The Effect of a Selective Phosphodiesterase Inhibitor, Rolipram, on Muricide in Olfactory Bulbectomized Rats

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Abstract—In order to evaluate the potential usefulness of the drug as an antidepressant, acute and chronic effects of rolipram, a selective inhibitor of Ca²⁺- and calmodulin-independent cyclic AMP phosphodiesterase were investigated on muricide in olfactory bulbectomized (OB) rats. Upon single administration to OB rats, rolipram at a dosage of 1 mg/kg body weight suppressed the muricide for 2 hr after its administration. As a consequence of daily administration of rolipram, however, the incidence of muricide at 24 hr after the administration was decreased, and more than 60% of the rats did not exhibit the muricide on the 12th day. After the cessation of the administration, the incidence of the muricide returned to the initial level. The suppression of the muricide was not antagonized by several kinds of neurotransmitter blockers. Administrations of phosphodiesterase inhibitors and dibutyryl cyclic AMP as well as desipramine and clomipramine also suppressed the muricide dose-dependently. Repeated administration of desipramine also gave results similar to those of rolipram; repetition of a short suppression on the muricide was followed by the appearance of a long-lasting suppression. Differently from rolipram and desipramine, dibutyryl cyclic AMP did not cause long-lasting suppression, and even the direct effect (75% suppression) observed 30 min after its administration on the first day disappeared during its repeated administration for 14 days. From these results, rolipram was considered to show an antidepressant effect through the inhibition of Ca²⁺- and calmodulin-independent cyclic AMP phosphodiesterase.

Rolipram is a novel dialkoxypyphenyl pyrroloidone derivative (Fig. 1) that was shown to inhibit Ca²⁺- and calmodulin-independent cyclic AMP phosphodiesterase (PDE) activity in vitro (1), causing an increase in the cyclic AMP content of discrete regions of rat brain in vivo (2). Based on its ability to reverse reserpine-induced hypothermia or hypokinesia, to potentiate yohimbine toxicity and to reduce the immobility time in a forced swimming test (3–6), rolipram is expected to possess antidepressant activity. Since rolipram was reported neither to have an anticholinergic effect nor to inhibit monoamine oxidase activity and monoamine reuptake (4), rolipram may be a new type of drug, which differs from the tricyclic antidepressants.

Muricide, mouse killing behavior, in olfactory bulbectomized rats has been shown to be pertinent for evaluating the effect of...
antidepressants (7, 8). In this test model, among various psychotherapeutic drugs, only typical antidepressants caused suppression of the muricide without causing muscle relaxation and ataxia, and this suppressive effect was potentiated and prolonged by repeated drug administration (9, 10). For this reason, the muricide model was used to evaluate the ability of rolipram as an antidepressant.

In this communication, we report that single and repeated administrations of rolipram effectively suppressed the muricide, as desipramine did. The effects of other phosphodiesterase inhibitors and dibutyryl cyclic AMP (Bt₂ cAMP) on the muricide were also investigated to clarify the mechanism of action of rolipram.

Materials and Methods

Materials: Drugs used were supplied from the following companies: (±)-rolipram (4-(cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidone), its (+) and (-)-isomer, and methysergide (methysergide bimeinate) from Schering AG; Ro 20-1724 (4-(3-n-butyloxy-4-methoxybenzyl)-2-imidazolidinone from Hoffmann-La Roche; IBMX (3-isobutyl-1-methylxanthine) and desipramine (desipramine hydrochloride) from Sigma; theophylline from Wako Pure Chemical Industries, Ltd.; papaverine (papaverine hydrochloride) from Dainippon Pharmaceutica; clomipramine (clomipramine hydrochloride) from Ciba-Geigy; propranolol (propranolol hydrochloride) from Sumitomo Pharmaceutica; phenoxybenzamine (phenoxybenzamine hydrochloride) from Nakarai Chemicals, Ltd.; bicuculline (bicuculline methiodide) from Cambridge Research Biochemicals, Ltd.

Rolipram, Ro 20-1724, theophylline, IBMX and phenoxybenzamine were suspended in an aqueous solution containing 0.5% carboxymethyl cellulose and 0.04% Tween 80. Bt₂ cAMP and other compounds were dissolved in distilled water and physiological saline, respectively.

Method: Male Wistar rats (Clea Japan, Inc.) weighing 180 to 220 g at the time of surgery were used. Three weeks after bilateral olfactory bulbectomy, which was performed as previously described (11), only rats exhibiting muricide were used: 5 to 60% of olfactory bulbectomized (OB) rats displayed muricide under our conditions. Throughout the experiment, each rat was kept in an isolated cage at a temperature of 22±2°C with a 12 hr light-dark cycle and allowed free access to food and water.

Muricide was tested before and at various times after intraperitoneal administration of drugs, unless otherwise mentioned, and was assessed as positive if the rat killed a mouse within 3 min after introducing the mouse into the rat’s homecage. The injection volume of drug solution was fixed at 0.1 ml per 100 g body weight.

Statistical analysis was done by Fisher’s exact probability test.

Results

Effect of single administration of rolipram on muricide: The effect of a single administration of 1 mg rolipram/kg body weight on the muricide was compared with those of 10 mg desipramine/kg body weight and 20 mg clomipramine/kg body weight (Fig. 2). These drugs suppressed the muricide 30 min after their administration. Rolipram suppressed it most effectively among the three drugs under this condition. However the antimuricide activity of rolipram disappeared within 2 hr, while those of clomipramine and desipramine lasted for a longer period of 4 to 6 hr. The effect of rolipram at 30 min after administration at dosages of 0.1 to 10 mg/kg body weight was found to be dose-dependent with an ED50 of 0.4 mg/kg body weight. Of the isomers of rolipram, the (-)-isomer suppressed the muricide markedly but the (+)-isomer did not at a dosage of 1 mg/kg body weight (Table 1). Although mild sedation of OB rats was observed after administration of these drugs at the dosages effectively suppressing the muricide, neither muscle relaxation nor ataxia appeared.

Possible involvement of a neurotransmitter in the effect of rolipram: The antimuricide effect of rolipram was not blocked by pretreatment of the rat with subcutaneous administration of phenoxybenzamine at a dosage of
Rolipram Suppresses Muricide in OB Rat

20 mg/kg body weight, which is known to inhibit the antimuricide effect of desipramine (Table 2). None of the other antagonists such as propranolol (10 mg/kg body weight, s.c.), methysergide (2 mg/kg body weight, i.p.), bicuculline (3 mg/kg body weight, i.p.) and theophylline (25 mg/kg body weight, i.p.) blocked the antimuricide activity of rolipram. At the dosage used, these antagonists did not affect the muricide by themselves.

Effect of single administration of various phosphodiesterase (PDE) inhibitors and Bt<sub>2</sub>cAMP on muricide: Since the well-known characteristic of rolipram is an inhibition of phosphodiesterase activity, some kinds of phosphodiesterase inhibitors were administered intraperitoneally to the OB rats to compare their antimuricide activities with that of rolipram. The muricide was suppressed in a similar extent with Ro 20-1724 and isobutylmethylxanthine at a dosage of 10 mg/kg body weight, theophylline at 100 mg/kg

Table 1. Effects of rolipram and other PDE inhibitors

<table>
<thead>
<tr>
<th>PDE inhibitors (mg/kg, i.p.)</th>
<th>Incidence of muricide&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>7/7</td>
</tr>
<tr>
<td>(+)-Rolipram</td>
<td>1/7**</td>
</tr>
<tr>
<td>(-)-Rolipram</td>
<td>0/5**</td>
</tr>
<tr>
<td>(+)-Rolipram</td>
<td>4/5</td>
</tr>
<tr>
<td>Ro 20-1724</td>
<td>1/6**</td>
</tr>
<tr>
<td>Isobutylmethylxanthine</td>
<td>3/6</td>
</tr>
<tr>
<td>Theophylline</td>
<td>6/6</td>
</tr>
<tr>
<td>Papaverine</td>
<td>5/6</td>
</tr>
<tr>
<td></td>
<td>2/6*</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number of rats exhibiting muricide/number of rats tested. Muricide was tested 30 min after drug administration. *P<0.05, **P<0.01, significantly different from the vehicle group.
body weight and papaverine at 40 mg/kg body weight, although the required dosages were rather high as compared to that of rolipram (Table 1). The rats treated with these PDE inhibitors showed mild sedation, but no muscle relaxation and ataxia.

These results prompted us to examine whether cyclic AMP suppresses the muricide. As shown in Fig. 3, 50 to 200 mg/kg body weight of Bt2 cAMP, a membrane penetrating analog of cyclic AMP, suppressed the muricide dose-dependently 30 min after intraperitoneal administration. The anti-muricide effect of Bt2 cAMP disappeared rather quickly; muricide was restored in the rats at 1, 2 and 4 hr after the administration at dosages of 50, 100 and 200 mg/kg body weight, respectively. Administration of Bt2 cAMP caused marked sedation.

Effect of repeated administration of drugs on muricide: In order to assess whether there is a long-lasting effect with repeated administration, the incidence of the muricide was tested 24 hr after daily administration of the drug. As shown in Fig. 4, the incidence of the muricide in daily administration of rolipram was decreased dose- and time-dependently. With a dosage of 0.35 mg/kg body weight, the incidence began to decrease from day 7 and reached about 50% at day 14. With 1 or 3 mg/kg body weight, the decreases were much quicker and greater than with 0.35 mg/kg body weight; the incidence began to decrease within a few days and almost complete inhibition of the muricide was already attained by day 7. It should be noted

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Dose mg/kg</th>
<th>Route</th>
<th>Incidence of muricidea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Saline</td>
</tr>
<tr>
<td>Saline</td>
<td></td>
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<td>7/7</td>
</tr>
<tr>
<td>Phenoxybenzamine b</td>
<td>20</td>
<td>s.c.</td>
<td>6/6</td>
</tr>
<tr>
<td>Propranolol b</td>
<td>10</td>
<td>s.c.</td>
<td>6/6</td>
</tr>
<tr>
<td>Methysergide c</td>
<td>2</td>
<td>i.p.</td>
<td>6/6</td>
</tr>
<tr>
<td>Bicuculline d</td>
<td>3</td>
<td>i.p.</td>
<td>6/6</td>
</tr>
<tr>
<td>Theophylline d</td>
<td>25</td>
<td>i.p.</td>
<td>6/6</td>
</tr>
</tbody>
</table>

aNumber of rats exhibiting muricide/number of rats tested. Muricide was tested 30 min after rolipram administration. b c d administered 30, 40, 15 min before intraperitoneal administration of rolipram (1 mg/kg body weight), respectively. **P<0.01, significantly different from the respective saline group.

![Graph](image-url)

**Fig. 3.** Effect of single administration of dibutyl adenosine 3',5'-cyclic monophosphate on muricide in olfactory bulbectomized rats. ○, saline; ●, 50 mg/kg, i.p.; △, 100 mg/kg, i.p.; ▲, 200 mg/kg, i.p. Eight animals were used in each experiment.
that neither noticeable sedation nor ataxia and muscle relaxation were observed at the testing time.

With a dosage of 1 mg rolipram/kg body weight, the suppressive effect on the muricide which was tested 30 min after administration was apparent already at the 1st day and tended to be strengthened day by day (Fig. 5). After the daily drug administration was terminated, muricide was restored in the rats within about one week.

Similar results were observed with the repeated administration of desipramine at a dosage of 10 mg/kg body weight (Fig. 5): progressive potentiation of the suppressive activity tested 1 hr after the drug administration and gradual appearance of a long-lasting suppression effect.

The repeated administration of Bt2 cAMP at a dosage of 100 mg/kg body weight for 15 days did not cause a long-lasting suppressive effect on the muricide (Fig. 6), and even the suppressive effect on the muricide tested 30 min after the administration (about 75% on the first day) disappeared gradually during the daily administration. The sedative effect observed also disappeared gradually.

**Discussion**

With a single administration of rolipram in OB rats, muricide was suppressed in a dose-dependent manner as was observed for desipramine and clomipramine. It was reported that the antimuricide effect of desipramine is antagonized by phenoxybenzamine but unaffected by sotalol and propranolol (10, 12), indicating that the effect of tricyclic anti-depressants results from an activation of the mechanism mediated via α-adrenergic receptor. In contrast to the effect of desipramine, the antimuricide effect of rolipram was prevented by none of the blockers for α- and β-adrenergic, serotonin, GABA and adenosine receptors. Taking these results together with the finding that rolipram did not possess any binding ability to various types of neurotransmitter receptors in rat brain (13), it is considered that the effect of rolipram is not ascribable to a direct effect on neurotransmitters such as those of typical antidepressants which increase the availability of monoamines in the synaptic cleft. Therefore, the antimuricide effect of rolipram probably results from either a direct or indirect effect which is derived from an increased level of cyclic AMP caused by the inhibitory action on brain cyclic AMP PDE. This consideration is supported by the following lines of evidence. First, suppression of the muricide was induced by (−)-rolipram but not by its (+)-isomer, the former being the isomer responsible for the inhibition of Ca2+- and
calmodulin-independent cyclic AMP PDE (14). In several kinds of behavior tests in animals, (−)-rolipram was also shown to be the active component of (±)-rolipram (4, 15). For instance, in the reserpine antagonism and the yohimbine potentiation tests, the (+)-isomer was only 1/10 to 1/15 as potent as the (−)-isomer (4). Similar results were also observed in a drug discrimination test (16, 17). Secondly, Ro 20-1724, which is known to be a specific inhibitor of Ca^{2+}- and CaM-independent cyclic AMP PDE like rolipram (1), was able to suppress the muricide. The relative potency of the antimuricide activity of rolipram and Ro 20-1724 appears to be proportional to the potency of inhibitory
action on PDE activity in vitro (1). For other PDE inhibitors at higher dosages, again there seems to be a correlation between their potencies in these two activities (1). Thirdly, a single administration of Bt2cAMP suppressed the muricide dose-dependently as rolipram did, and rolipram has been reported to increase cyclic AMP levels in particular regions of the brain such as the frontal cortex and striatum of the rat in vivo (2).

It is worthy to mention that during repeated administration of rolipram and desipramine, the long-lasting suppression effect on the muricide became evident. This effect demonstrated here can not be regarded as a direct effect of rolipram, since the effect appeared a few days after the treatment, and since it took about a week for the effect to disappear after termination of the daily treatment. Our unpublished data showed that there was no accumulation of rolipram in the body to which the long-lasting effect can be ascribed.

The effect of Bt2cAMP under its repeated administration was interesting. Differing from rolipram and desipramine, potentiation of the antimuricide activity of Bt2cAMP did not occur and even tolerance to the antimuricide effect developed. These characteristics resemble those of neuroleptics (9).

Since tricyclic antidepressants such as desipramine caused the antimuricide action on OB rats via a noradrenergic mechanism, it may be concluded from these lines of evidence that the effect of rolipram on the muricide is ascribable to an increase of cyclic AMP level in a neural pathway in which the noradrenergic mechanism also participates. The fact that not only desipramine but also rolipram caused a long-lasting effect on the muricide, therefore, further indicates that rather than those at the receptor site, alterations in the post-receptor site may be a common mechanism for a long-lasting suppression of the muricide.

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