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MECHANISM OF ACTION OF PIRIBEDIL ON NORADRENERGIC NEURONS IN RAT BRAIN

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Abstract—Piribedil at 30 mg/kg intraperitoneally (i.p.) induces a marked increase of MOPEG-SO_4 without affecting NA levels. At higher doses (60 mg/kg) both an increase at MOPEG-SO_4 and a decrease of NA levels can be observed. The ratio MOPEG-SO_4–NA is 0.9. The Piribedil effect on MOPEG-SO_4 levels is mainly confined to the cortex-hippocampus. These effects of Piribedil on MOPEG-SO_4 are similar to those described for amphetamine. Reserpine (1.25 mg/kg i.p.) given 24 hr before Piribedil reduces but does not abolish the Piribedil induced increase of MOPEG-SO_4. On the other hand, DMI (10 mg/kg i.p.) at a dose blocking the increase of MOPEG-SO_4 induced by amphetamine but not that by reserpine, inhibits this effect of Piribedil. The possibility that Piribedil has an amphetamine-like mechanism of action on noradrenergic neurons is discussed.

Piribedil (1-(2-pyrimidyl)-4-piperonyl piperazine) has profound effects on animal behavior. It induces stereotyped and circling behavior in rats and decreases body temperature in rats and rabbits [1, 2]. These are considered as dopaminergic effects and are blocked by pimozide [1–4].

Piribedil induces also ferocity and agressivity in rats, antagonized by propranolol. These effects may be due to the activation of the noradrenergic system [1]. Piribedil, in fact, has been shown to decrease NA and increase MOPEG-SO_4 levels [2, 4, 5]. Whether these changes could reflect on activation of NA system similarly to that induced by amphetamine remained to be elucidated.

The present report describes the effects of Piribedil on NA and MOPEG-SO_4 concentrations in the attempt to elucidate the mechanism of its action on NA neurons. The results obtained suggest that Piribedil has an amphetamine-like activity on noradrenergic neurons.

MATERIALS AND METHODS

Male Charles–River rats weighing 150–200 g, kept in makrolon cages at constant room temperature (21–22°) and relative humidity (60 per cent) were used. The animals were kept in a controlled 12 hr dark light cycle environment. Rats were sacrificed by decapitation, brain removed and brain regions (whole brain without cerebellum, cortex-hippocampus and medulla-pons) dissected and frozen on dry-ice. Brain tissues were kept at −80° overnight and the morning following dissection, homogenized in 4–7 ml of $ZnSO_4$ and an equivalent amount of $Ba(OH)_2$. Supernatant of 30,000 *g* centrifugation was filtered and either directly applied to Sephadex DEAE A-25 columns or stored frozen at −80° until assayed. Internal standards of authentic MOPEG-SO_4 were added to aliquots of brain supernatants and run similarly to unknown brain samples. MOPEG-SO_4 was isolated and assayed as previously described [6] following the method of Meek and Neff [7].

In parallel experiments NA was assayed fluorometrically according to Chang [8]. Brain tissue was homogenized in 9.7 ml of 0.4 N $HClO_4$ containing 0.2 ml of 10% EDTA and 0.1 ml of 6% cysteine. After centrifugation, limpid supernatant was adjusted to pH 8.0 with Tris buffer pH 9.0, NA adsorbed on alumina, eluted and oxidized with iodine. Fluorescence was measured using an Aminco–Bowman Spectrophotofluorimeter.

In order to gain further indications on the fate of NA released by drugs or other stimuli we have calculated an arbitrary index considering the nmole of NA released and the nmole of MOPEG-SO_4 when new steady-state levels are reached.

The index is the ratio of Δ MOPEG-SO_4 (nmole accumulated—nmole control) divided for Δ NA (nmole control—nmole released).

This arbitrary index would only serve as an approximate estimation of the NA metabolism and it is here used to compare the activity of different drugs or stimuli (such as electrical stimulation) on NA disposition.

RESULTS

Piribedil at a dose of 30 mg/kg i.p. significantly increases MOPEG-SO_4 brain concentrations (Table 1). NA levels were only slightly decreased. At 60 mg/kg the effect on MOPEG-SO_4 levels was higher and also NA levels were significantly decreased (Table 1). The peak time of these effects was at 2½ hr (Table 2). The MOPEG-SO_4/NA ratio at 2½ hr was 0.9. This value is similar to that seen after amphetamine and much higher than that found for Reserpine [9–11]. The Piribedil-induced increase of MOPEG-SO_4 was localized mainly in the cortex-hippocampus region (Table 2) similarly to that seen for amphetamine [10].

Abbreviations—NA—Noradrenaline, MOPEG-SO_4—3-methoxy-4-hydroxyphenylglycolsulfate.

Table 1. Effect of Piribedil on NA and MOPEG-SO_4 concentrations in the rat whole brain

Treatment	Dose mg/kg	Hr after injection	NA nmole/g ± S.E.	% Cont.	MOPEG-SO_4 nmole/g ± S.E.	% Cont.	Δ MOPEG-SO_4 ‡ / Δ NA
Controls	—	—	2.80 ± 0.09 (12)	100	0.56 ± 0.02 (12)	100	
Piribedil	30	2½	2.68 ± 0.10 (5)	90	0.91 ± 0.08* (5)	162	
	60	1	2.00 ± 0.08* (5)	70	1.10 ± 0.05* (5)	196	
		2	1.97 ± 0.06* (5)	70	1.20 ± 0.08* (5)	214	
		2½	1.90 ± 0.10* (5)	72	1.37 ± 0.09* (5)	245	0.9
		3	2.10 ± 0.11† (4)	75	1.10 ± 0.11* (4)	196	
		4	2.30 ± 0.12 (5)	83	0.75 ± 0.06* (5)	130	

* $P < 0.01$ vs controls, Dunnett's test.
† $P < 0.05$ vs controls, Dunnett's test.
‡ See Methods for detail on ratio calculation.

Table 2. Effect of Piribedil on MOPEG-SO_4 different brain areas

	Medulla-Pons	Mid-brain	Cortex-hippocampus
Control	0.71 ± 0.03 (5)	0.40 ± 0.02 (5)	0.53 ± 0.02 (5)
Piribedil (60 mg/kg i.p.)	0.85 ± 0.04$^{\Delta}$ (5)	0.53 ± 0.04$^{\Delta}$ (5)	1.43 ± 0.10* (5)

* $P < 0.001$ Student's 't' test.
$^{\Delta}$ $P < 0.05$ Student's 't' test.

Piribedil's behavioural effects such as aggressivity are reduced but not abolished by reserpine pretreatment [1]. This suggests that Piribedil releases NA in part from the reserpine-sensitive storage pool. After depleting NA storage-pool with reserpine we have seen that the Piribedil-induced increase of MOPEG-SO_4 levels was reduced (Table 3). However a large (42 per cent) and significant increase of MOPEG-SO_4 was still present. This corresponds to the increase induced by amphetamine, which is supposed to act only on the mobile NA pool. Amphetamine action on MOPEG-SO_4, in fact, was not blocked by reserpine treatment (Table 3).

DMI has been shown to decrease the firing rate of NA neurons and the rate of MOPEG-SO_4 formation [12–14]. DMI (10 mg/kg i.p.) completely blocks the amphetamine-induced increase of MOPEG-SO_4 but not that induced by reserpine (Table 4).

We have found that DMI significantly reduces the increase of MOPEG-SO_4 induced by Piribedil (Table 4), but the inhibition of Piribedil effect is not complete.

DISCUSSION

The mechanism of action of Piribedil on NA neurons shows some similarities with that of amphetamine.

In both cases there is a high MOPEG-SO_4–NA ratio; the effect on MOPEG-SO_4 is restricted to the cortex-hippocampus region and it is not blocked by reserpine pretreatment but it is decreased by DMI.

These results support the view that while some of the behavioral effects of Piribedil, such as stereo-

Table 3. Interaction between reserpine and amphetamine or Piribedil on MOPEG-SO_4 concentrations of rat whole brain

Treatment	Dose mg/kg i.p.	Time after first drug, hr	Time after second drug, hr	MOPEG-SO_4 nmole/g ± S.E.	% Cont.	% Res.
Controls	—	—	—	0.56 ± 0.02 (13)	100	
Reserpine	2.5	24	—	0.44 ± 0.03 (5)	80	100
Piribedil	60	—	2	1.20 ± 0.08 (5)	214	—
Reserpine	2.5	22	2	*0.64 ± 0.06 (5)	114	142
Piribedil*	60					
Amphetamine	5.0	—	2	0.79 ± 0.02 (10)	146	—
Reserpine	2.5	22	2	0.62 ± 0.03 (5)	102	141
Amphetamine*	5.0					

* $P < 0.05$ compared to reserpine untreated group, Student's 't' test.

Table 4. Interactions of DMI (10 mg/kg) with Piribedil (60 mg/kg) and amphetamine (5 mg/kg) on MOPEG-SO_4 concentration in the rat whole brain

Treatment	MOPEG-SO_4 nmole/g ± S.E.
Piribedil	1.34 ± 0.09 (5)
DMI + Piribedil	*0.68 ± 0.05 (5)
Amphetamine	0.79 ± 0.02 (10)
DMI + amphetamine	*0.60 ± 0.02 (5)
Reserpine	0.98 ± 0.14 (8)
DMI + reserpine	0.91 ± 0.09 (5)

* $P < 0.01$ compared to DMI untreated groups (Student's 't' test). DMI was given 30 min before the test drug. Animals were sacrificed 2 hr after the test drug.

typed behavior, are linked to an activation of DA system, other effects, such as aggressivity, are due to a noradrenergic stimulation [1, 4].

However, our findings suggest that the mechanism of action of Piribedil on NA is complex. In fact, depleting NA storage pool with reserpine does not affect the amphetamine-induced increase of MOPEG-SO_4 whereas it does antagonize the Piribedil-induced increase.

This agrees with the findings of a reduced behavioral activity of Piribedil by reserpine [1]. Thus, we may assume that Piribedil is acting on both the storage and mobile pool of NA. In the case of Piribedil the MOPEG-SO_4–NA ratio (0.9) is higher than either that after amphetamine (0.50) or reserpine (0.27) [10, 11].

High values of this ratio (0.52–0.62) have been found when NA is released as an 'active' neurotransmitter, such as after stress, electrical stimulation or amphetamine, while lower values have been found when it is released in a non-active form such as after reserpine [10, 11]. These findings support the view that MOPEG-SO_4 is an index of extraneuronal metabolism of NA. This has been confirmed by data showing that DMI decreases the MOPEG-SO_4 levels by depressing the neuronal impulse flow and not by a direct consequence of uptake inhibition [14].

It may be therefore deduced that MOPEG-SO_4–NA ratio reflects in many instances various mechanisms of NA release and disposition. Since Piribedil induces the highest values of the ratio this drug should be considered active through both an amphetamine and a reserpine-like mechanism.

The amphetamine-like mechanism of Piribedil action is confirmed by the results of the interactions with DMI.

The amphetamine-induced increase of MOPEG-SO_4 is blocked by DMI, which indicates that a normal impulse flow in NA neurons is required for the amphetamine action. DMI, in fact, has been shown to depress the rate of NA neurons rate of firing [12]. DMI does not reduce the reserpine-induced increase of MOPEG-SO_4, but does reduce the increase induced by Piribedil.

Our findings indicate therefore that Piribedil releases NA in part as an active transmitter with an amphetamine-like activity. On the other hand Piribedil seems to release NA also from the storage pool with a reserpine-like mechanism.

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