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BEHAVIOURAL AND NEUROCHEMICAL INTERACTIONS BETWEEN CHRONIC RESERPINE AND CHRONIC ANTIDEPRESSANTS

A POSSIBLE MODEL FOR THE DETECTION OF ATYPICAL ANTIDEPRESSANTS

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Abstract—Chronic treatment with a low dose of reserpine (0.1 mg/kg) caused rats to become hyperactive in the "open field" apparatus. When mianserin (5 mg/kg) or the selective serotonin uptake inhibitor ORG. 6582 (5 mg/kg) was chronically administered in combination with reserpine, the hyperactivity was attenuated. Both antidepressants were found to reverse the reduction in the noradrenaline concentration of the amygdaloid cortex caused by chronic reserpine treatment. It is proposed that changes in the activity of the noradrenergic system in the amygdaloid cortex may be causally related to the changes in activity of the rats in the "open field" apparatus.

The effects of an acute high dosage of reserpine (10 mg/kg) on the behaviour of rodents and the reversal of these effects by high acute doses of tricyclic antidepressants is widely used in the selection of compounds with potential antidepressant activity. Under such conditions reserpine causes a maximal depletion of biogenic amine neurotransmitters in the brain. It has also been shown that following chronic administration, reserpine causes an increase in the specific binding of [^{3}H]-dihydroalprenolol and in the activity of the noradrenaline sensitive adenylate cyclase in the cortex of the rat brain [1–3]. This suggests that following chronic reserpine treatment, the sensitivity of post synaptic adrenoreceptors is enhanced. As there is experimental evidence to show that following chronic administration of antidepressants the sensitivity of post synaptic adrenoreceptors is decreased (see review by Leonard [4]), it is not unreasonable to postulate that such drugs may reverse the neurochemical and behavioural effects seen following the chronic administration of reserpine.

The aim of the present investigation was to see what effects two atypical antidepressants had on the behaviour of rats treated chronically with low dosage of reserpine. By using this approach, it was hoped to deplete central biogenic amines without causing the severe debilitating effects normally seen after the acute administration of high dosage of the drug.

METHODS

In these experiments, male Sprague–Dawley rats (280–300 g; $N = 7$) were injected daily for 12 days with reserpine alone (0.1 mg/kg i.p.) saline alone, a combination of reserpine together with either mianserin (5 mg/kg i.p.) or ORG. 6582 (5 mg/kg i.p.)* and drugs control.

Twelve days after the commencement of reserpine treatment, the behaviour of the rats were assessed individually during the first three minutes exposure to the "open field" apparatus [5]. The following variables were measured: ambulation (the number of squares crossed), rearing (the number of times the animal lifted its forepaws and raised itself from the floor). Following the behavioural testing, the animals were maintained for a further two days on the appropriate drug treatment and then decapitated.

The brains were rapidly removed and the midbrain and amygdaloid cortex removed according to Popov *et al.* [6]. The amines and some of their metabolites were determined following separation on Sephadex G-10 columns by the method of Earley and Leonard [7]. The statistical significance of the changes was evaluated by means of a two-tailed Student's *t*-test, the significance being set at $P \leq 0.05$.

RESULTS AND DISCUSSION

In the first experiment, it was found that when reserpine was administered on every fourth day for two weeks, no change occurred in the behaviour of the rats in the "open field". However, in subsequent experiments, in which reserpine was administered once daily for two weeks, it was found that the ambulation and rearing scores increased (Table 1). Under these conditions chronically administered mianserin or ORG. 6582 normalized these behavioural changes (Table 1). By contrast the acute administration of ORG. 6582 to the reserpinized rats, while decreasing the rearing scores, did not attenuate the hyperactivity (Table 2). From these

* (5α,9α,11S*)-2-chloro-5,6,9,10-tetrahydro-5,9-methanobenzocyclooctane-11-amine-HCl.

Table 1. The effects of reserpine (0.1 mg/kg i.p.) administered once daily for two weeks on the behavioural parameters measured in the "open field" and the reversal of these effects by chronically administered mianserin (5 mg/kg i.p.) and ORG. 6582 (5 mg/kg i.p.)

	Treatment group					
	Saline	Reserpine	Mianserin	ORG. 6582	Mianserin + reserpine	ORG. 6582 + reserpine
Ambulation	73.5 ± 1.8	104.0 ± 9.2*	73.0 ± 1.6	68.6 ± 4.5	69.6 ± 4.5	78.1 ± 1.7†
Rearing	7.5 ± 0.9	12.3 ± 0.4*	5.5 ± 1.2	5.8 ± 0.6	5.2 ± 1.1†	6.2 ± 0.8†

$N = 7$, mean ± S.E.M.
* $P \leq 0.05$ vs saline.
† $P < 0.05$ vs reserpine; by two-tailed Student's t-test.

Table 2. The effect of an acute dose of ORG. 6582 (5 mg/kg i.p. 90 min) on the behaviour of the chronically reserpinized rats in the "open field"

	Treatment group			
	Saline	Reserpine	ORG. 6582	ORG. 6582 + reserpine
Ambulation	84.2 ± 4.1	117.6 ± 8.4*	76.3 ± 5.1	106.0 ± 9.8†
Rearing	7.2 ± 1.0	16.3 ± 1.6*	6.1 ± 0.9	10.6 ± 1.7†

$N = 7$, mean ± S.E.M.
* $P \leq 0.05$ versus saline.
† $P < 0.05$ versus reserpine by two-tailed Student's t-test.

experimental results it is apparent that the hypermotility resulting from chronic administration of a low dosage of reserpine is sensitive to atypical antidepressants.

As would be anticipated, the concentration of biogenic amine neurotransmitters decreased following reserpine administration (Table 3). Chronic treatment with mianserin reversed the effect of reserpine on the concentrations of noradrenaline (NA) and 5-hydroxyindole acetic acid (5-HIAA) in both brain areas studied, without altering that of serotonin (5-HT; Table 3). ORG. 6582, when administered chronically to the reserpinized animals, increased the concentration of 5-HT and normalized that of 5-HIAA in both brain areas. In addition, this drug also reversed the effect of reserpine on the steady-state concentration of NA in the amygdaloid cortex (Table 3). However, acutely administered ORG. 6582 failed to reverse these neurotransmitter changes induced by reserpine. The only acute effect of ORG. 6582 observed was on the concentration of 5-HIAA in the mid-brain (Table 4).

The reversal of the effects of reserpine on the noradrenergic system by mianserin and on the

Table 3. The effects of chronic administration of ORG. 6582 (5 mg/kg, i.p.) or (±)-mianserin (5 mg/kg, i.p.) on the neurotransmitter metabolism of chronically reserpinized animals

Amygdaloid cortex						
	Saline	Reserpine	ORG. 6582	Mianserin	Reserpine + mianserin	Reserpine + mianserin
DA	0.29 ± 0.04	0.17 ± 0.03*	0.27 ± 0.03	0.31 ± 0.05	0.18 ± 0.01*	0.17 ± 0.01*
NA	0.36 ± 0.04	0.19 ± 0.02*	0.36 ± 0.04	0.44 ± 0.01*	0.29 ± 0.03†	0.27 ± 0.01†
5-HT	0.25 ± 0.01	0.18 ± 0.01*	0.29 ± 0.01*	0.25 ± 0.01	0.19 ± 0.01*	0.25 ± 0.02†
5-HIAA	0.51 ± 0.02	0.39 ± 0.02*	0.57 ± 0.05	0.55 ± 0.05	0.46 ± 0.02†	0.48 ± 0.03†
Midbrain						
	Saline	Reserpine	ORG. 6582	Mianserin	Reserpine + mianserin	Reserpine + ORG. 6582
DA	0.21 ± 0.01	0.16 ± 0.01*	0.20 ± 0.01	0.20 ± 0.01	0.17 ± 0.01*	0.17 ± 0.01*
NA	0.34 ± 0.01	0.18 ± 0.01*	0.37 ± 0.03	0.42 ± 0.02*	0.24 ± 0.02†	0.19 ± 0.02*
5-HT	0.35 ± 0.01	0.26 ± 0.01*	0.40 ± 0.01*	0.36 ± 0.01	0.25 ± 0.01*	0.30 ± 0.01†
5-HIAA	0.49 ± 0.04	0.36 ± 0.04*	0.47 ± 0.05	0.51 ± 0.03	0.47 ± 0.04†	0.47 ± 0.04†

The values are expressed as μg/g ± S.E.M. $P \leq 0.05$; * vs control; † vs reserpine.

Table 4. The effects of acute administration of ORG. 6582 (5 mg/kg, i.p., 90 min) on the neurotransmitter metabolism of chronically reserpinized (0.1 mg/kg) animals

Amygdaloid cortex	Saline	Reserpine	ORG. 6582	Reserpine + ORG. 6582
NA	0.51 ± 0.04	0.28 ± 0.02†	0.47 ± 0.02	0.29 ± 0.02†
5-HT	0.35 ± 0.02	0.24 ± 0.02†	0.38 ± 0.02	0.27 ± 0.03†
5-HIAA	0.30 ± 0.01	0.25 ± 0.01†	0.13 ± 0.01	0.24 ± 0.01†
Midbrain				
	Saline	Reserpine	ORG. 6582	Reserpine + ORG. 6582
NA	0.43 ± 0.02	0.32 ± 0.02†	0.42 ± 0.02	0.29 ± 0.01†
5-HT	0.44 ± 0.03	0.27 ± 0.02†	0.45 ± 0.03	0.31 ± 0.03†
5-HIAA	0.41 ± 0.01	0.40 ± 0.02	0.36 ± 0.01†	0.34 ± 0.01†

The values are expressed as $\mu g/g \pm$ S.E.M.
† $P \leq 0.05$ vs saline.

serotonergic system by ORG. 6582 was anticipated from the effects of these drugs on central neurotransmitter metabolism *in vivo*. Thus mianserin has been shown to be a pre-synaptic α_2-adrenoreceptor antagonist [8], which increases the release of noradrenaline, whereas ORG. 6582 acts as a selective inhibitor of 5-HT uptake [9]. However, irrespective of the specificity of the effects of these drugs on the noradrenergic or serotonergic system *in vivo*, both attenuate the hyperactivity of the reserpinized rats in the "open field". In addition, both drugs normalize the decrease in the concentration of noradrenaline in the amygdaloid cortex. Whereas this effect is not unexpected in the case of mianserin which has been shown to facilitate central noradrenergic transmission *in vivo*, the explanation for the effect of ORG. 6582 is a matter of speculation. One possibility is that the facilitation of central serotonergic transmission by ORG. 6582 increases the activity of serotonergic receptors situated in the proximity of noradrenergic terminals; this may result in an enhanced release of noradrenaline and ultimately a normalization of both the serotonergic and noradrenergic systems [4]. Of the two brain regions examined, it would appear that most of the changes observed occur in the amygdaloid cortex. This supports the view that the amygdaloid cortex may play a role in regulating locomotor activity of the rat in a stressful environment [10, 11].

It may be concluded that the animal model which results from the chronic administration of a low dose of reserpine may offer an alternative method for the detection of antidepressant activity. However, the value of this model can only be fully assessed after other psychotropic drugs, with and without antidepressant activity, have been evaluated.

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REFERENCES

1. A. A. Roscher, U. N. Wiesmann and U. E. Honegger, *J. Pharmac. Exp. Therap.* **216**, 419 (1981).
2. D. A. Greenberg and S. H. Snyder, *Molec. Pharmac.* **14**, 38 (1979).
3. M. Hermoni, B. Lerer, R. P. Ebstein and R. H. Belmaker, *J. Pharm. Pharmac. (Communications)* **32**, 510 (1980).
4. B. E. Leonard, *Int. Review of the Triazolopyridine Derivatives*. Royal Society of Medicine Int. Congress and Symposium Series, **46**, 3 (1981).
5. J. A. Gray and B. Lalljee, *Anim. Behav.* **22**, 856 (1974).
6. N. Popov, W. Pohle, V. Roster and H. Matthies, *Acta Med. Biol.* **18**, 695 (1969).
7. C. J. Earley and B. E. Leonard, *J. Pharmac. Methods* **1**, 66 (1978).
8. J. M. Fludder and B. E. Leonard, *Psychopharmacology* **64**, 329 (1979).
9. W. E. Kafoe and B. E. Leonard, *Biochem. Pharmac.* **26**, 1083 (1977).
10. J. S. Richardson, *Acta. Neurobiol. Exp.* **34**, 543 (1974).
11. P. Gloor and J. Field (Eds.), *Handbook of Physiology, 2, Amydala.* Washington, Am. Physiol. Soc. (1960).