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BRL 13776: A NOVEL ANTIHYPERTENSIVE AGENT WITH INTERESTING MONOAMINE DEPLETING PROPERTIES

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1 Oral doses of 10–100 mg/kg of BRL 13776 lowered the blood pressure of both deoxycorticosterone acetate (DOCA)/NaCl-treated hypertensive rats and untreated normotensive rats.

2 BRL 13776 (100 mg/kg, orally) also reduced the blood pressure of renal hypertensive cats (cellophane perinephritis model).

3 No tolerance developed to the blood-pressure lowering action of BRL 13776 when an oral daily dose of 100 mg/kg was administered repeatedly for up to 15 days to hypertensive rats and cats.

4 The fall in blood pressure to BRL 13776 in rats was associated with a reduction of tissue catecholamines.

5 The catecholamine depletion occurred in all the peripheral tissues examined but in the brain was restricted to certain regions, these being the hind-brain on single dosing and the hind-brain, hypothalamus and mid-brain on repeated dosing. Catecholamine levels in the cerebral hemispheres were not affected by either single or repeated doses of BRL 13776.

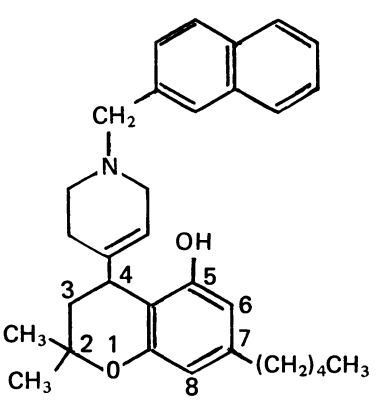
6 BRL 13776 caused some reduction of the 5-hydroxytryptamine content of the heart but not of whole brain or any brain region.

7 Neither single doses (up to 900 mg/kg orally) nor repeated doses (100–300 mg/kg orally) of BRL 13776 produced any significant behavioural effects in animals.

8 BRL 13776 is a new type of agent to display both antihypertensive and monoamine-depleting properties. The reduction of noradrenaline in certain brain regions may be a cause of the antihypertensive response but depletion in the periphery could contribute in a major or minor way. The differential action on noradrenaline in the brain together with the lack of effect on 5-hydroxytryptamine might also explain the apparent absence of behavioural effects.

Introduction

Synthesis, firstly by Arthur D. Little Inc. and then subsequently in our own laboratories, of compounds structurally related to the tetrahydrocannabinols has produced a series of 4-(tetrahydropyrid-4-yl)chromen- and chroman-5-ols with antihypertensive activity (Fake, Gardner, Melton & Miller, 1974; Poyser & Palfreyman, 1975). One of the most promising compounds of this series is BRL 13776 (7-*n*-pentyl-4-[1-(2-naphthylmethyl)- 1,2,5,6-tetrahydro-4-pyridyl]-2,2-dimethylchroman-5-ol) whose structure is shown alongside. BRL 13776 was found to have interesting monoamine-depleting properties, and these together with the antihypertensive effects are described in this paper. A preliminary communication of this work was presented to the British Pharmacological Society (Melrose, Palfreyman, Poyser & Whiting, 1976).



BRL 13776

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Methods

Deoxycorticosterone acetate (DOCA)/NaCl-treated hypertensive rats

Hypertension was induced by subcutaneous implantation of DOCA (50 mg), into male Sprague Dawley rats weighing 60–80 g, together with unilateral nephrectomy and replacement of the drinking water with 1% w/v NaCl solution for the first 5 weeks. The rats were left at least 2 months after the operative procedure at which time their body weights were between 300–450 g and their blood pressures had usually attained a stable level. A minimum value for systolic blood pressure of 160 mmHg (1 mmHg $\approx$ 133 Pa) was taken for selection of animals as hypertensive. Systolic blood pressure was recorded by the tail cuff method (Friedman & Freed, 1949) using a W + W B.P. Recorder, Model No. 8002. For all measurements of blood pressure the rats were held in restraining cages in a heated environment (33.5 ± 0.5°C) and each determination was the mean of at least 6 readings.

Hypertensive cats

Hypertension was induced in male cats (2.5–3.5 kg) by a modification of the cellophane perinephritis method of Page (1939). This involved wrapping the left kidney in cellophane with contralateral nephrectomy. Cats treated in this way usually developed a suitable level of hypertension (diastolic blood pressure > 105 mmHg) within 4–6 weeks (see also Poyser, Shorter & Whiting, 1974). Direct blood pressure measurements were made in the conscious animal via an indwelling arterial catheter (Day & Whiting, 1972).

Endogenous monoamine determinations

Male Sprague Dawley rats were used throughout. Their body weights were 200–250 g in experiments involving only normotensive animals and 300–450 g in experiments where both normotensive and hypertensive rats were used.

Animals were killed by cervical dislocation, decapitation and exsanguination. The tissues were rapidly removed, dissected free of connective tissue and fat on a chilled plate and then frozen on solid carbon dioxide (Cardice). The brain was either used intact or dissected into four regions, viz: the cerebral hemispheres, mid-brain, hypothalamus and pons/medulla (hind-brain) according to the parameters of Glowinski & Iversen (1966).

Tissues were weighed and homogenized in 0.4 M perchloric acid containing 0.05% w/v disodium edetate (EDTA) and 0.1% w/v sodium metabisulphite. The homogenate was centrifuged at 20,000 *g* for 20 min at 0°C and the catecholamines were determined, after alumina adsorption, by the fluorometric method of Shellenberger & Gordon (1971). 5-Hydroxytryptamine (5-HT) was determined in the same tissue samples by the method of Snyder, Axelrod & Zweig (1965). All assays were checked for interference by BRL 13776 by including internal standards. Statistical significance between drug-treated and vehicle-treated (control) groups was assessed by the Student's *t* test for unpaired data, and the results were usually expressed as a percentage of control in order to compare effects on monoamine levels in more than one tissue and/or different monoamines in the same tissue.

Behavioural studies

Examination of BRL 13776 on the general behaviour and response of mice and rats was performed by the use of a modification of Irwin's multidimensional observation technique (Irwin, 1968). This was used in studies involving single dosing in both mice (CFLP weight range 20–25 g) and rats (Sprague Dawley 200–250 g) and repeated daily dosing in mice. For single oral dosing, groups of at least 3 animals were used at each dose level and these were observed at hourly intervals for up to 6–8 h and then again at 24 hours. In the repeat dose study in mice, test compounds and vehicle controls were administered orally to groups of 5 mice for 14 days. Each group was observed on days 1, 4, 8, 11 and 15 of the experiment. In addition the body weights of the mice in each group were recorded throughout the experiment.

The other tests employed to examine behavioural effects of BRL 13776 in mice were as follows: potentiation of hexobarbitone hypnosis according to the method of Jacobsen (1964) except that a quantal method of measuring drug effect was used; effect on maximal electro-shock convulsions using the method of Swinyard, Brown & Goodman (1952) with a slight modification in that an Ediswan electro-convulsive therapy unit was used with a 22 kΩ resistance in series with a subcutaneous needle electrode; leptazol-induced convulsions according to the method of Goodman, Grewal, Brown & Swinyard (1953); clonidine-induced fighting by the method of Morpurgo (1968), again with the slight modification of using an intravenous dose of 25 mg/kg of clonidine to induce the attacking behaviour and a dead mouse rather than another clonidine-dosed mouse as the recipient of the attack, and finally prevention of reserpine-induced hypothermia by the method of Garattini & Jori (1967).

All these behavioural tests were carried out in a temperature-controlled room at 20°C.

Drugs

BRL 13776 was synthesized in our laboratories by Mr C.S. Fake. Reserpine (base) and syrosingopine (base)

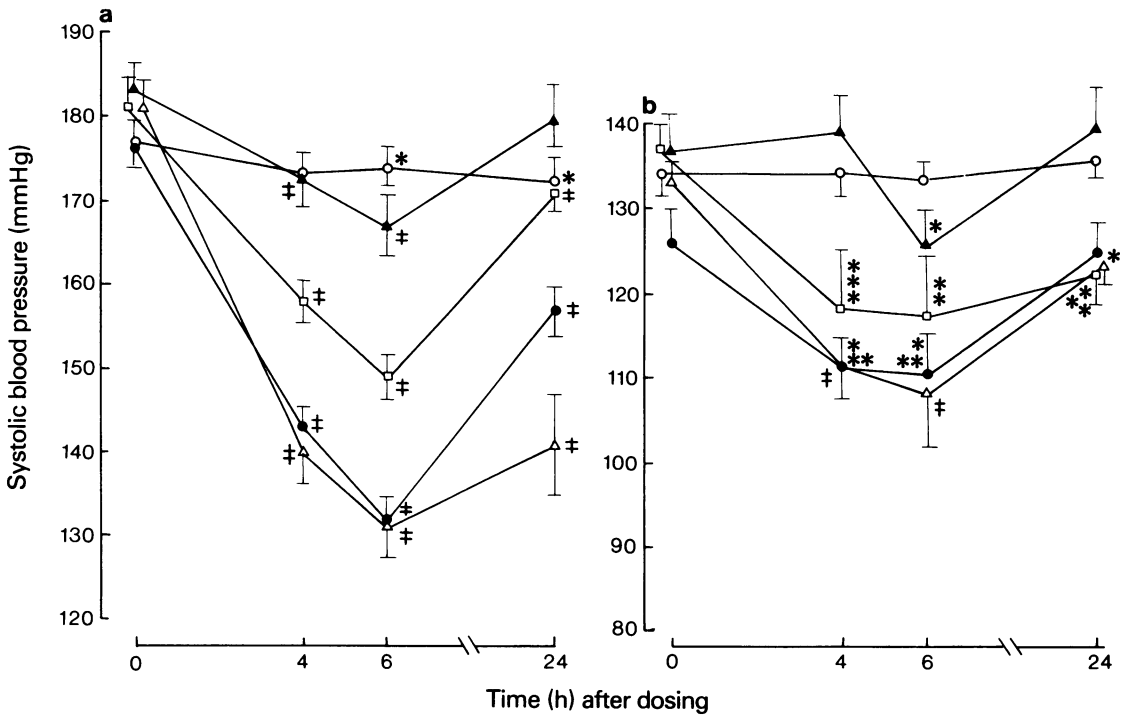

Figure 1 Effect of oral doses of 10 (▲), 30 (□), 100 (●) and 300 (△) mg/kg of BRL 13776 on the systolic blood pressure of (a) deoxycorticosterone acetate/NaCl-treated hypertensive rats and (b) normotensive (i.e. untreated) rats. Control rats (○) in each study received the suspending agent (1% w/v methyl cellulose solution) alone. For each group of rats ($n \geqslant 24$ in (a) and $n = 12$ in (b)) the mean systolic blood pressure is shown at various times after dosing. Vertical lines show s.e. mean. The significance of the blood pressure change from the pre-dose value (paired t test) is shown as: * $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$ and ‡ $P < 0.001$.

were kindly supplied by Ciba Laboratories. In all studies in rats and mice these drugs were administered orally as a suspension in 1% w/v methyl cellulose solution. In cats, the BRL 13776 was administered as a dry powder in gelatin capsules. Clonidine was a gift of C.H. Boehringer Sohn, Ingelheim. Leptazol (pentylenetetrazol) was purchased from Sigma. Δ^8-Tetrahydrocannabinol, which was used in the behavioural tests, was synthesized in our laboratories by Drs D.V. Gardner and T. Melton. This was administered orally dissolved in the oil phase of an arachis oil in water emulsion (prepared using acacia powder).

Results

DOCA/NaCl-treated hypertensive rats

Single oral doses of BRL 13776 lowered the blood pressure of DOCA/NaCl-treated hypertensive rats, the effect being dose-related over the range 10 to 100 mg/kg (Figure 1a). A dose of 300 mg/kg was longer acting but caused no greater effect than the 100 mg/kg dose. At all doses the maximum response was recorded at 6 h with the blood pressure having returned to or towards the pre-dose value at 24 hours. A slight fall in blood pressure occurred in the control group but an analysis of variance test between all groups confirmed the significance ($P < 0.001$) of the dose-response to BRL 13776. No significant change ($P > 0.05$) in heart rate was observed at any time after any of the dose levels of BRL 13776 used. In addition no behavioural or adverse effects were apparent.

The antihypertensive response to BRL 13776 in DOCA/NaCl-treated hypertensive rats was evident during 14 days repeated administration with a daily oral dose of 100 mg/kg (Figure 2). However, the initial fall in blood pressure in this particular study was not so marked as that previously seen in the single-dose studies and there was also variation from day to day in the response. Nevertheless, no definite evidence of tolerance was apparent and the blood pressure returned within 2 days to the pre-dose value

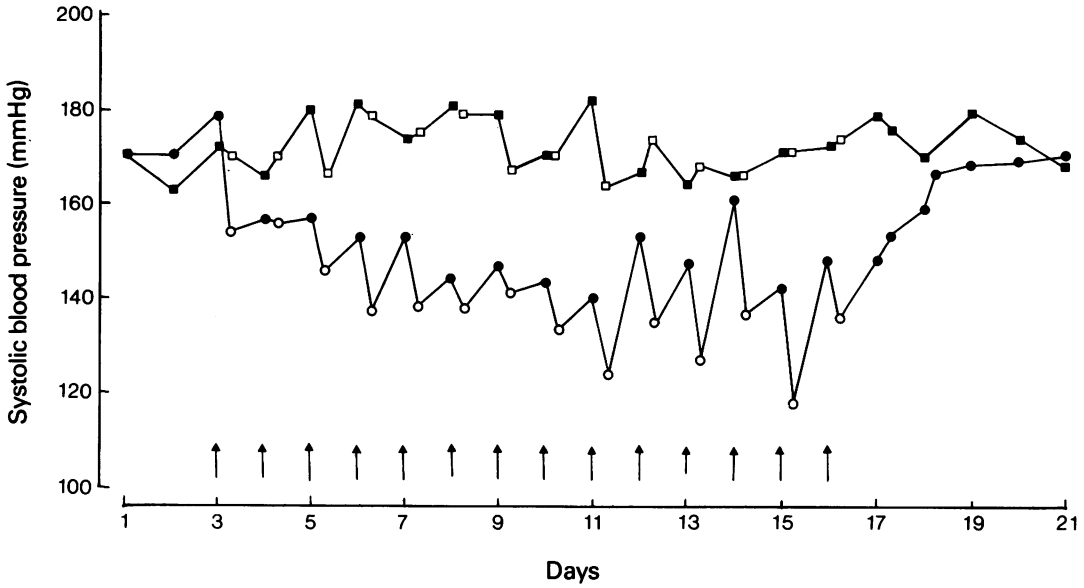

Figure 2 Effect of a repeated daily oral dose of 100 mg/kg of BRL 13776 on the systolic blood pressure of deoxycorticosterone acetate/NaCl-treated hypertensive rats. Circles denote the group ($n=6$) receiving BRL 13776; squares represent the control group ($n=6$) which received the suspending agent (1% w/v methyl cellulose) alone. Either BRL 13776 or suspending agent was given at the arrows and the open symbols show the blood pressure at 6 h after each treatment.

when dosing was discontinued. As with the single dose experiments, no consistent change in heart rate, or evidence of behavioural or adverse effects, was apparent in this repeat-dose study.

Normotensive rats

The blood pressure of normotensive rats was also lowered by single oral doses of BRL 13776 (Figure 1b). However, the falls in blood pressure were less than those obtained in hypertensive rats. Again, there was no significant change in heart rate and no behavioural or adverse symptoms were observed. A repeat-dose study (7 days) in normotensive rats was similar to that in hypertensive rats in showing that no tolerance occurred to the hypotensive action of BRL 13776 (data not shown).

Hypertensive cats

BRL 13776 was effective in hypertensive cats over the same dose-range as that used in rats. The maximum response in this model occurred 4 to 5 h after dosing and a typical experiment using an oral dose of 100 mg/kg is shown in Figure 3. It was of interest to note that a change in posture of the cat (i.e. sitting to standing) did not affect the fall in blood pressure. Heart rate was usually slightly reduced, this being in contrast to the results in rats. In a group of 6 hypertensive cats the dose of 100 mg/kg of BRL 13776 lowered the systolic blood pressure from 205 ± 8 mmHg (mean $\pm$ s.e. mean) to 160 ± 6 mmHg and the diastolic blood pressure from 140 ± 4 mmHg to 110 ± 4 mmHg. This effect was highly significant ($P < 0.01$).

In a repeat-dose study in cats an oral dose of 100 mg/kg of BRL 13776 caused a fall in blood pressure on most days of dosing (Figure 4). In addition, taking the dosing period as a whole, there was a general reduction in blood pressure values. The heart rate also fell as dosing continued. No tolerance was apparent, and following cessation of dosing both blood pressure and heart rate returned over a period of 1 week to the pre-dose values.

No behavioural effects such as diminution of spontaneous activity, ataxia, raucous mewing or mydriasis, which are typical of tetrahydrocannabinols (Lipparini, de Carolis & Longo, 1969), were observed with the single or repeated doses of BRL 13776 used in cats.

Endogenous monoamine levels

An oral dose of 100 mg/kg of BRL 13776 produced, at 6 h after dosing, a significant decrease in the noradrenaline content of the heart, spleen and

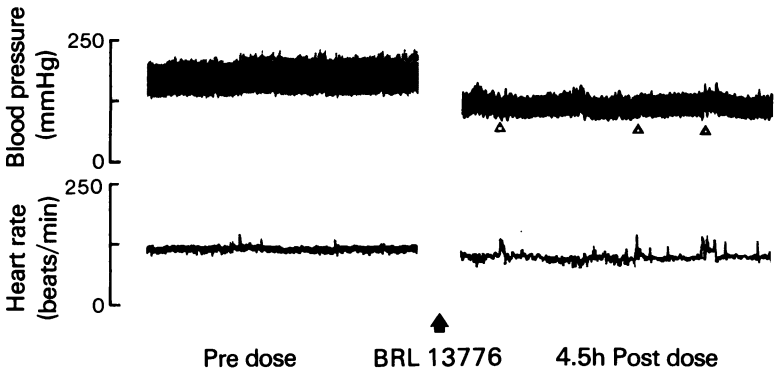

Figure 3 Reduction of blood pressure in response to an oral dose of BRL 13776 100 mg/kg in a conscious hypertensive cat (cellophane perinephritis model). Heart rate is also shown and is slightly reduced. During the recording the cat remained in the sitting posture except at the triangles when it was induced to stand up for a few seconds.

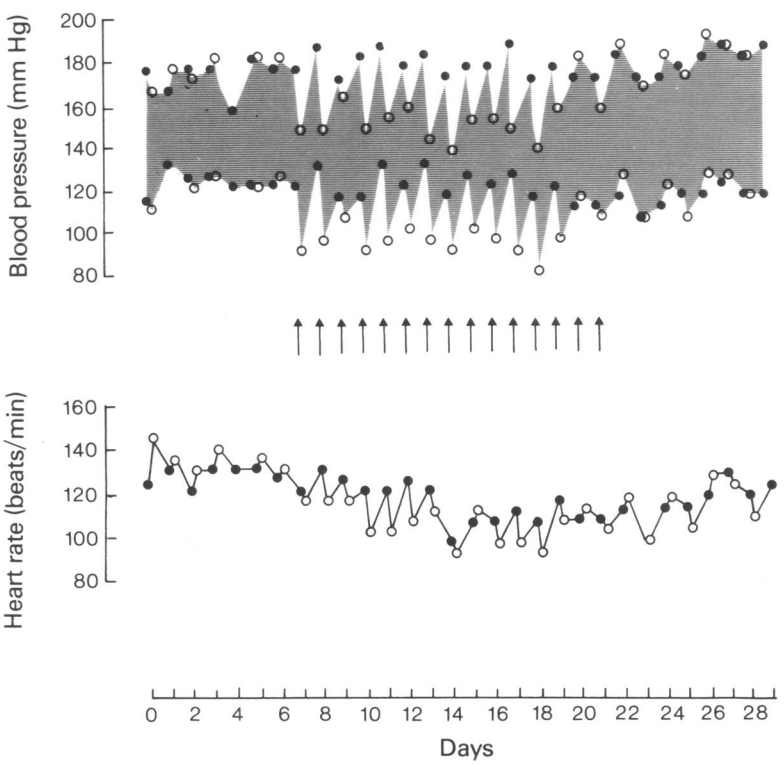

Figure 4 Effect of a repeated daily oral dose of BRL 13776 100 mg/kg on the blood pressure of a group of 3 hypertensive cats. BRL 13776 (as a dry powder in gelatin capsules) was administered at the arrows. Capsules containing lactose were administered during the periods before and after the BRL 13776 treatment. The closed symbols denote the blood pressure and heart rate values at the times of dosing with BRL 13776 or lactose and the open symbols the values at 5 h after dosing.

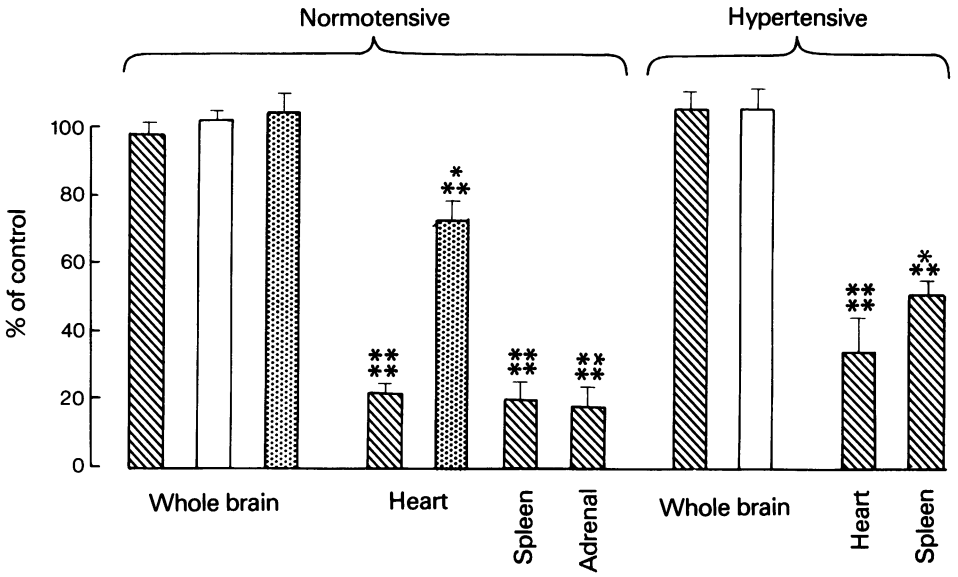

Figure 5 Effect of an oral dose of BRL 13776 100 mg/kg on the endogenous levels of noradrenaline (hatched columns), dopamine (open columns) and 5-hydroxytryptamine (stippled columns) in some organs (noradrenaline plus adrenaline measured in adrenals) of hypertensive and/or normotensive rats. Monoamine levels were determined at 6 h post dose, with a group of normotensive and hypertensive animals receiving the suspending agent alone (1% w/v methyl cellulose) serving as the controls. Groups of at least 6 animals were used and the results are expressed as percentages of controls. Mean values are shown; vertical lines indicate s.e. mean. The degree of significance of the change from control (Student's *t* test) is represented by the asterisks: ***$P<0.01$; ****$P<0.001$.

adrenals of normotensive rats and the heart and spleen (adrenals not examined) of hypertensive rats without a concomitant decrease in the content of this amine in the whole brain (Figure 5). Dopamine and 5-hydroxytryptamine (5-HT) concentrations were similarly unaffected in whole brain, but in the heart (normotensive rats) some decrease (–27%, $P<0.01$) in 5-HT content was observed.

The decrease in noradrenaline in rat heart occurred over the same dose-range as the antihypertensive effect and followed a similar time course (Figures 6 and 7, and see also Figure 1). In contrast the small decrease in the heart concentration of 5-HT did not correlate in either a time- or dose-related manner with the antihypertensive effect.

Because BRL 13776 did not alter the monoamine concentrations in whole brain after a single dose, we examined the effect of repeated administration on tissue amines. Rats were dosed daily by the oral route with 30 or 100 mg/kg BRL 13776 or with the suspending agent alone (1% w/v methyl cellulose). The rats were killed 24 h after the last dose and the results are shown in Table 1. Repeated administration of BRL 13776 for 28 days was still without effect on the whole brain concentrations of noradrenaline, dopamine or 5-HT, but the depletion of noradrenaline

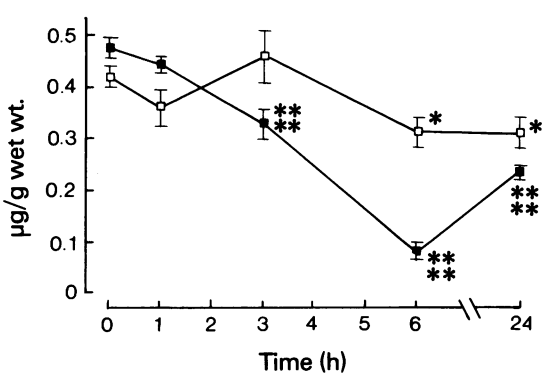

Figure 6 Concentrations of noradrenaline (■) and 5-hydroxytryptamine (□) in the rat heart at various times after an oral dose of 100 mg/kg of BRL 13776. A group of 6 normotensive rats was used at each time. Results are expressed as μg/g wet weight, vertical lines show s.e. mean. The degree of significance of the change (Student's *t* test) as compared to time 0 h is represented by the asterisks: *$P<0.05$; ****$P<0.001$. Administration of vehicle (1% w/v methyl cellulose) alone to a further group of 6 rats produced no temporally-related changes in noradrenaline or 5-hydroxytryptamine (not shown).

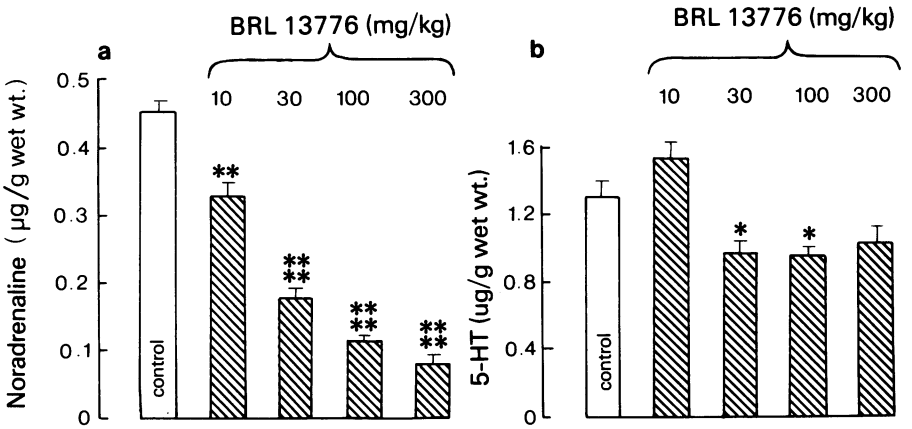

Figure 7 Concentration of (a) noradrenaline and (b) 5-hydroxytryptamine (5-HT) in the rat heart 6 h after various oral doses of BRL 13776. A group of 5 rats was used at each dose level with a further group of 5 rats receiving the suspending agent alone (1% w/v methyl cellulose) serving as the control. Results are expressed as μg/g wet weight, vertical lines show s.e. mean. The degree of significant difference from control (Student's *t* test) is represented by the asterisks: $*P<0.05$; $**P<0.02$; $****P<0.001$.

and 5-HT was still apparent in the heart. Decreases in these monoamines in the latter tissue were found to be dose-related. No behavioural nor adverse toxic symptomology was observed in this repeat-dose study.

Although both single and repeat-dose studies with BRL 13776 failed to show a change in the monoamine content of whole brain, acute experiments on the effect of BRL 13776 on the content of monoamines in discrete areas of the brain revealed that the compound decreased the noradrenaline content of the pons/medullary or hind-brain region of both normotensive and hypertensive rats without a concomitant decrease in the noradrenaline content of the cerebral hemispheres, mid-brain or hypothalamus (Figure 8). The dopamine content of the pons/medulla was similarly reduced in hypertensive rats but was not altered in this region in normotensive animals. However in these normotensive animals the dopamine content of the hypothalamus was reduced, an effect not seen in the hypertensive rats.

In contrast to the effect of BRL 13776 on catecholamines of the pons/medulla and hypothalamus the 5-HT content of these different brain areas was not significantly altered ($P>0.05$) by similar doses of the compound (data not shown).

Because this selectivity of effect of BRL 13776 on hind-brain noradrenaline content appeared to be novel we examined the effect of the classical monoamine depleting agent reserpine and one of its analogues, syrosingopine, on the catecholamine content of the same four areas of the brain. We chose an oral dose of 3 mg/kg of reserpine and 100 mg/kg of syrosingopine since we had found that these were approximately equivalent to 100 mg/kg of BRL 13776 in lowering blood pressure. The reserpine group was examined at 20 h after dosing, and the syrosingopine-treated rats, like the BRL 13776 rats, were killed at 6 hours. Reserpine, in contrast to BRL 13776, produced a fairly generalized decrease in the catecholamine content of the four areas of the brain examined (Figure 9). Interestingly, syrosingopine which is claimed to be a selective peripheral monoamine depleting agent (Orlans, Finger & Brodie, 1960; Leroy & De Schaepdryver, 1961; Pham-Huu-Chanh & De Schaepdryver, 1965) produced a similar decrease in hind-brain noradrenaline content to BRL 13776. Syrosingopine did differ in one respect from BRL 13776 in that it also decreased the dopamine content of the pons/medullary region of these normotensive rats; an effect only seen in hypertensive rats administered BRL 13776.

Because BRL 13776 and syrosingopine had similar actions after single dosing, their effects on brain catecholamines were compared following repeated administration. A twice daily dosing schedule of 100 mg/kg orally of BRL 13776 (chosen because of the compounds shorter duration of action) was compared with a once daily dosing schedule of 100 mg/kg orally of syrosingopine. Rats dosed with syrosingopine on this schedule showed typical reserpine-like side effects, both central and peripheral, such as ptosis, sedation and diarrhoea from day 3 onwards and were in such poor condition that monoamine estimations were undertaken on day 7. In contrast rats on the twice daily dosing schedule of BRL 13776 displayed no ill effects throughout the dosing period and were therefore killed after 14 days dosing.

Table 1 Monoamine concentrations in the heart and/or brain of normotensive rats following 28 daily oral doses of BRL 13776

Daily dose of BRL 13776 (mg/kg)	Monoamine concentration (μg/g) with % of control in parentheses			
	Noradrenaline		Dopamine	5-Hydroxytryptamine
	Brain	Heart	Brain	Brain	Heart
Control (vehicle-dosed)	0.348 ± 0.019	0.577 ± 0.061	0.910 ± 0.035	1.38 ± 0.084	1.17 ± 0.116
30	0.349 ± 0.011 (100%)	0.288 ± 0.089* (50%)	0.970 ± 0.032 (107%)	1.39 ± 0.040 (101%)	0.916 ± 0.092 (78%)
100	0.326 ± 0.007 (94%)	0.157 ± 0.033**** (27%)	0.881 ± 0.030 (97%)	1.37 ± 0.062 (99%)	0.676 ± 0.130* (58%)

A group of 6 rats was used for each dose level of BRL 13776 with a further group of 6 rats receiving the suspending agent alone (1% w/v methyl cellulose solution) serving as the control. All animals were killed 24 h after the last dose. Results are means ± s.e. means and the degree of significance of the change from control (Student's t test) is represented by the asterisks: *$P < 0.05$; ****$P < 0.001$.

With the repeated dosing of BRL 13776 the pons/medullary noradrenaline content was reduced to about the same extent as that seen on single dose administration (Figure 10, compare with Figure 8). However, this repeat-dosing was also associated with a decrease in the hypothalamic and midbrain content of noradrenaline, although the latter was not statistically significant at the 5% level. The repeat-dosing schedule still did not produce any decrease in the noradrenaline content of the cerebral hemispheres.

In contrast to the effect of BRL 13776 on monoamines in the brain, syrosingopine given only once daily for 7 days at 100 mg/kg produced a much more marked and generalized decrease in noradrenaline throughout the brain (Figure 10).

The repeat-dose schedules of both BRL 13776 and syrosingopine caused a marked (approximately 80–90%) reduction in the noradrenaline content of the heart.

Specific tests for behavioural effects

BRL 13776 in single oral doses up to 900 mg/kg did not modify the overt behaviour of mice or rats (apart from the animals being a little excited when touched) as measured in a multi-dimensional observation test (Irwin, 1968). In contrast, oral doses of 9 mg/kg and above of reserpine and 30 mg/kg and above of Δ^8-tetrahydrocannabinol (Δ^8-THC, which is not as active as the Δ^9-isomer) produced depressant effects (e.g. ataxia, depression of spontaneous locomotor activity, hypothermia) in this test. Both reserpine (20 mg/kg orally) and Δ^8-THC (100 mg/kg orally) also caused a significant ($P < 0.05$) prolongation of hexobarbitone sleeping time in mice whereas BRL 13776 (100 mg/kg orally) was again inactive.

BRL 13776 (100 mg/kg orally) was likewise inactive in various other tests for effects on mood and behaviour. Thus the compound did not protect against maximal electroshock or leptazol-induced convulsions and did not antagonize clonidine-induced aggression, in mice. These tests will detect anticonvulsant and/or tranquillizing activity, and in addition it has been shown in our laboratories that the clonidine test is sensitive for tetrahydrocannabinols and like agents. No antidepressant activity for BRL 13776 was suggested as the oral dose of 100 mg/kg did not prevent reserpine hypothermia in mice.

Apart from some increased reactivity, no behavioural nor adverse effects were apparent when oral doses of 30, 100 and 300 mg/kg of BRL 13776 were administered daily for 14 days to mice. The increased reactivity to stimulation was evident on the first few days but subsided as dosing continued. In contrast, daily oral dosing with reserpine (3 mg/kg) or syrosingopine (30, 100 and 300 mg/kg) resulted in sedation accompanied by a hunched posture, ptosis, miosis, diarrhoea and a reduction in body weight and temperature. All mice receiving 100 and 300 mg/kg of

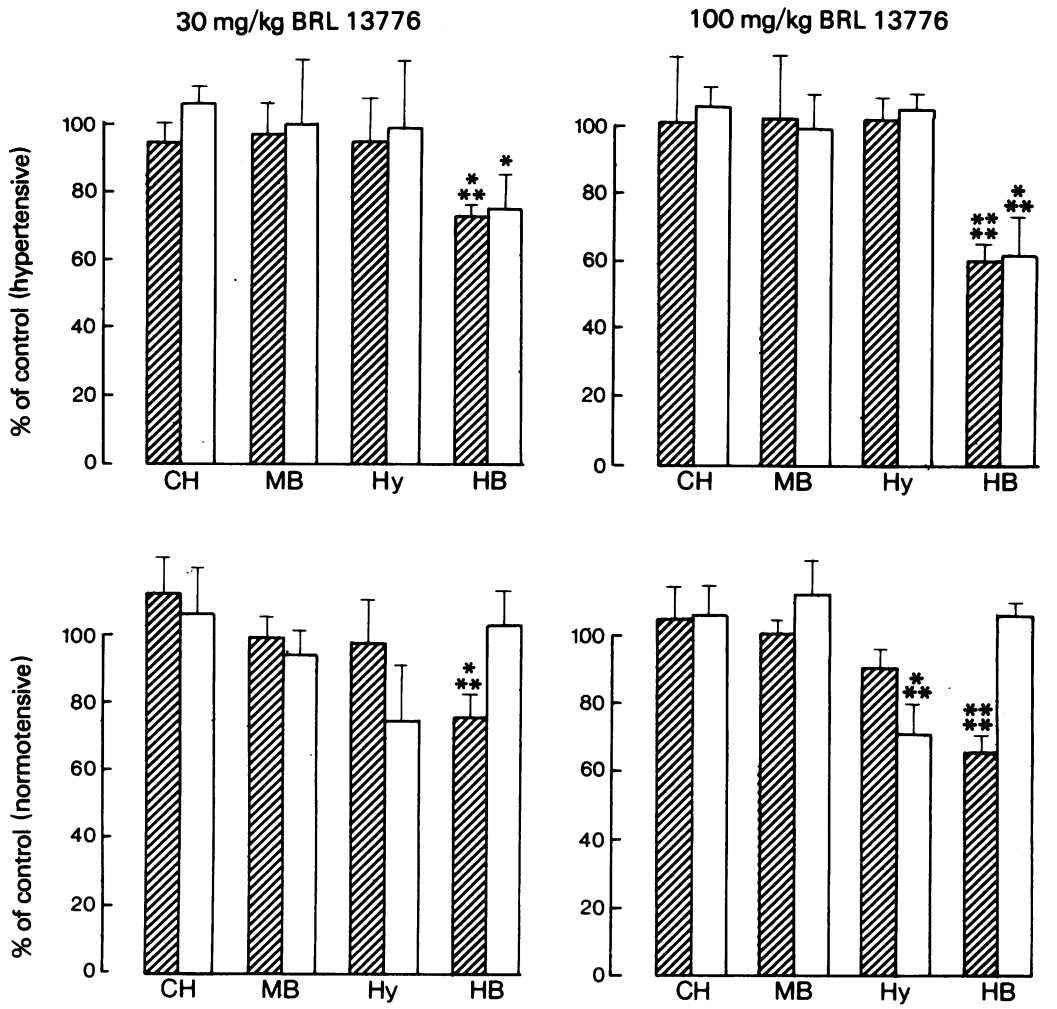

Figure 8 Noradrenaline (hatched columns) and dopamine (open columns) concentrations in the cerebral hemispheres (CH), mid-brain (MB), hypothalamus (Hy) and hind-brain (HB) regions at 6 h following single oral doses of BRL 13776 in hypertensive and normotensive rats. Groups of ten rats were used including control groups which received the suspending agent (1% w/v methyl cellulose) alone. Mean values in BRL 13776-treated groups are expressed as a percentage of control; vertical lines show s.e. mean. The degree of significance of the change from control (Student's *t* test) is represented by the asterisks: $*P<0.05$; $***P<0.01$; $****P<0.001$.

syrosingopine were dead by day 11 but the 30 mg/kg group survived the 14 days dosing. One mouse (out of 5) in the reserpine group died (at day 11) during the treatment period.

Discussion

BRL 13776 is an effective antihypertensive agent being capable of lowering blood pressure in both DOCA/NaCl-treated hypertensive rats and renal hypertensive cats (cellophane perinephritis model). Some fall in blood pressure also occurs in normotensive rats but this is less than that seen in hypertensive animals. No definite tolerance to the blood pressure lowering effect is evident when the compound is administered repeatedly to rats and cats although some day to day variation in the response is apparent. This variation may be due to differences in gastro-intestinal absorption as we have shown in our

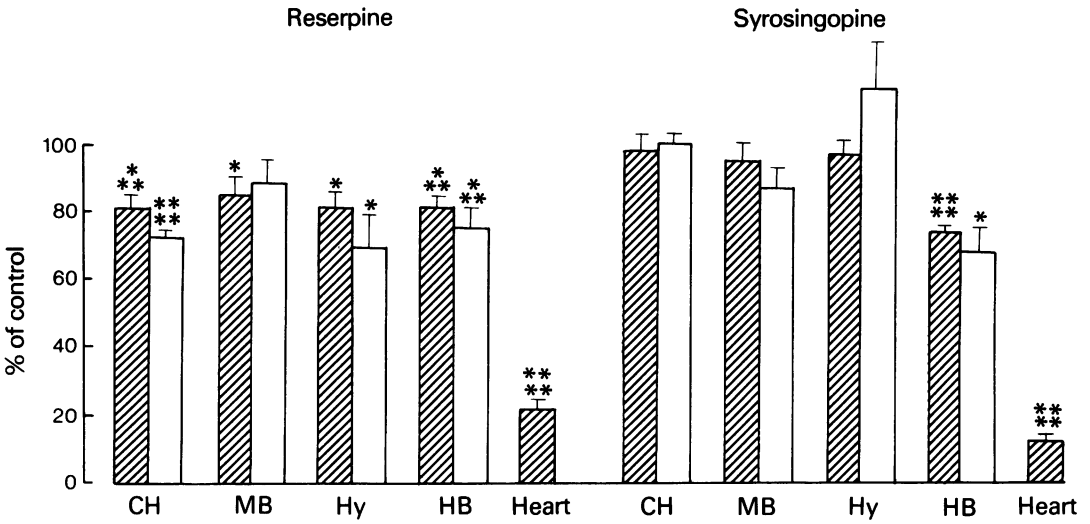

Figure 9 Noradrenaline (hatched columns) and dopamine (open columns) concentrations in the cerebral hemispheres (CH), mid-brain (MB), hypothalamus (Hy), hind-brain (HB) and heart at 20 h following an oral dose of 3 mg/kg of reserpine and 6 h following an oral dose of 100 mg/kg of syrosingopine, in normotensive rats. Mean values are expressed as a percentage of control, vertical lines show s.e. mean. Groups of six rats were used with the controls receiving the suspending agent (1% w/v methyl cellulose) alone. The degree of significant change from control (Student's t test) is represented by the asterisks: $*P<0.05$; $***P<0.01$; $****P<0.001$.

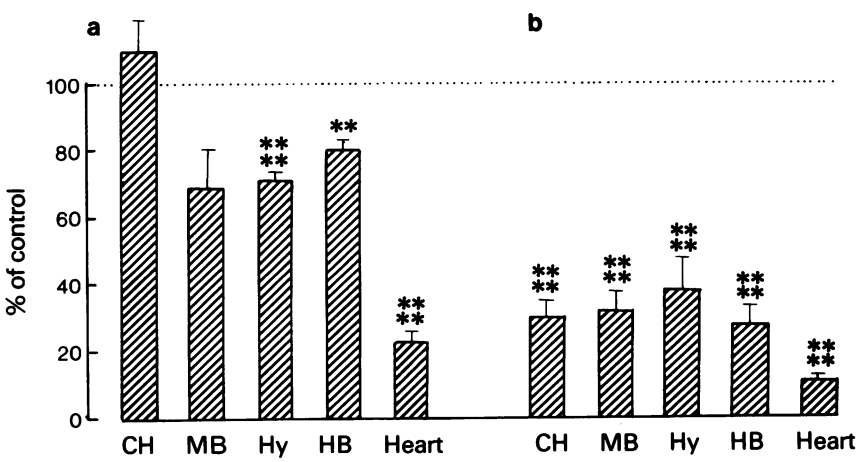

Figure 10 Noradrenaline concentrations in the cerebral hemispheres (CH), mid-brain (MB), hypothalamus (Hy), hind-brain (HB) and heart after oral dosing with 100 mg/kg of BRL 13776 twice daily for 14 days (a) or 100 mg/kg of syrosingopine once daily for 7 days (b). Mean values are expressed as a percentage of control, vertical lines show s.e. mean. Groups of six normotensive rats were used with the control groups receiving the suspending agent (1% w/v methyl cellulose) alone. The degree of significant change from control (Student's t test) is represented by the asterisks: $*P<0.05$; $**P<0.02$; $****P<0.001$.

laboratories that BRL 13776 is not particularly well absorbed in animals by the oral route of administration.

The blood pressure lowering effect of BRL 13776 in rats is associated with a reduction of tissue catecholamines. This was apparent in all the peripheral tissues examined but in the brain was restricted to certain areas, notably the hind-brain on single dosing and the hind-brain, hypothalamus and mid-brain (i.e. cerebral hemispheres not affected) on repeated dosing. The classical monoamine-depleting agent reserpine did not display such selectivity in the brain and although we found syrosingopine (a reserpine analogue) to affect only the hind-brain on single dosing, it produced a marked depletion in all brain areas on repeated dosing. BRL 13776 therefore shows some differences from both these agents and these could possibly explain the absence of behavioural effects in animals. The lack of effect on 5-HT levels throughout the brain may also be relevant in this respect.

It seems likely in the rat that the effect of BRL 13776 on tissue catecholamines, particularly noradrenaline, is responsible for the antihypertensive response. Reduction of noradrenaline in the heart and hind-brain is dose-related and occurs over the same dose-range as that required for the depression of blood pressure. Moreover, similar time courses are obtained for the cardiac depletion of noradrenaline and the antihypertensive effect. Additional evidence for the involvement of noradrenaline depletion is provided by findings (unpublished) in our laboratories that BRL 13776 is devoid of α- and β-adrenoceptor blocking, ganglion blocking, adrenergic neurone blocking, direct vasodilator and diuretic properties.

Provided catecholamine depletion is involved in the blood pressure reduction to BRL 13776 then the question arises as to which effect, central or peripheral, is the more important. It is logical to suppose that the peripheral depletion may play a role but on the other hand we found no evidence of general impairment of peripheral adrenergic function. For example, there was no ptosis in rats or relaxation of the nictitating membrane or postural hypotension in cats. Also, despite noradrenaline depletion in the heart of rats, there was no reduction in heart rate. Consequently, it could be that the action of BRL 13776 at specific brain sites, e.g. hind-brain, is important and results in a reduction of sympathetic tone to the vasculature. Catecholamines in the hind-brain and also the hypothalamus have been implicated in blood pressure control (see Nakamura, Gerold & Thoenen (1971) and de Champlain & van Ameringen (1975) for involvement of catecholamines at these sites in DOCA/NaCl-hypertension in the rat; see also reviews by Chalmers (1975) and Haeusler (1975)).

BRL 13776 arose from a series of compounds derived from THC. It possesses the 2,2-dimethyl-7-*n*-pentyl-chroman-5-ol structure of THC but in place of the fused carbocyclic ring at positions 3 and 4, it has a 1-(2-naphthylmethyl)-1,2,5,6-tetrahydropyrid-4-yl substituent at position 4 (see structure in Introduction). Whether or not the structural relation of BRL 13776 to THC is of any importance, with regard to its pharmacological effects, is uncertain at the present time. Δ^9-THC has been reported to lower blood pressure in various forms of hypertension in rats but tolerance to this effect usually develops on repeated dosing (Nahas, Schwartz, Adamec & Manger, 1973; Birmingham, 1973; Williams, Ng, Lamprecht, Roth & Kopin, 1973; Varma & Goldbaum, 1975). Studies on the effects of the tetrahydrocannabinols on catecholamine levels have yielded conflicting results. Holtzman, Lovell, Jaffe & Freedman (1969) reported that the concentration of noradrenaline in whole brain of mice was decreased after low doses but increased after high doses of Δ^9-THC. Decreases in noradrenaline in various tissues of rats were observed by Bensemana & Gascon (1974) but various other workers using rats found that the tetrahydrocannabinols had no effect on whole brain and/or heart levels of catecholamines (Maitre, Staehelin & Bein, 1970; Ho, Taylor, Englert & McIsaac, 1971; Leonard, 1971; Schildkraut & Efron, 1971; Maitre, Baumann & Delini-Stula, 1972; Taylor & Fennessy, 1975) though in some of these studies an increase in the turnover of noradrenaline was apparent. When discrete brain areas of rats were examined, Yagiela, McCarthy & Gibb (1974) reported that Δ^9-THC (50 mg/kg i.p.) caused a short term reduction of brain stem but not hypothalamic noradrenaline, whereas Bracs, Jackson & Chester (1975) found oral doses of 20–60 mg/kg had no effect on catecholamine levels or turnover in various different brain regions.

Thus the tetrahydrocannabinols appear capable of lowering blood pressure in hypertensive animals and also could possibly have some effect on catecholamines. The difference in results with regard to the latter may be due to variation in the doses used, time of examination after dosing, environmental and possibly other conditions employed. However, it is conceivable that the chemical manipulation to produce BRL 13776 has resulted in an intensification of a certain action on catecholamines (with a consequent effect on blood pressure) whilst at the same time some other properties inherent in the THC molecule (e.g. behavioural effects) have been lost. In this connection it is interesting to note that Graham, Lewis & Li (1974) have shown that Δ^9-THC can be taken up by the sympathetic nerves of the isolated vas deferens of the rat and released upon stimulation. At the same time noradrenaline release was reduced. This suggests that Δ^9-THC can displace noradrenaline in adrenergic neurones and could act as a false transmitter substance. The reduction in noradrenaline we have

observed in various peripheral tissues, and even certain brain regions, with BRL 13776 could be a consequence of a similar action and we intend to examine this possibility in further work.

In conclusion, BRL 13776 is a novel antihypertensive agent displaying interesting noradrenaline-depleting properties with no apparent behavioural effects.

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