

## Effect of Rolipram, a Phosphodiesterase Inhibitor and Potential Antidepressant, on the Firing Rate of Central Monoaminergic Neurons in the Rat

J. SCUVÉE-MOREAU, I. GIESBERS AND A. DRESSE

Department of Pharmacology, University of Liège,  
B-4000 Sart-Tilman, Belgium

**Abstract**—Rolipram is a potent phosphodiesterase inhibitor, active in classical pharmacological tests used in the screening of antidepressants (AD). In contrast with classical AD, rolipram does not block the reuptake of monoamines, but its action on the metabolic degradation of cyclic AMP may enhance adenylate-cyclase linked catecholaminergic and serotonergic transmission. Biochemical studies showed that rolipram induces various modifications in the turnover of monoamines but the net effect of these modifications on the electrical activity of monoaminergic neurons remained to be elucidated.

Thus, the influence of rolipram on the firing rate of central locus coeruleus (LC) noradrenergic neurons, mesolimbic ( $A_{10}$ ) dopaminergic neurons and dorsal raphe (DR) serotonergic neurons was investigated. When rolipram was perfused into the jugular vein, it produced a long-lasting excitatory effect on LC neurons, a prolonged but usually partial inhibitory effect on  $A_{10}$  neurons and no consistent effect on DR neurons. The action of rolipram on monoaminergic neurons contrasts with that of most classical and new AD in which the electrical activity of LC and/or DR neurons decreases.

### Introduction

Rolipram, 4-(3-cyclopentyloxy-4-methoxy-phenyl)-2-pyrrolidone (Fig. 1), is a potent inhibitor of adenosine cyclic 3',5'-monophosphate (cAMP) phosphodiesterase (Schwabe *et al.*, 1976). This drug is active in classical

pharmacological tests used in the screening of antidepressants (AD): it antagonizes reserpine-induced hypothermia and hypokinesia and potentiates yohimbine-induced lethality (Wachtel, 1983a). Contrary to classical AD, rolipram does not block the reuptake of monoamines in nerve terminals. This compound is an inhibitor of the metabolic degradation of cAMP and therefore should enhance adenylate-cyclase linked catecholaminergic and serotonergic transmission. Biochemical studies *in vivo* show that rolipram induces various modifications in catecholamines and 5-hydroxytryptamine (5-HT) turnover, e.g. rolipram increases noradrenaline (NA) synthesis and metabolism, increases dopamine (DA) synthesis but decreases DA utilization in the striatum, decreases 5-hydroxytryptophan (5-HTP) accumulation in some brain regions despite an increase in tryptophan levels (Kehr *et al.*, 1985). The net effect of these modifications on the electrical activity of monoaminergic neurons remains to be elucidated. Electrophysiological techniques are useful for the study, *in vivo*, of the influence of drugs on the electrical activity of central monoaminergic neurons. It has been previously demonstrated that classical tricyclic AD and several new AD more or less selectively decrease the firing rate of central noradrenergic and/or serotonergic neurons (Scuvée-Moreau and Dresse, 1979; 1982). Thus, we studied the effects of rolipram on the firing rates of the central noradrenergic neurons of the locus coeruleus (LC), the central dopaminergic neurons of the mesolimbic system (group A10) and the central serotonergic neurons of the dorsal raphe nucleus (DR).

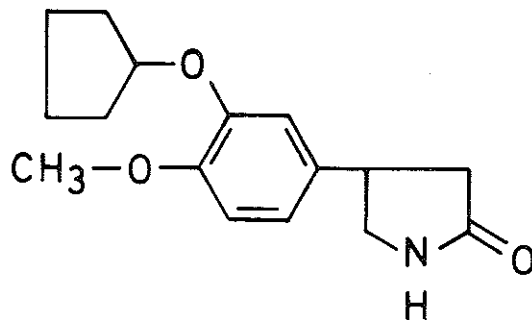


FIG. 1  
Chemical structure of rolipram.

## Methods

Experiments were performed on male Wistar rats weighing 200–300 g. The animals were anaesthetized with chloral hydrate (400 mg/kg *i.p.*) and

mounted in a stereotaxic atlas and maintained at 36–37°C by a heating blanket removed above the incision. The brain was cut coronally. The electrodes were inserted into the brain through micropipettes filled with a saline solution. The electrodes were inserted into the posterior, 1.1–1.3 mm from the midline, cerebellar surface; A10 group neurons were located 7.2–8 mm under the surface, lateral to lambda and the dorsal raphe nucleus. The electrodes were passed through a stereotaxic atlas, a Tektronix oscilloscope, a pulse generator, a discriminator and a digital counter.

LC neurons were identified by their characteristic discharge of 0.5–5 spikes/sec (Graham and Aghajanian, 1977). A neuron was characterized by bursts of 3–9 spikes/sec of discharge of 3–9 spikes/sec characterized by a regular firing rate of 0.2–2 spikes/sec (Aghajanian, 1977). The firing rate was recorded during the baseline firing rate of the neuron by means of a perfusion of a saline solution in a spot of fast green wa-

The animal was perfused with a saline solution. The brain was removed for histological studies. A minimum of 6 animals were used. The studies had shown that the firing rate of monoaminergic neurons was not affected by the perfusion.

For quantitative analysis, the firing rate of the cell (expressed as a percentage of the control period) was compared immediately after the application of rolipram. Statistical analysis was performed on related samples.

## Results

### LC neurons

Rolipram at 1.0 mg/kg increased the frequency of firing of LC neurons.

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mounted in a stereotaxic apparatus. Their body temperatures were maintained at 36–37°C by means of a heating pad. A small piece of bone was removed above the implantation point and the venous sinus was tied and cut. The electrical activity was recorded by means of extracellular glass micropipettes filled with a solution of NaCl 2M saturated with fast green. The electrodes were implanted at the following coordinates: LC 1.7–2.2 mm posterior, 1.1–1.3 mm lateral to lambda and 5.5–6.5 mm under the cerebellar surface; A10 1.8–2.2 mm anterior, 0.4–0.6 mm lateral to lambda and 7.2–8 mm under the cortical surface; DR 0.0–0.5 mm anterior, 0.0 mm lateral to lambda and 4.5–5.5 mm under the cortical surface. Action potentials were passed through an impedance adapter and an amplifier into a Tektronix oscilloscope. Signals were also passed into an amplitude discriminator and a digital counter.

LC neurons were characterized by a regular firing rate, a frequency of discharge of 0.5–5 spikes/sec and a typical response to noxious stimuli (Graham and Aghajanian, 1971; Korf *et al.*, 1974); A10 neurons were characterized by bursts of spikes of decreasing amplitude and a frequency of discharge of 3–9 spikes/sec (Bunney *et al.*, 1973); DR neurons were characterized by a regular firing rate and a low frequency of discharge of 0.2–2 spikes/sec (Aghajanian *et al.*, 1978). The electrical activity of the cell was recorded during a control period of a few min in order to determine the baseline firing rate. Rolipram was administered into the jugular vein by means of a perfusion pump (flow 6 ml/hr). At the end of the experiment a spot of fast green was deposited at the tip of the electrode.

The animal was perfused with a solution of formaldehyde 4% and the brain was removed for histological control of the position of the electrode. A minimum of 6 animals was used to study each brain area. Rolipram was dissolved in a mixture of propylene glycol and water (1/6). Preliminary studies had shown that this vehicle had no effect on the firing rate of monoaminergic neurons.

For quantitative analysis of the results the mean frequency of discharge of the cell (expressed as the mean number of spikes/10 sec) during the control period was compared with the frequency of discharge of the same cell immediately after the administration of a cumulative dose of 1 mg/kg of rolipram. Statistical analysis was performed using Student's *t*-test for related samples.

## Results

### *LC neurons*

Rolipram at 1.0 mg/kg cumulative dose, produced a highly significant increase in the frequency of discharge of LC neurons (Table I). At the dose

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of 0.1 mg/kg/min during 4 min no modification of the frequency of discharge of the cell was observed. A dose of 0.2 mg/kg/min during 6 and 10 min was tested on 2 rats and a slight increase in the frequency of discharge was observed in both cases. Four experiments were performed with 0.5 mg/kg/min (Fig. 2A): in these experiments rolipram also increased the frequency of discharge. This increase was maximal after 3 min of perfusion and no further activation of the firing rate was induced by continuing the perfusion. The activating effect of rolipram persisted after the end of the administration for a period of at least 10 min. No return to base line values was observed due to the long-lasting action of rolipram.

#### *A<sub>10</sub> neurons*

At a cumulative dose of 1.0 mg/kg rolipram significantly decreased the frequency of discharge of A<sub>10</sub> neurons (Table I). At doses of 0.2 and 0.3 mg/kg/min during 10 min a slight decrease of the frequency of discharge was observed. As shown in Fig. 2B, at the dose of 0.5 mg/kg/min, the inhibitory effect of rolipram was more pronounced. In 2 out of 6 experiments a complete inhibition of the firing rate was observed, but this complete inhibition was of short duration. In most cases the firing rate was stabilized at a level inferior to 50 % of the control activity. The mean cumulative dose required to produce a 50 % inhibition of the firing rate was  $0.47 \pm 0.12$  mg/kg ( $n = 6$ ). The inhibitory effect of rolipram continued for several min after the end of the perfusion.

#### *DR neurons*

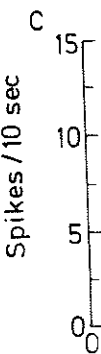
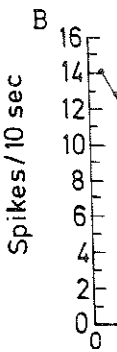
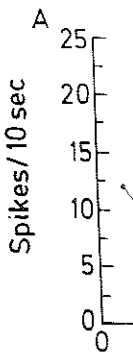
Rolipram did not induce any significant modification of the frequency of discharge of DR neurons at a cumulative dose of 1.0 mg/kg (Table I). Furthermore, as shown in Fig. 2C, a slight activation of firing rate was

TABLE I

*Effect of a total dose of 1 mg/kg of rolipram perfused i.v. on the frequency of discharge of central monoaminergic neurons*

Region	Number of spikes/10 sec (mean $\pm$ E.S.)		
	Controls	After rolipram	n
L.C.	$14.5 \pm 2.7$	$21.5 \pm 3.5^{**}$	6
A <sub>10</sub>	$24.2 \pm 4.8$	$10.0 \pm 3.7^{***}$	8
D.R.	$13.3 \pm 2.9$	$15.5 \pm 3.2$	6

Statistical analysis was performed using Student's *t*-test for related samples.  $n$  = number of experiments.  $^{**} p < 0.005$ ;  $^{***} p < 0.001$ .



(A): I.v. perfusion of noradrenergic neuron; (B) the firing rate of this A<sub>10</sub> neuron; (C) temporary activation of this DR neuron every min is represented

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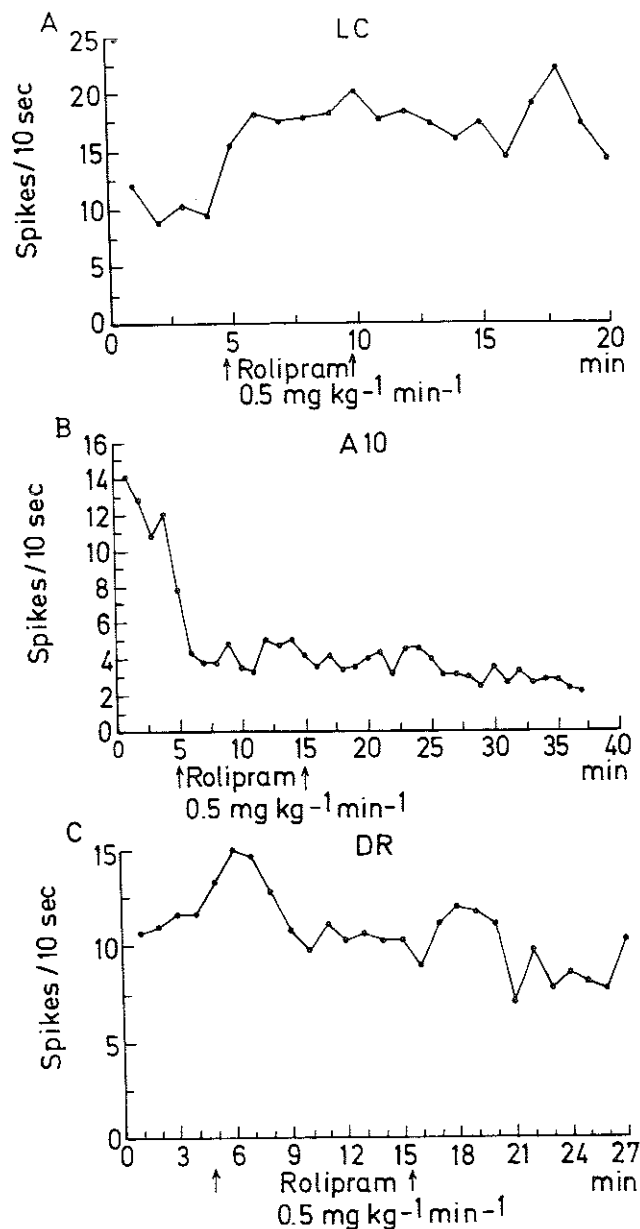


FIG. 2

(A): I.v. perfusion of rolipram induces a long-lasting activation of the firing rate of this LC noradrenergic neuron; (B) i.v. perfusion of rolipram induces a long-lasting partial inhibition of the firing rate of this A<sub>10</sub> dopaminergic neuron; (C) i.v. perfusion of rolipram induces a temporary activation of this DR serotonergic cell. The mean number of spikes/10 sec calculated every min is represented against time.

observed at the beginning of the perfusion in 6 out of 10 animals independently of the dose perfused (0.05 to 0.5 mg/kg/min). In 4 of these 6 experiments the duration of the perfusion was sufficient to establish that the activation was of short duration and was followed by a return to control activity. Rolipram did not modify the frequency of discharge in 3 animals. A slight reduction of the electrical activity was observed in 1 animal.

### Discussion

Our present knowledge is not sufficient to elucidate the relationship between the electrophysiological effects of rolipram and the inhibition of phosphodiesterase activity.

The excitatory effect of rolipram on the electrical activity of LC neurons correlates well with biochemical data, in the sense of a stimulation of NA synthesis and turnover by this compound (Kehr *et al.*, 1985). With regard to dopaminergic mechanisms, an increase in DA synthesis but a decrease in DA utilization in striatum following rolipram was observed (Kehr *et al.*, 1985). The inhibitory effect of rolipram on A<sub>10</sub> neurons is in agreement with the inhibition of DA utilization. Data concerning the influence of rolipram on serotonergic mechanisms are more complex: biochemical studies show a decrease of 5-HTP accumulation in several brain regions despite an increase in tryptophan levels (Kehr *et al.*, 1985). Behavioural studies show that rolipram induces head-twitches in rats (Wachtel, 1983b), which is generally considered as reflecting an increased 5-HT postsynaptic transmission. In view of the occurrence of these head-twitches, the rolipram-induced reduction of 5-HT metabolism could be due to feedback inhibition of the activity of 5-HT neurons. However, our electrophysiological studies do not show an inhibition of the electrical activity of DR neurons by rolipram. Additional experiments are indicated.

The influence of rolipram on the firing rate of central monoaminergic neurons is different from the effect of most classical and new AD which more or less selectively decrease the firing rate of LC and/or DR neurons (Scuvée-Moreau and Dresse, 1979; 1982).

This inhibitory effect of classical and new AD is closely related to their ability to block amine uptake. Several regulatory mechanisms seem to be implicated: a postsynaptic mechanism involving putative uni- or multineuronal feedback loops and a presynaptic mechanism involving increased stimulation of presynaptic receptors (Quinaux *et al.*, 1982). If the therapeutic effectiveness of AD is due to an enhancement of monoaminergic transmission, as suggested by the monoaminergic hypothesis of depression, these regulatory mechanisms tend to oppose this facilitation of transmission and could be partly responsible for the therapeutic delay

observed in AD therapy. Like rolipram which enhances the activity of the interesting therapeutic

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