724 [4-(3-n-butoxy-4-methoxybenzyl)-2-imidazolidinone] (Hoffmann-La Roche), anhydrous theophylline (Sigma Chemical Co.), and anhydrous caffeine (Yoneyama Yakuhin Kogyo Co., Ltd.)

All drugs were suspended in 1% methylcellulose (MC) and administered i.p. in a constant volume of 1 ml/kg.

Apparatus: The Skinner box equipped with two levers was used. The levers were disposed 17 cm apart at a height of 5.5 cm above the floor grid, with a feeder being mounted between the levers.

Procedure: Each rat was first trained to learn how to obtain one food pellet (about 50 mg, Muromachi Kikai Co., Ltd.) from the feeder as a positive reinforcement (Fixed Ratio 1: FR 1) by pressing either one of the two levers. After an establishment of the lever-pressing in FR 1, the ratio was gradually increased until FR 10. After the establishment of the FR10 lever-pressing condition, drug discrimination training was initiated.

Each rat was immediately put into the Skinner box. ($\pm$)-Rolipram or saline were injected i.p. 30 min prior to the training sessions. A possible effect of lever preference was counter-balanced by randomly assigning the right lever to be the drug lever for 5 rats and the left lever to be the drug lever for the other 5 rats. Discrimination training between ($\pm$)-rolipram (0.32 mg/kg) and saline was performed at FR 5 in sessions 1 to 11 and at FR 10 in session 12 and beyond. Discrimination training after alternative administration of saline or ($\pm$)-rolipram was conducted once a day, and each session ended when 50 reinforcements had been taken or after 30 min. The experiment was performed six days per week. Generalization tests were conducted only in the rats that consistently responded with incorrect lever-pressing of less than 15 in both ($\pm$)-rolipram- and saline-training sessions.

For the generalization test, the rat was put in the Skinner box immediately after i.p. administration of the drug and the test was started 30 min later. In the generalization test schedule, the rat was able to obtain food by responding 10 times on either of the two levers. The generalization test was also

terminated when the rat had obtained the 50th reinforcement or at the end of a test time of 30 min.

Results

1. The acquisition curve for ($\pm$)-rolipram:

The FR 10 response was established after about 10 training sessions.

The upper part of Fig. 1 shows the acquisition curve for ($\pm$)-rolipram discrimination. Trained rats achieved at least 80% correct response in the consecutive generalization tests with both saline and ($\pm$)-rolipram after 33 sessions at the earliest and 55 sessions at the latest. The discriminative learning was established after a mean of 42 sessions and was well maintained thereafter. The lower part of Fig. 1 shows the changes in response rate during discrimination training. Throughout the training period, the response rate in the ($\pm$)-rolipram-session was about 27% less than the rate in the saline session.

After completion of discriminative learning, generalization tests using various doses of ($\pm$)-rolipram were performed (N=10). The ($\pm$)-rolipram-appropriate response was expressed as the total number of responses shown by each rat until it took the 50th pellet or until a test time of 30 min had expired. The appropriate response to ($\pm$)-rolipram was found to increase in a dose-dependent manner (Figs. 2 and 3). Thus the ($\pm$)-rolipram-appropriate response was 0.7% for the vehicle alone, 23.8% at 0.032 mg/kg, 58.7% at 0.1 mg/kg, and 98.9% at the training dose of 0.32 mg/kg (Fig. 3). On the other hand, the response rate was found to decline in a dose-dependent manner (Fig. 3).

2. Generalization tests with the optical isomers of rolipram: The results of generalization tests with the optical isomers of rolipram are shown in Fig. 3. Both the (+)-rolipram (N=6) and (-)-rolipram (N=6) showed the ($\pm$)-rolipram-appropriate response in a dose-dependent manner, but there was a difference in the dose required. Namely, (+)-rolipram at 3.2 mg/kg was generalized to the ($\pm$)-rolipram stimulus (99.7%), whereas only 0.1 mg/kg of (-)-rolipram was required for generalization

Fig. 1. Acquisition of a discrimination between (±)-rolipram 0.32 mg/kg (i.p.) and saline in rats (upper panel: N=10). Values are the mean percent with S.E.M. of (±)-rolipram for appropriate responding during 3 min following initiation of the test period. The response rate is expressed as the mean number of lever pressing responses per min (lower panel).

(81.2%). On the other hand, both isomers slightly decreased the response rate (Fig. 3).

3. Generalization tests with phosphodiesterase inhibitors: Figure 4 shows results of generalization of phosphodiesterase inhibitors caffeine (N=4), theophylline (N=6) and Ro20-1724 (N=5) to the (±)-rolipram stimulus. Rats treated with all three different phosphodiesterase inhibitors showed (±)-rolipram-appropriate responses in a dose-dependent manner. The generalization doses were 3.2 mg/kg for Ro20-1724 and 56 mg/kg for caffeine and theophylline. Likewise, response rate was decreased by these phosphodiesterase inhibitors at these generalization doses.

Discussion

The present experiment showed that (±)-rolipram at a small dose (0.32 mg/kg) served as a discriminative cue and that its discrimination was stably maintained. Overtone and Batta (12) succeeded in training animals to discriminate doxepin, imipramine or amitriptyline from saline at relatively high

doses in a shock-escape T-maze task. In a two-lever operant task, desipramine (10 mg/kg) has served as a discriminative stimulus (5). However, even in this case, only about one-half of the rats learned to discriminate. Furthermore, this dose produced an increase in mortality among the experimental subjects before extensive transfer testing could be accomplished (5). Jones et al. (13) reported that imipramine at 10 mg/kg failed to be discriminated from saline in rats. From these findings, it has generally been considered that antidepressants produce drug discrimination with difficulty, and when discrimination is achieved, it can not be reliably maintained. In this respect, (±)-rolipram is qualitatively different from imipramine and desipramine.

Rolipram is a racemic drug which can be resolved into its optically active forms (+ and -). In the present study, the (-)-isomer was generalized at a dose equal to about one-third of the training dose of the (±)-compound, but the (+)-isomer had to be used at a dose 10 times that of the (±)-

Fig. 2. Cumulative response records of performance under FR 10 after the administration of ($\pm$)-rolipram (upper panel) and Ro 20-1724 (lower panel) in ($\pm$)-rolipram-trained rats. The response-marking pen (D) moves one step upward in turn with each ($\pm$)-rolipram-appropriate response (pressing ($\pm$)-rolipram-lever). The event-marking pen (S) indicates the saline appropriate response (pressing saline-lever). Positive reinforcement (a 50 mg food pellet) is indicated by a diagonal hash marked on the cumulative record.

compound to achieve the same effect. In the reserpine antagonism and yohimbine potentiation assays, the ($\pm$)-rolipram and its ($-$)-isomer showed approximately the same potency, but the (+)-compound was only 1/10 to 1/15 as potent as the ($-$)-compound (6, 14). Present results of the generalization tests are substantially consistent with this finding. It is thus clear that the discriminative stimulus of rolipram is stereospecific and that the ($-$)-rolipram is more potent than the (+)-rolipram as a discriminative stimulus.

There are at least three types of phosphodiesterases: i) one is present in the membrane and hydrolyzes both cyclic AMP

and cyclic GMP, but is insensitive to Ca^{++} and calmodulin (CaM); ii) the second is a low affinity enzyme ($K_m=300 \mu\text{M}$ for cyclic AMP) which is present in the cytoplasm, and it is activated by Ca^{++} and CaM and hydrolyzes both cyclic AMP and cyclic GMP; and iii) the last is a high affinity form ($K_m=4 \mu\text{M}$ for cyclic AMP) which is also present in the cytoplasm, but is insensitive to Ca^{++} and CaM and selectively hydrolyzes cyclic AMP (15). Rolipram has been reported to inhibit only the cyclic AMP-selective phosphodiesterase (16, 17). Ro20-1724, which also inhibits only the cyclic AMP-selective phosphodiesterase (16, 17), was generalized to the ($\pm$)-rolipram stimulus. Caffeine and

Fig. 3. Generalization to optical isomers of rolipram by rats trained to discriminate (±)-rolipram (0.32 mg/kg, i.p.) from saline.

Fig. 4. Generalization to various phosphodiesterase inhibitors by rats trained to discriminate (±)-rolipram (0.32 mg/kg, i.p.) from saline.

theophylline differ from rolipram in that their inhibitory action shows no substrate specificity (16-18). However, both xanthines at a dose of 56 mg/kg were generalized

almost 100% to ($\pm$)-rolipram. These results suggest that the discriminative stimulus of ($\pm$)-rolipram may be related to an increase in cyclic AMP, but unrelated to the substrate-specificity of phosphodiesterase inhibition.

Ro20-1724 induces hypothermia and hypoactivity similar to ($\pm$)-rolipram (6, 19, 20). Ro20-1724 is approximately 15 times less potent than ($\pm$)-rolipram in these effects. Moreover, in the present study, the required generalization dose of Ro20-1724 was about 10 times higher than the ($\pm$)-rolipram training dose. Ro20-1724 possess both reserpine-antagonistic and yohimbine-potentiating effects similar to antidepressants (6, 7). Furthermore, the potent phosphodiesterase inhibitor IBMX, another xanthine derivative, also antagonizes reserpine-induced hypothermia and hypoactivity and reduces immobility time in the behavioral despair test similar to antidepressants (7). Although there are no clinical reports which have directly discussed the antidepressant effects of these phosphodiesterase inhibitors, these results suggest that phosphodiesterase inhibition may cause the antidepressant effects observed clinically (6, 7).

($\pm$)-Rolipram suppressed the response rate as compared with the saline injection. It is very interesting that Ro20-1724, caffeine and theophylline reduced the response rate concurrently at the dose of generalization to ($\pm$)-rolipram, although an atypical antidepressant, mianserin, also suppressed the response rate in ($\pm$)-rolipram-trained rats. However, mianserin failed to generalize to ($\pm$)-rolipram (T. Yamamoto et al., unpublished data). This finding indicates that the response rate suppression itself is not due to generalization to ($\pm$)-rolipram.

In conclusion, it was found that rats can be trained to discriminate 0.32 mg/kg ($\pm$)-rolipram (i.p.) from saline, and that phosphodiesterase inhibition may be an important pharmacological action of ($\pm$)-rolipram in drug discrimination.

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