The effect of L-dopa and (+)-amphetamine on the locomotor activity after pimozide and phenoxybenzamine

Previous experiments with L-dopa, apomorphine and amantadine (Maj, Grabowska & Mogilnicka, 1971; Maj, Grabowska & Gajda, 1972; Maj, Sowińska & Baran, 1972) showed that in the stimulation of locomotor activity induced by these compounds both central catecholamines, dopamine and noradrenaline, play a role. Experiments studying the interactions between pimozide and phenoxybenzamine, on one hand, and L-dopa and (+)-amphetamine on the other, may support this hypothesis. The former two compounds block specifically central dopamine (Andén, Butcher & others, 1970) and noradrenaline receptors respectively (Andén, Dahlström & others, 1966). L-Dopa and amphetamine stimulate both the receptors (Ernst & Smelik, 1966; Andén, Engel & Rubenson, 1971; Randrup & Scheel-Krüger, 1966; Svensson, 1970).

The locomotor activity of adult Wistar rats and Albino-Swiss mice of either sex in groups of at least 10 animals was measured in a photoresistor actometer for 15 min. The drugs were given before the test as follows: pimozide (i.p.) 4 h, phenoxybenzamine (i.p.) 2 h, L-dopa (i.p.) 1 h 45 min, (+)-amphetamine sulphate (s.c.) 30 min. An inhibitor of extracerebral decarboxylase, Ro 4-4602 [N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl) hydrazine] (50 mg/kg i.p., 30 min before L-dopa) preceded all injections of L-dopa. The control groups were treated with appropriate solvents. Pimozide, phenoxybenzamine and L-dopa were administered as suspensions in 3% aqueous solution of Tween 80; (+)-amphetamine and Ro 4-4602 was dissolved in saline. Statistical evaluation was made using Student's *t*-test.

Pimozide, 1 and 2 mg/kg, depressed the locomotor activity of rats (Table 1). The

Table 1. *Effect of* L-*dopa* (*with Ro 4-4602*), *amphetamine, pimozide and phenoxybenzamine on locomotor activity in rats.* Drugs were given before the test as follows: pimozide (i.p.) 4 h, phenoxybenzamine (i.p.) 2 h, Ro 4-4602 (i.p.) 2 h 15 min, L-dopa (i.p.) 1 h 45 min, (+)-amphetamine sulphate (s.c.) 30 min. Locomotor activity was measured in single animals for 15 min. Statistical significance was calculated using Student's *t*-test.

Group	Drugs mg/kg	Activity counts mean ± s.e.	*P*	Groups
I	Control	111·3 ± 11·7	—	—
II	Ro 4-4602 + L-dopa 100	242·9 ± 22·0	<0·001	II/I
III	Ro 4-4602 + L-dopa 200	316·0 ± 34·9	<0·001	III/I
IV	Pimozide 1	38·2 ± 5·6	<0·001	IV/I
V	Pimozide 1 + Ro 4-4602 + L-dopa 100	121·0 ± 16·0	<0·001	V/IV
VI	Pimozide 1 + Ro 4-4602 + L-dopa 200	138·7 ± 52·0	>0·05	VI/IV
VII	Pimozide 2	15·5 ± 2·5	<0·001	VII/I
VIII	Pimozide 2 + Ro 4-4602 + L-dopa 100	10·4 ± 3·9	>0·2	VIII/VII
IX	Pimozide 2 + Ro 4-4602 + L-dopa 200	10·9 ± 2·3	>0·1	IX/VIII
X	Phenoxybenzamine 25	10·1 ± 2·9	<0·001	X/I
XI	Phenoxybenzamine 25 + Ro 4-4602 + L-dopa 200	11·0 ± 4·1	>0·8	XI/X
XII	Amphetamine 2·5	184·1 ± 23·8	<0·02	XII/I
XIII	Amphetamine 5·0	332·2 ± 17·0	<0·001	XIII/I
XIV	Pimozide 1 + amphetamine 2·5	37·3 ± 12·2	>0·9	XIV/IV
XV	Pimozide 1 + amphetamine 5	36·4 ± 7·1	>0·8	XV/IV
XVI	Pimozide 2 + amphetamine 2·5	7·7 ± 1·3	<0·05	XVI/VII
XVII	Pimozide 2 + amphetamine 5	35·6 ± 3·8	<0·001	XVII/VII
XVIII	Phenoxybenzamine 25 + amphetamine 2·5	30·0 ± 7·7	<0·05	XVIII/X
XIX	Phenoxybenzamine 25 + amphetamine 5	127·1 ± 21·0	<0·001	XIX/X

L-dopa-stimulation and that induced by (+)-amphetamine (2·5 and 5 mg/kg) was antagonized by pimozide. Phenoxybenzamine (25 mg/kg) which markedly depressed the motor activity of normal rats, produced complete or partial inhibition of hyperactivity induced by L-dopa or (+)-amphetamine.

Table 2. *Effect of amphetamine, pimozide and phenoxybenzamine on locomotor activity in mice* (see Table 1 for key)

Group	Drugs mg/kg	Activity counts mean ± s.e.	*P*	Groups
I	Control	160·8 ± 18·8	—	—
II	Amphetamine 2·5	304·2 ± 36·3	<0·01	II/I
III	Amphetamine 5	543·1 ± 54·0	<0·001	III/I
IV	Pimozide 1	44·9 ± 10·0	<0·001	IV/I
V	Pimozide 1 + amphetamine 2·5	47·5 ± 7·7	>0·8	V/IV
VI	Pimozide 2	25·2 ± 4·7	<0·001	VI/I
VII	Pimozide 2 + amphetamine 2·5	18·4 ± 3·9	>0·2	VII/VI
VIII	Pimozide 1	64·2 ± 12·6	<0·001	VIII/I
IX	Pimozide 1 + amphetamine 5	64·1 ± 14·3	>0·9	IX/VIII
X	Pimozide 2	65·2 ± 14·1	<0·001	X/I
XI	Pimozide 2 + amphetamine 5	80·9 ± 19·5	>0·5	XI/X
XII	Phenoxybenzamine 25	22·9 ± 8·8	<0·001	XII/I
XIII	Phenoxybenzamine 25 + amphetamine 2·5	19·1 ± 8·8	>0·7	XIII/XII
XIV	Phenoxybenzamine 25 + amphetamine 5	66·9 ± 19·0	<0·05	XIV/XII

Similar results were obtained in mice (Table 2). Pimozide completely protected the animals against locomotor stimulation induced by amphetamine. Phenoxybenzamine abolished the effects of the lower amphetamine dose and markedly inhibited the effects of the high dose. Both pimozide and phenoxybenzamine depressed the locomotor activity of normal mice but phenoxybenzamine did not inhibit the stereotypy induced by (+)-amphetamine or L-dopa.

The results show that blockade of one type of the central catecholamine receptors (dopamine receptors by pimozide or noradrenaline receptors by phenoxybenzamine) antagonizes the increase of locomotor activity induced by compounds that stimulate these two receptors, (+)-amphetamine or L-dopa.

These results confirm our previous findings that both catecholamines, noradrenaline and dopamine, are important for the locomotor activity. A similar conclusion based on different experiments was drawn by Andén, Corrodi & others (1970), Corrodi, Fuxe & others, (1970), Svensson (1970) and Svensson & Waldeck (1970).

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Δ^9-Tetrahydrocannabinol and its metabolites in monkey brains

The metabolism of (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC), the major active constituent of marihuana, has been studied primarily in peripheral tissues of animals and man. The major metabolite has been identified as 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-Δ^9-THC), a product of liver microsomal hydroxylation (Agurell, Nilsson & others, 1969, 1970; Ben-Zvi, Mechoulam & Burstein, 1970; Burstein, Menezes & others, 1970; Foltz, Fentiman & others, 1970; Lemberger, Silberstein & others, 1970; Nilsson, Agurell & others, 1970; Wall, Brine & others, 1970; Christensen, Freudenthal & others, 1971). Further hydroxylation of 11-OH-Δ^9-THC was shown *in vitro* to form 8,11-dihydroxy-Δ^9-tetrahydrocannabinol (8,11-$(OH)_2$-Δ^9-THC) (Wall & others, 1970; Christensen & others, 1971). The monohydroxylated metabolite has been reported to be behaviorally and pharmacologically as active as Δ^9-THC, whereas the dihydroxylated product is inactive (Wall & others, 1970; Truitt, 1970; Christensen & others, 1971).

In vitro incubation of mouse brain homogenate with ^{14}C-Δ^9-THC in the presence of a NADPH regenerating system did not produce an appreciable degree of hydroxylation (Christensen & others, 1971). In the only *in vivo* work thus far reported, both the 11-OH- and 8,11-$(OH)_2$-Δ^9-THC were found in brains of mice injected intravenously or intracerebrally with 0·2 mg of Δ^9-THC (Christensen & others, 1971).

The long retention of Δ^9-THC and its metabolites in rat brains (Ho, Fritchie & others, 1970; Layman & Milton, 1971), and the long-lasting behavioral effects of the compound in monkeys (Scheckel, Boff & others, 1968) prompted us to examine the metabolic alteration of Δ^9-THC in squirrel monkey brains at various times after its administration. Several thin-layer chromatography systems were developed to aid in the identification of Δ^9-THC and its metabolites.

Eight male squirrel monkeys (average 650 g) were injected intravenously *via* the saphenous vein with 10 or 1·5 mg/kg of ^{3}H-Δ^9-THC (146·3 μCi/mg) in 4% Tween 80 in saline. The animals were anaesthetized with ether and killed by bleeding at designated times. The brains were removed immediately, washed with saline and homogenized in four volumes of water. Triplicate aliquots (25 μl) of each homogenate were pipetted into counting vials; mixed with methanol then scintillation fluor, and assayed for tritium by liquid scintillation spectrometry. All values were corrected for 100% efficiency (channel ratio) and recovery.

For chromatography, each brain homogenate was extracted three times with five volumes of anhydrous methanol. The extracts were evaporated to dryness under nitrogen and the residue treated with a small volume of hot methanol. After centrifugation, the methanol solution was concentrated to less than one ml, and again centrifuged. The final supernatant, containing from 98 to 100% of the original radioactivity, was streaked on Silica Gel G precoated plates (Brinkmann Co.) and developed, along with reference compounds, in the solvent systems in Table 1. The distribution of radioactivity in the chromatograms was determined by scraping sections of silica (0·5 cm × 1 cm) and assaying for tritium. The percentage of each metabolite was calculated by a mapping technique which involved the plotting of per cent of radioactivity in each section *vs* R_F values.