Rolipram and Its Optical Isomers, Phosphodiesterase 4 Inhibitors, Attenuated the Scopolamine-Induced Impairments of Learning and Memory in Rats

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ABSTRACT—We investigated the effects of (±)-rolipram, a phosphodiesterase (PDE) 4 inhibitor, and its isomers on scopolamine-induced deficits of learning and memory in rats using an 8-arm radial maze task and a passive avoidance task. 1) In the 8-arm radial maze task, (±)-rolipram (0.02-0.2 mg/kg, p.o.), (−)-rolipram (0.01-0.02 and 0.2-0.5 mg/kg, p.o.) and (+)-rolipram (20-50 mg/kg, p.o.) attenuated the scopolamine-induced deficits of spatial cognition. As for the minimum effective dose of each drug, (−)-rolipram was 2 and 2000 times as potent as (±)-rolipram and (+)-rolipram, respectively. (−)-Rolipram produced a biphasic dose-response and (±)-rolipram produced a broad dose-response. 2) (±)-Rolipram and its isomers also attenuated the scopolamine-induced deficits in the passive avoidance response. Also for the minimum effective dose, (−)-rolipram (0.01-0.02 mg/kg) was 2 and 200 times as potent as (±)-rolipram (0.02-0.1 mg/kg) and (+)-rolipram (2 mg/kg). 3) The behaviorally effective doses of (±)-rolipram and its isomers also enhanced the oxotremorine-induced tremors in mice. Comparing these racemic isomers, (−)- and (±)-rolipram have more potent effects than (+)-rolipram on scopolamine-induced deficits in the 8-arm radial maze task and passive avoidance task. Especially (±)-rolipram has a wide dose range in these behavioral study. These results suggest that the ameliorating effects of rolipram might result from the indirect potentiation of various transmitters including cholinergic and noradrenergic systems by an increase in cAMP with the inhibition of PDE4.

Keywords: Rolipram, Radial arm maze, Passive avoidance, Tremor, Scopolamine

The dysfunction of central cholinergic systems has been apparent in patients suffering from dementia (1, 2). In addition, the cholinesterase inhibitors are effective in treating Alzheimer's disease (3-6) and in experimental animals (7, 8). Therefore, numerous animal models used in the search for potential nootropic agents are related to the modulation of acetylcholine systems (9, 10). Recent studies indicated that the monoamines and the second messengers including adenosine 3′5′-cyclic monophosphate (cAMP) play critical roles in learning and memory (11). A relationship between the monoaminergic system and the cholinergic pathway has also been suggested (12-14). In fact, monoaminergic agents such as monoamine oxidase inhibitors, which have an anti-depressive effect, improved cognitive function in animal models (15, 16). The hypothesis has been put forward that cognitive disruption might arise not only from a dysfunction of the cholinergic system, but also from a dysfunction of central monoaminergic systems, including neuronal second messenger systems (17, 18).

Rolipram is an inhibitor that is selective for the Ca^{2+}/calmodulin-independent and cAMP-specific isozyme of phosphodiesterase (PDE4) (19). It has been demonstrated to be effective against depressive disorders in clinics and in experimental animals (20-22) and has different effects on the monoaminergic system compared with those of the known monoaminergic agents. It has been suggested that rolipram has an accelerating effect on noradrenergic transmission by both an enhancement of noradrenergic turnover presynaptically (23) and an inhibition of cAMP degradation postsynaptically (24). However, the effect of rolipram on learning and memory is as yet unknown. In

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the present study, to examine the differences in the pharmacological properties of rolipram and its stereoisomers, we investigated 1) whether (±)-rolipram and its isomers could attenuate the scopolamine-induced deficits of learning and memory in rats by using a radial arm maze and a passive avoidance test and 2) whether these agents would potentiate the oxotremorine-induced tremors in mice and have a stimulating effect on the cholinergic system.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 200–250 g and male ddY mice weighing 20–25 g were obtained from the Kyu-Do Co. (Saga); they were housed in groups of 4 to 5 per cage, in a room with a controlled temperature of 23±2℃ and relative humidity of 60±10% with lights on from 7:00 to 19:00. The rats used in the 8-arm radial maze task were placed under restricted food intake (10–12 g/day) (CE-2; Clea Japan, Tokyo). Their body weights were maintained at approximately 80% of the free feeding level during the experimental period. Water was freely available in their home cages. Animal care and the experimental procedures were based on the regulations dictated by the Animal Care and Use Committee at Fukuoka University.

Drugs

The following drugs were used: Scopolamine hydrobromide and oxotremorine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA). Scopolamine and oxotremorine were dissolved in saline (0.9% NaCl in distilled water). (±)-Rolipram ((±)-4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidinone, Fig. 1) and its optical isomers were from Schering AG (Berlin, Germany); they were suspended in 0.5% carboxymethylcellulose (Sigma).

Measurement of radial arm maze performance

The behavioral testing was conducted on an 8-arm radial maze (Neuroscience Co., Tokyo), a modification of the maze originally developed by Olton and Samuelson (25). It was elevated 50 cm from the floor. The maze consisted of a central platform 24 cm in diameter, with eight arms extending radially. Each arm was 50 cm in length, 10 cm in width and 50 cm in height with transparent plastic side walls. Food cups for the reinforcers were located near the end of each arm. The maze was located in a room containing many extra-maze visual cues. For the behavioral analysis, an image motion analyzer, AXIS-30 (Neuroscience), was used to quantify the task performance of rats in the maze. The high speed analyzer had an automatic tracking system which was able to track each rat's movement in the maze with a CCD camera linked to a personal computer, which then analyzed the movement in real time and assessed the length of the route, frequency of arm visits, velocity of walking and time required to accomplish the task.

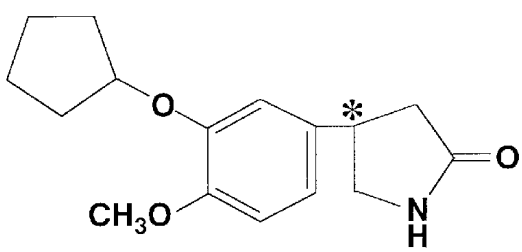

Fig. 1. Chemical structure of rolipram. * indicates an asymmetrical carbon atom.

At first, the test animals were habituated to the apparatus for a 10-min period; this was done 3 times a day at an interval of more than 1 hr. In these periods, the animal was allowed to move freely within the maze and to get pellets. The habituation was carried out for 3 consecutive days before the training period. For each training session, the test animal was placed in a circular plastic ring on the platform at the middle of the maze. Then, after 1 min, the ring was lifted, and the test animal was allowed to move freely in the maze. The session continued until the test animal entered all eight arms or 10 min had elapsed. The performance of the test animal in each training session was assessed by two parameters: the number of correct choices of the first 8 arms chosen and the number of errors, that was defined as choosing an arm that had already been visited. If a test animal reached the criterion of more than 7 correct choices and less than 1 error in 3 successive sessions, scopolamine (0.5 mg/kg, i.p.) was administered to the test animal 30 min prior to the test. Rolipram or an isomer was administered p.o. 30 min before the test animals were treated with scopolamine (3–9 rats per group). Test performance was also assessed with the above two parameters. Spatial cognition in the affected animals was considered to be improved when the drug-state rats made significantly increased correct choices and significantly decreased errors as compared with untreated affected animals.

Measurement of passive avoidance response

The experiments were carried out using a step-through type passive avoidance apparatus (Neuroscience) consisting of an illuminated compartment (20×10×12 cm) with a 6 W tungsten lamp and a dark compartment (30×30×30 cm) with a grid floor (15 parallel steel rods). The compartments were separated by a guillotine door (8×8 cm). Electroshocks (3 mA-AC) were delivered through the grid floor in the dark compartment by a

shock scrambler (Model SGS-102; Muromachi, Tokyo) for 5 sec.

The experiments consisted of training (acquisition) and retention tests. In the training session, each rat was placed in the illuminated compartment and allowed 1 min for habituation. The guillotine door was then opened. The guillotine door was closed immediately when the animal entered the dark compartment, and 10 sec later, an electric shock was delivered through the grid floor. In the retention test, 24 hr after the training test, each animal was again placed in the illuminated compartment, and its latency (step-through latency) to enter the dark compartment was measured (the cut-off time was 600 sec). Scopolamine at a dose of 3 mg/kg was used for this experiment, because scopolamine at 0.5 mg/kg which is a disruptive dose of the radial maze task was not impaired in the retention of the passive avoidance task. Scopolamine was injected i.p. 30 min before the retention test, and rolipram or an optical isomer was administered p.o. 30 min before the treatment of scopolamine (4–10 rats per group).

Measurement of oxotremorine-induced tremors

Mice were used for the investigation of the effects of rolipram and its isomers on oxotremorine-induced tremors. Immediately after an intraperitoneal injection of oxotremorine at 0.3 mg/kg, each mouse was placed into a glass container (12 cm in diameter) and the intensity of tremors was recorded as one of the following scores: 0: no abnormal behavior was observed, 1: intermittent slight tremors, 2: occasional moderate tremors besides intermittent slight tremors, 3: persistent moderate tremors, 4: persistent severe tremors.

Rolipram or an optical isomer was administered orally 50 min before the injection of oxotremorine (10 mice per group). Observation of the tremors was made at 5-min intervals starting 30 min after the oxotremorine treatment, and the intensity of tremors was determined as the mean of the total score during a 30-min test.

Statistical analyses

The results of the radial maze task and the oxotremorine tremor were expressed as means±S.E.M., and medians and interquartile ranges were calculated for the passive avoidance task. The following statistical analyses were made to assess the differences in values between groups: Wilcoxon's rank sum test for the 8-arm radial maze task and oxotremorine-induced tremors and the Mann-Whitney *U*-test for the passive avoidance task.

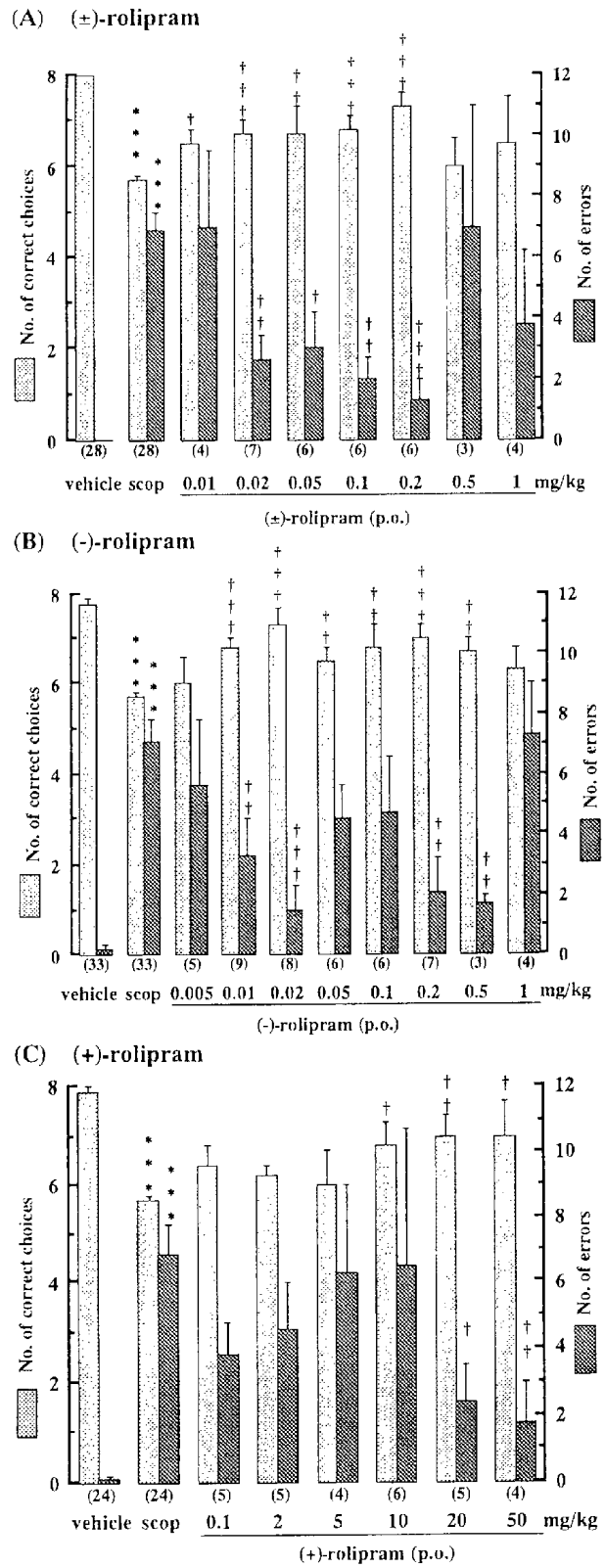

Fig. 2. Effects of rolipram and its isomers on the radial arm maze performance in rats. Each value represents the mean±S.E.M. ***P<0.001, significantly different from the vehicle-treated group. †P<0.05, ††P<0.01, †††P<0.001, significantly different from the scopolamine-treated group (Wilcoxon's rank sum test).

RESULTS

Effects of rolipram and its optical isomers on scopolamine-induced deficits of spatial cognition in the radial arm maze task

Figure 2 shows the effects of rolipram and its isomers on the radial arm maze performance in rats. After scopolamine (0.5 mg/kg) was injected to the test animals that had acquired spatial cognition, the numbers of correct choices were significantly decreased and the number of errors were significantly increased, indicating that the spatial cognition was impaired.

(±)-Rolipram at a dose of 0.02–0.2 mg/kg reversed the decrease in the number of correct choices and the increase in the number of errors induced by scopolamine. The dose-response curve of the effect of (±)-rolipram was an inverted U-shape. (−)-Rolipram reduced the decrease in the number of correct choices at the lower dose of 0.01 mg/kg and the increase in the number of errors at doses of 0.01, 0.02, 0.2 and 0.5 mg/kg. (−)-Rolipram had a biphasic effect, since that at low doses of 0.01 and 0.02 mg/kg and at high doses of 0.2 and 0.5 mg/kg, it improved the scopolamine-induced disruption of spatial cognition. (+)-Rolipram at higher doses of 20–50 mg/kg improved the scopolamine-induced deficits of spatial cognition. The results showed that although (−)-rolipram was twice as potent as (±)-rolipram with regards to the minimum effective dose, (±)-rolipram showed a more clear dose-response curve than (−)-rolipram.

Effects of rolipram and its optical isomers on scopolamine-induced deficits of the passive avoidance task performance

As shown in Fig. 3, scopolamine (3 mg/kg, i.p.) remarkably shortened the step-through latency in the retention test, indicating that scopolamine impaired the retention process of the passive avoidance performance. (±)-Rolipram at 0.02–0.1 mg/kg dose-dependently attenuated the shortening of the step-through latency induced by scopolamine. (−)-Rolipram at 0.01–0.02 mg/kg and (+)-rolipram at 2 mg/kg prolonged the step-through latency in comparison with the scopolamine-treated group. Also, (−)-rolipram was twice as potent as (±)-rolipram with respect to the minimum effective dose.

Effects of rolipram and its isomers on the oxotremorine-induced tremors

Figure 4 shows the effects of rolipram and its isomers on oxotremorine-induced tremors in mice. Oxotremorine (0.3 mg/kg, i.p.) induced slight tremors with a mean score of 2.8±0.6. (±)-Rolipram at doses of 0.2 and 1 mg/kg potentiated the oxotremorine tremors. (−)-Rolipram also enhanced the oxotremorine tremors at the

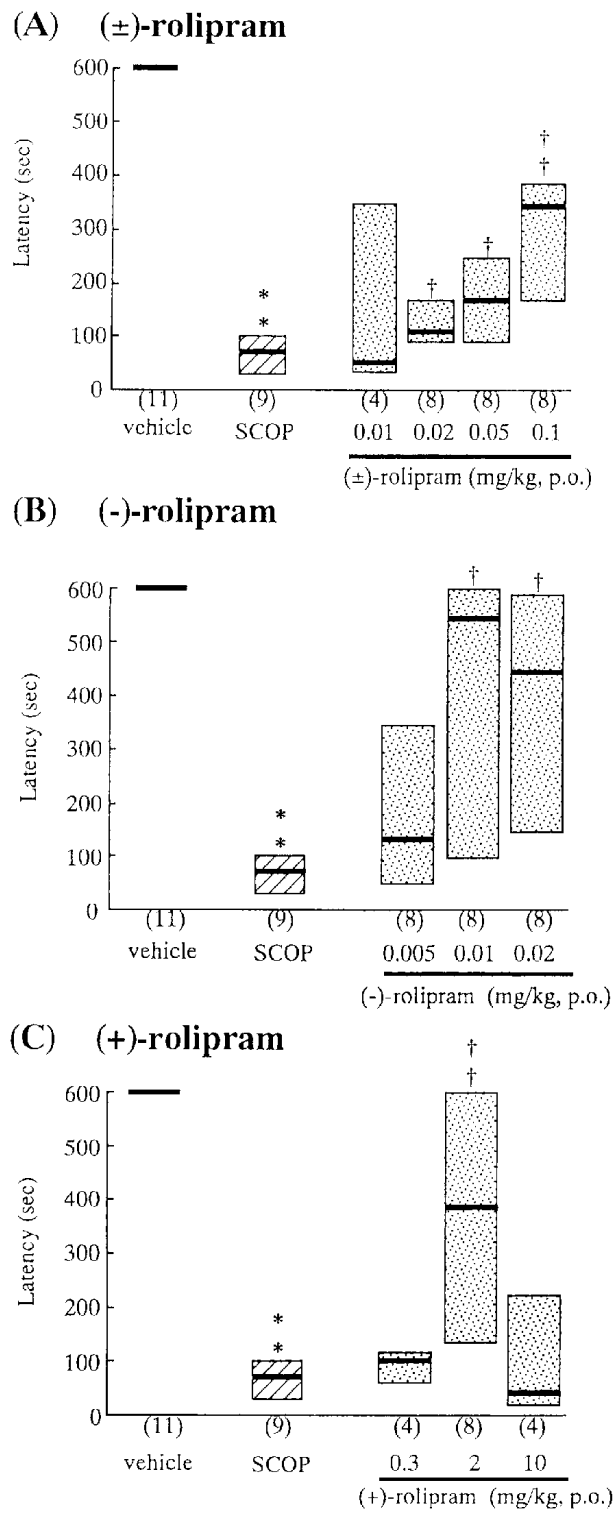

Fig. 3. Effects of rolipram and its isomers on the passive avoidance task in rats. Each value represents the median of latency (horizontal bold bar) and the interquartile range (column) in the retention test. $^{**}P<0.01$ significantly different from the vehicle-treated group. $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$, significantly different from the scopolamine-treated group (Mann-Whitney *U*-test).

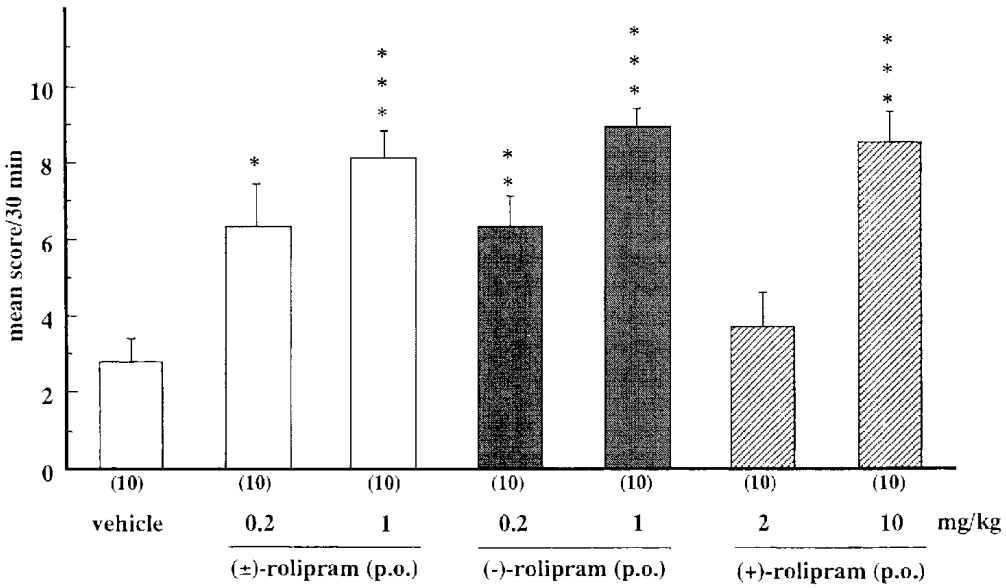

Fig. 4. Effects of rolipram and its isomers on the oxotremorine tremor in mice. Each value represents the mean ± S.E.M. of the total score during a 30-min test. *P < 0.05, **P < 0.01, ***P < 0.001, significantly different from the vehicle-treated group (Wilcoxon's rank sum test).

same dose as (±)-rolipram. (+)-Rolipram enhanced the tremors at a higher dose (10 mg/kg).

DISCUSSION

It is well-known that brain cholinergic hypofunction can cause dementia such as memory loss and disorientation in cerebrovascular or Alzheimer's disease (3, 4). An acetylcholinesterase inhibitor, tetrahydro-aminoacridine (THA), has been shown to improve these symptoms in patients with dementia (5-7). Based on these findings concerning the cholinergic systems, scopolamine, a muscarinic receptor antagonist, has been useful for the evaluation of the effects of drugs on learning and memory deficits in animals and humans (9, 10). We also have reported that scopolamine impaired the performance in the radial maze task and passive avoidance task, and cholinergic enhancers such as physostigmine and THA improved the deficits of memory performance (26). Moreover, it has been reported that scopolamine decreased not only the acetylcholine (ACh) contents but also the noradrenaline (NA) contents in the frontal cortex (FC), and non-cholinergic drugs such as low-dose amantadine or L-threo-DOPS also improved the scopolamine-induced deficits of spatial cognition and reversed the decrease in the NA contents of the FC (27, 28). These results suggest that the scopolamine-induced deficits of spatial cognition in rats originate from the dysfunction of multiple neurotransmitter.

In the present study, to examine the pharmacological properties of rolipram, we investigated whether rolipram and its isomers attenuate scopolamine-induced learning and memory deficits in rats. Rats treated with rolipram or one of its isomers showed improvement in the scopolamine-induced deficits in the radial arm maze task and passive avoidance task. In the maze task, (±)-rolipram (0.02-0.2 mg/kg, p.o.), (-)-rolipram (0.01-0.02 and 0.2-0.5 mg/kg, p.o.) and (+)-rolipram (20-50 mg/kg, p.o.) each improved the scopolamine-induced deficits of spatial cognition in rats. In the passive avoidance task, (±)-rolipram (0.02-0.1 mg/kg, p.o.), (-)-rolipram (0.01-0.02 mg/kg, p.o.) and (+)-rolipram (2 mg/kg, p.o.) all improved the scopolamine-induced deficits of passive avoidance response. Comparing these racemic isomers, the effective range of (-)- and (±)-rolipram on the scopolamine-induced deficits of spatial cognition in the 8-arm radial maze is approximately 2000 times more potent than that of (+)-rolipram at their minimum effective dose. Furthermore, the effective range of (-)- and (±)-rolipram on the passive avoidance task is also approximately 200 times more potent than that of (+)-rolipram. Although the exact reason why the racemic isomer's effects is still unknown, (-)- or (±)-rolipram would seem to be good candidates for the treatment of cholinergic deficiencies. Therefore, comparing these two drugs, (±)-rolipram has a wide range of efficacy in scopolamine-induced deficit of spatial cognition. These results confirmed the previous finding using a discrimination task that the rank order potency of each of the rolipram stereoisomers as the discriminative stimulus was

(−)-rolipram = (±)-rolipram > (+)-rolipram, although the potency for both tasks was different from the IC_{50} for inhibiting PDE4. Moreover, several PDE inhibitors were found to substitute for (±)-rolipram's cue, their potencies in the discrimination task were reported to be well-correlated with their potency to displace [^{3}H]-rolipram from forebrain binding sites in vivo (29). It was also reported that the action of (−)-rolipram is very potent in increasing cAMP levels in rat brain (30). These results suggest that the wide ameliorating efficacy of (±)-rolipram might be useful for clinical use.

Recent pharmacological studies demonstrated that cAMP enhanced hippocampal long-term potentiation (31, 32), which is widely regarded as a possible candidate for a synaptic basis of learning and memory in the central nervous system (33). These findings suggested that the enhancement of signal trasduction via adenylate cyclase in the hippocampus may play a major role in learning and memory (11). From these facts, present findings that (±)-rolipram attenuates the scopolamine-induced deficits of learning and memory may result from an increase in cAMP via the inhibition of PDE4. Therefore, to investigate whether (±)-rolipram had a direct cholinergic stimulating effect, we examined the effect of (±)-rolipram and its isomers on oxotremorine-induced tremor. Oxotremorine-induced tremor in mice has been reported to be a useful model for the initial identification of drugs with the central cholinergic potency because these drugs have a direct stimulating effect on central muscarinic receptors (27). The present results showed that (−)- and (±)-rolipram significantly potentiated the oxotremorine-induced tremor at doses that appeared to improve on the maze task. In addition, analogues of cAMP have been reported to promote the activity of cholinergic neurons and to potentiate acetylcholine responses (34, 35). However, it is also reported that rolipram enhances NA turnover presynaptically and accumulates cAMP by inhibiting PDE postsynaptically (36). Since NA-related drugs can attenuate the scopolamine-induced deficits of learning and memory, our results suggest that (±)-rolipram possesses a stimulating effect on the central monoaminergic systems. From these results it is considered that (±)-rolipram may increase cAMP via the inhibition of PDE4 and secondarily activate the cholinergic and noradrenergic systems. It is necessary to consider that rolipram affects other systems that are disrupted by scopolamine via an increase in cAMP. Indeed, we already reported that many cognitive enhancers can improve scoplamine-induced disruption of spatial cognition in rats without activating directly the cholinergic and noradrenergic systems (26, 27).

In conclusion, the results showed that the treatments with (±)-rolipram attenuate the scopolamine-induced impairments of learning and memory in the radial arm maze performance and passive avoidance response of rats with a wide dose range and that these agents enhanced oxotremorine-induced tremors in mice. Therefore, the ameliorating effects of rolipram might result from the indirect potentiation of central cholinergic and noradrenergic systems by an increase in cAMP with the inhibition of PDE4.

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