J. Pharm. Pharmac., 1972, **24**, 735

LETTERS TO THE EDITOR

The effect of L-dopa on neuroleptic-induced catalepsy

We have found previously that DL-dopa, a catecholamine precursor, antagonizes the catalepsy induced by a number of phenothiazine and butyrophenone neuroleptics given intraperitoneally to rats and mice previously treated with the monamine oxidase inhibitor nialamide (Maj & Szurska, 1967; Maj & Wielosz, 1967). The inhibition of MAO blocks the metabolism of both central and peripheral catecholamines and 5-hydroxytryptamine. We wished to establish if the anticataleptic action of dopa is present under conditions in which the selective increase of brain catecholamines appears. For this purpose L-dopa was given to the animals pretreated with an inhibitor of peripheral decarboxylase, Ro 4-4602 [N^1-(DL-seryl)-N^2-2,3,4-trihydroxybenzyl)-hydrazine]. Two phenothiazine and two butyrophenone neuroleptics were used: chlorpromazine and fluphenazine (both in saline), pimozide and spiroperidol (both in 3% aqueous Tween 80) all given intraperitoneally.

The experiments were made on male Wistar rats of 140–220 g. The catalepsy was scored for 2 h at 30 min intervals after L-dopa administered intraperitoneally in 3% Tween 80 according to Delini-Stula & Morpurgo (1968) with a modified system of scoring (double). The degree of catalepsy was expressed as the sum of four observations i.e. the maximal value was 24. Times and routes of administration of the drugs are given in the Tables.

Ro 4-4602 (50 mg/kg, in saline) alone did not affect the catalepsy induced by any neuroleptic used.

L-Dopa (100 and 200 mg/kg, i.p.) given 30 min before the test after pretreatment with Ro 4-4602 (50 mg/kg) 30 min previously weakened or abolished the catalepsy caused by chlorpromazine (8 mg/kg) given 75 min or pimozide (4 mg/kg) given 210 min before the test (Table 1).

The catalepsy induced by fluphenazine (4 mg/kg) given 165 min before the test was antagonized to a lesser degree by L-dopa given with Ro 4-4602 30 min before the test (Table 2). Stronger antagonism was observed after treatment with L-dopa and nialamide (100 mg/kg, i.p. 18 h before the test). A similar anticataleptic effect of L-dopa given to nialamide-pretreated rats was seen in animals previously receiving an inhibitor of 5-hydroxytryptamine synthesis, *p*-chlorophenylalanine (316 mg/kg, i.p. 72 h before the test). Nialamide alone did not change the catalepsy induced by fluphenazine both in normal and in *p*-chlorophenylalanine-pretreated rats.

Catalepsy induced by spiroperidol (0·4 mg/kg, 195 min before the test) was not changed by L-dopa (doses up to 400 mg/kg) injected after Ro 4-4602 (Table 3), but was

Table 1. *Effect of* L-*dopa* (*with Ro 4-4602 50 mg/kg*) *on catalepsy induced by chlorpromazine* (*8 mg/kg*) *or pimozide* (*4 mg/kg*).

Group (n=10)	Drugs doses mg/kg	Cataleptic effect mean ± s.e.		*P* (*t*-test)
I	Chlorpromazine	23·0 ± 0·8		—
II	Chlorpromazine + Ro 4-4602 + L-dopa 100	9·0 ± 2·9	II/I	<0·001
III	Chlorpromazine + Ro 4-4602 + L-dopa 200	3·8 ± 1·9	III/I	<0·001
IV	Pimozide	22·9 ± 1·1		—
V	Pimozide + Ro 4-4602 + L-dopa 50	21·4 ± 1·8	V/IV	n.s.
VI	Pimozide + Ro 4-4602 + L-dopa 100	4·0 ± 1·5	VI/IV	<0·001
VII	Pimozide + Ro 4-4602 + L-dopa 200	2·2 ± 1·3	VII/IV	<0·001

antagonized partly or completely by L-dopa (50, 100 and 200 mg/kg respectively) given after nialamide. In rats pretreated with *p*-chlorophenylalanine the anticataleptic action of nialamide and L-dopa was similar to that in normal rats. Nialamide alone did not influence spiroperidol catalepsy.

Therefore, as in nialamide-pretreated animals, L-dopa given after Ro 4-4602 antagonizes the catalepsy induced by three of the neuroleptics used but not spiroperidol. The anticataleptic action of L-dopa is, however, less potent in Ro 4-4602 than in nialamide-pretreated rats. In the latter group the catalepsy induced by spiroperidol is also abolished. The experiments with *p*-chlorophenylalanine indicate that the difference between nialamide and Ro 4-4602 pretreatment is not related to 5-hydroxytryptamine, although nialamide increases its brain level and L-dopa may release it (Ng, Chase & others, 1970). The difference may be related to the fact that the brain levels of catecholamines are higher in rats receiving L-dopa after inhibition of MAO than after inhibition of peripheral decarboxylase. The brain catecholamine

Table 2. *Effect of* L-*dopa* (*with Ro 4-4602 50 mg/kg or nialamide 100 mg/kg*) *on catalepsy induced by fluphenazine* (*0·4 mg/kg*) *in normal and p-chlorophenylalanine* (*316 mg/kg*) *pretreated rats.*

Group (n=10)	Drugs doses mg/kg	Cataleptic effect mean ± s.e.	*P* (*t*-test)	
I	Fluphenazine	22·5 ± 1·5		—
II	Fluphenazine + Ro 4-4602 + L-dopa 50	18·5 ± 2·2	II/I	n.s.
III	Fluphenazine + Ro 4-4602 + L-dopa 100	13·2 ± 2·4	III/I	<0·01
IV	Fluphenazine + Ro 4-4602 + L-dopa 200	12·4 ± 2·2	IV/I	<0·01
V	Fluphenazine + Ro 4-4602 + L-dopa 400	4·2 ± 1·3	V/I	<0·001
VI	Fluphenazine + nialamide + L-dopa 50	14·0 ± 0·3	VI/I	<0·01
			VI/II	n.s.
VII	Fluphenazine + nialamide + L-dopa 100	10·4 ± 0·3	VII/I	<0·001
			VII/III	n.s.
VIII	Fluphenazine + nialamide + L-dopa 200	0	VIII/I	<0·001
			VIII/IV	<0·001
IX	*p*-Chlorophenylalanine + fluphenazine	18·5 ± 0·9	IX/I	<0·05
X	*p*-Chlorophenylalanine + fluphenazine + + nialamide + L-dopa 100	9·2 ± 1·7	X/IX	<0·001
			X/VII	n.s.
XI	*p*-Chlorophenylalanine + fluphenazine + + nialamide + L-dopa 200	1·5 ± 0·5	XI/IX	<0·001
			XI/VIII	<0·01

Table 3. *Effect of* L-*dopa* (*with Ro 4-4602 50 mg/kg or nialamide 100 mg/kg*) *on catalepsy induced by spiroperidol* (*0·4 mg/kg*) *in normal and p-chlorophenylalanine* (*316 mg/kg*) *pretreated rats.*

Group (n=10)	Drugs doses mg/kg	Cataleptic effect mean ± s.e.	*P* (*t*-test)	
I	Spiroperidol	22·9 ± 0·7		—
II	Spiroperidol + Ro 4-4602 + L-dopa 200	20·4 ± 2·1	II/I	n.s.
III	Spiroperidol + Ro 4-4602 + L-dopa 400	23·4 ± 0·4	III/I	n.s.
IV	Spiroperidol + nialamide + L-dopa 50	14·6 ± 1·3	IV/I	<0·01
V	Spiroperidol + nialamide + L-dopa 100	9·2 ± 1·3	V/I	<0·001
VI	Spiroperidol + nialamide + L-dopa 200	0	VI/I	<0·001
			VI/II	<0·001
VII	*p*-Chlorophenylalanine + spiroperidol	24·0 ± 0	VII/I	n.s.
VIII	*p*-Chlorophenylalanine + spiroperidol + + nialamide + L-dopa 50	16·0 ± 1·4	VIII/VII	<0·01
			VIII/IV	n.s.
IX	*p*-Chlorophenylalanine + spiroperidol + + nialamide + L-dopa 100	11·6 ± 1·3	IX/VII	<0·001
			IX/V	n.s.

levels may be increased for a longer time in the former group than in the latter, since we observed that the anticataleptic action of L-dopa was shorter in rats pretreated with Ro 4-4602, and the action decreased during the 2 h observation period.

Spiroperidol-induced catalepsy seems to be particularly resistant to the antagonistic action of L-dopa and is not antagonized by apomorphine (unpublished results).

We would like to thank Dr. Janssen for generous gifts of spiroperidol and pimozide.

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April 29, 1972

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Plasma levels in man of nitroglycerin after buccal administration

A major problem in the therapy of angina pectoris is how to prolong the undisputed beneficial effects of buccal nitroglycerin. For evaluation of retard preparations and so-called long acting nitrates, knowledge of the plasma levels of different preparations would be an advantage. Results in man have been reported for pentaerythritol tetranitrate (Davidson, Miller & DiCarlo, 1971); for nitroglycerin valid data are only available in animals (Lang, Johnson & Needleman, 1972); the few papers on plasma levels in man are unacceptable due to the lack of specificity and sensitivity of the method used (Berry & Roach, 1968; Ritschel & Clotten, 1970).

We recently reported a sensitive method for specific determination of nitroglycerin, using gas chromatography with electron capture detection (Rosseel & Bogaert, 1972). The procedure can be used for determination of nitroglycerin in plasma. After centrifugation of the blood at 4°, the plasma (*ca* 5 ml) is removed and extracted three times with 5 ml of ethyl acetate of high purity (Carlo Erba, pro pesticidi). The extracts are passed through Norite filters and evaporated to near dryness under nitrogen, at room temperature. The residue from the three extractions is dissolved in 0·5 ml ethyl acetate, filtered again through a Norite filter, evaporated and dissolved in 10 μl of benzene. 1 to 2 μl of this solution is injected in the gas chromatograph.

Before the extraction procedure, isosorbide dinitrate is added to the plasma as internal standard. The ratio of the peak area of nitroglycerin to peak area of isosorbide dinitrate shows a linear relation to the ratio of the quantities of the two injected substances, as ascertained by control experiments. With this method, concentrations of nitroglycerin as low as 0·5 ng/ml of plasma can be specifically measured.

The method was used for measurement of plasma levels in man after buccal administration of nitroglycerin; this route was chosen because of its importance as a reference treatment of angina. Doses of nitroglycerin from 600 to 2500 μg, dissolved in 0·12 ml of ethanol were introduced in the mouth of young healthy volunteers; they were asked to circulate, without swallowing, their saliva in the mouth for 3½ min, at that moment they expelled their saliva, rinsed their mouth, and venous blood samples were taken at different times thereafter.

Fig. 1 shows the results in 5 volunteers: plasma levels are clearly higher for the