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Behavioral Effects of Rolipram and Structurally Related Compounds in Mice: Behavioral Sedation of cAMP Phosphodiesterase Inhibitors

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GRIEBEL, G., R. MISSLIN, E. VOGEL AND J.-J. BOURGUIGNON. *Behavioral effects of rolipram and structurally related compounds in mice: Behavioral sedation of cAMP phosphodiesterase inhibitors.* PHARMACOL BIOCHEM BEHAV **39**(2) 321–323, 1991.—The behavioral effects of specific cAMP phosphodiesterase inhibitors (PDE-I) such as rolipram and structurally related compounds were investigated in mice. Selected PDE-I induced a potent dose-dependent decrease in locomotion and in rearing of mice confronted with a free exploratory procedure, these effects being considered as a behavioral sedation. However, in the light/dark choice test especially conceived to reveal disinhibitory and/or anxiolytic action, they did not show obvious effects. These results suggest that the increase of cAMP probably does not account for our previously observed anxiolytic properties of BW A78U, an adenine derivative PDE-I (20).

Sedation Anxiolysis Locomotion Rears Mice Rolipram cAMP phosphodiesterase inhibitors Adenine

AMONG the novel psychotropic drugs, rolipram has been shown to induce behavioral effects such as hypoactivity, forepaw shaking, grooming and head twitches in rats (17,18). Furthermore, this drug has been found to exhibit some potential antidepressant-like effects in animal models (9,19) as well as in preliminary clinical observations (5). It induces a specific increase in brain cyclic AMP (cAMP) levels in rats without affecting cyclic GMP levels (4,13). Recently, we have described in mice sedative and anxiolytic properties of BW A78U (20). Such purine derivatives, like xanthines, have been described as cyclic nucleotides phosphodiesterase inhibitors (PDE-I) with a poor selectivity (15). Moreover, BW A78U was found as potent as rolipram in inhibiting in vascular smooth tissues the specific cAMP PDE (Lugnier, C.; unpublished data). This latter isoenzyme, which is at present called the rolipram-sensitive phosphodiesterase (1), has been characterized in rat brain (8,12). Thus it can be hypothesized that the sedative and anxiolytic effects of BW A78U might be mediated by its ability to increase cAMP in the brain. To examine this hypothesis, the effects of several doses of selective cAMP PDE-I such as rolipram [4-(3-cyclopentyloxy-4-methoxy-phenyl)-2-pyrrolidone], AAL 122 (the 3,4-dimethoxyphenyl analog of rolipram) (14) and another structurally related analog of rolipram, AAL 250 [6-(3-cyclopentyloxy-4-methoxyphenyl)-4,5-dihydro-3 (2H)-pyridazinone] were investigated on the behavior of mice. We used the same behavioral procedures as those used with BW A78U, a free exploratory test especially adapted to reveal behavioral sedation (Experiment 1) and an unconditioned conflict situation, the light/dark choice procedure, described by Crawley and Goodwin (3) and behaviorally validated by Misslin et al. (7) (Experiment 2).

METHOD

Animals

Male Swiss albino mice from Centre d'Elevage R. Janvier, 12 weeks old at time of testing, were used. Prior to experimental testing, they were housed five to a standard cage containing a constant supply of food and water, and kept on a 12/12-h light-dark cycle with lights on at 1 a.m. in order to observe animals in their high activity period, that is, when lights are off.

Drugs

The compound AAL 250 has been prepared following a literature procedure (16). All drugs were dissolved in saline and administered intraperitoneally, 20 minutes before testing, at different concentrations in a volume of 10 ml/kg body wt. There was no repeated testing of subjects.

Statistical Analysis

Statistical significance of differences between control and treated groups was ascertained by a combined analysis of variance followed by the Dunnett test or, in the case of unequal variances, by the Bonferroni test.

Experiment 1

The apparatus consisted of a polyvinyl box (30 × 20 × 20 cm) subdivided into six equal, square units and covered with Plexi-

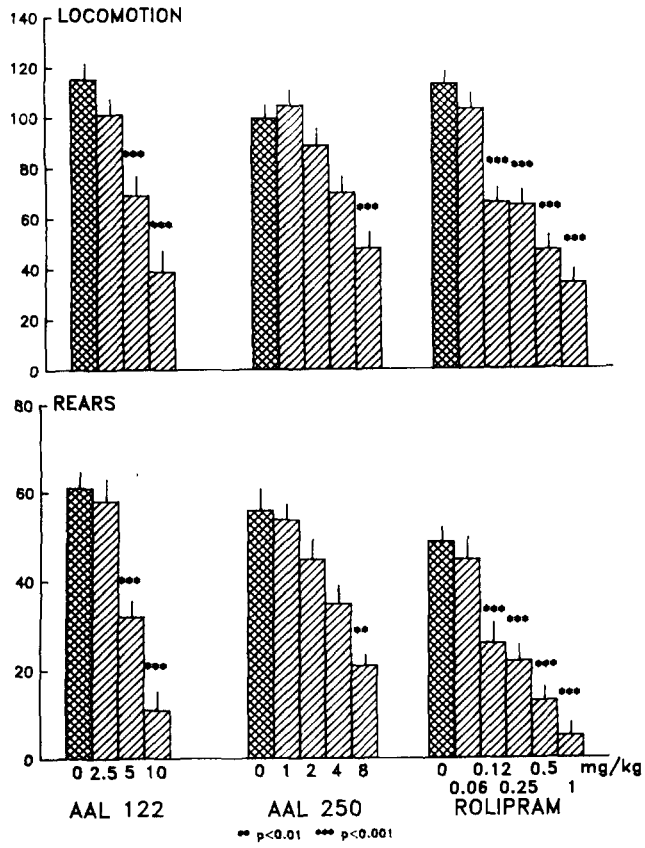

FIG. 1. Effects (mean ± SEM) of cAMP phosphodiesterase inhibitors on the behavior of mice confronted with a free exploratory test (AAL 122: n = 15; AAL 250, rolipram: n = 10).

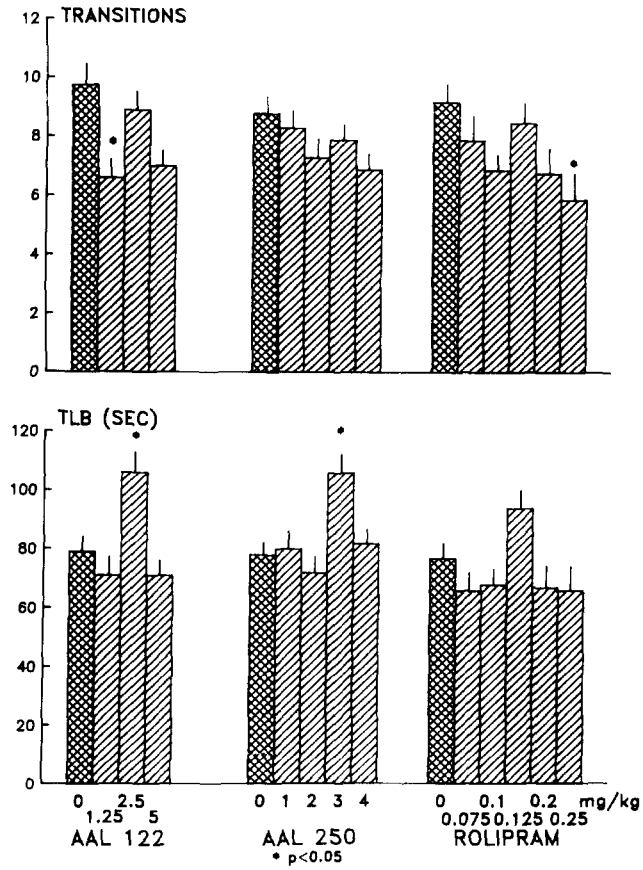

FIG. 2. Effects (mean ± SEM) of cAMP phosphodiesterase inhibitors on the behavior of mice in the light/dark choice procedure (AAL 122: n = 12; AAL 250: saline, n = 15; drug, n = 15; rolipram, n = 15). TLB: time in the lit box; TRANSITIONS: number of tunnel crossings.

glas. It could be temporarily divided in half by means of three partitions. Each mouse was placed for approximately 24 h in one-half of the apparatus with the temporary partitions inserted. Approximately 24 h after this familiarization, each mouse was exposed to both the familiar and novel environments by the removal of the temporary partitions. The subject was then observed in red light for ten minutes. The number of units entered by the subject (locomotion) as well as the number of rearing responses were recorded. A dose-dependent decrease of both parameters characterized a behavioral sedation.

Mice were randomly allocated to the following groups: a) AAL 122: vehicle control (saline; n = 15) and drug (2.5, 5 and 10 mg/kg in saline; n = 15); b) AAL 250: vehicle control (saline; n = 10) and drug (1, 2, 4 and 8 mg/kg in saline; n = 10); c) rolipram: vehicle control (saline; n = 10) and drug (0.06, 0.12, 0.25, 0.5 and 1 mg/kg in saline; n = 10).

Experiment 2

The apparatus consisted of two polyvinyl chloride boxes (20 × 20 × 14 cm) covered with Plexiglas. One of these boxes was kept darkened. A light from a 100-watt desk lamp above the other provided the only room illumination. An opaque plastic tunnel (5 × 7 × 10 cm) separated the dark box from the illuminated one. The subjects were individually tested in five-minute sessions in the apparatus described above. All mice were placed in the illuminated box to initiate the test session. The amount of time spent by mice in the illuminated box (TLB) and the number of tunnel crossings (transitions) were recorded minute by minute, during 5 minutes after the first entry into the dark box. A dose-dependent increase of both parameters characterized an anticonflict or anxiolytic action.

Mice were randomly allocated to the following groups: a) AAL 122: vehicle control (saline; n = 12) and drug (1.25, 2.5 and 5 mg/kg in saline; n = 12); b) AAL 250: vehicle control (saline; n = 15) and drug (1, 2, 3 and 4 mg/kg in saline; n = 15); c) rolipram: vehicle control (saline; n = 15) and drug (0.075, 0.1, 0.125, 0.2 and 0.25 mg/kg in saline; n = 15).

RESULTS

Experiment 1

Analysis of variance revealed significant differences among the groups: a) for locomotion in mice treated with all drugs [AAL 122: $F(3,56) = 18.40$, $p<0.001$; AAL 250: $F(4,45) = 8.92$, $p<0.001$; rolipram: $F(5,54) = 18.92$, $p<0.001$]; and b) for the rearing behavior in mice treated with all drugs [AAL 122: $F(3,56) = 22.93$, $p<0.001$; AAL 250: $F(4,45) = 9.04$, $p<0.001$; rolipram: $F(5,54) = 23.07$, $p<0.001$]. All drugs significantly decreased locomotion as well as number of rears in a dose-dependent manner (Fig. 1).

Experiment 2

Analysis of variance revealed significant differences among the groups with respect to the time spent by mice in the illuminated box in animals treated with AAL 122, $F(3,44)=8.11$, $p<0.001$, AAL 250, $F(4,70)=4.58$, $p<0.002$, but not in mice treated with rolipram, $F(5,84)=2.18$. Further, there were significant group differences for the number of transitions in mice treated with rolipram, $F(5,84)=2.73$, $p<0.02$, and AAL 122, $F(3,44)=3.99$, $p<0.01$, but not with AAL 250, $F(4,70)=1.82$. AAL 122 and AAL 250 significantly increased TLB at the intermediate doses. Finally, AAL 122, at the low dose, and rolipram, at the highest dose, significantly decreased the number of transitions (Fig. 2).

DISCUSSION

The present data clearly demonstrate that PDE-I exhibited strong behavioral sedative properties insofar as these drugs produced a dose-dependent decrease in locomotion as well as in rearing in mice confronted with a free exploratory test. These data are in agreement with the report of hypoactivity in rats induced by rolipram (17,18). It has also been shown that rolipram as well as AAL 122 are able to potentiate the pentobarbital-induced sleep in rats (14). As we have previously shown that AAL 122 was ten times less active than rolipram in inhibiting the rat brain cAMP PDE (Lugnier, C.; unpublished data), these results, taken together, support the hypothesis that substances that increase cAMP in brain are potent sedative compounds, as has been suggested by Wachtel (17). However, other mechanisms accounting for the sedative effects of rolipram and its analogs cannot be ruled out since AAL 250, which was thirty times more potent than AAL 122 in vitro, is about equiactive in vivo. High-affinity binding sites have been characterized for rolipram in the brain (11), and their distribution highly corresponds to that of adenosine A_1 receptors (6). Moreover, rolipram has been proved to be a potent adenosine uptake inhibitor (10).

In contrast, the results obtained in the light/dark choice procedure, especially adapted to reveal anticonflict or anxiolytic effects, are not conclusive, since, in this test, PDE-I did not simultaneously increase in a dose-dependent manner both the time spent by mice in the lit box and the number of transitions, as classically observed with benzodiazepines (2). Since sedative as well as anxiolytic effects of BW A78U have been observed in mice confronted with the same behavioral procedures as those used here (20), it can be suggested from the present results that the behavioral effects of BW A78U are not related to its ability to inhibit, like rolipram, AAL 122 and AAL 250, cAMP PDE in brain. As we have already suggested (20), some of the behavioral effects of BW A78U may likely be related to its other putative action on the adenosinergic neuromodulatory system.

In conclusion, although the strong sedative effects of rolipram may be mediated through an intracellular mechanism involving cAMP increase, this mechanism is not unequivocal. An extracellular action of both rolipram and BW A78U cannot be disregarded.

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