J. Pharm. Pharmac., 1972, **24**, 690–695 *Received January* 17, 1972

Effects of L-dopa and inhibitors of decarboxylase and monoamine oxidase on brain noradrenaline levels and blood pressure in spontaneously hypertensive rats

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Acute administration of L-dopa (200 mg/kg) resulted in a significant decrease in blood pressure of the spontaneously hypertensive (SH) rat. Simultaneous treatment with a peripheral decarboxylase inhibitor (3,4-dihydroxyphenyl-2-hydrazino-2-methylpropionic acid; MK 485) potentiated this effect while treatment with a decarboxylase inhibitor that penetrates the central nervous system (4-bromo-3-hydroxybenzyloxyamine; NSD 1055) tended to block the effect of L-dopa. In general, only treatments that increased brainstem noradrenaline were effective in reducing blood pressure. In young SH rats chronic treatment with L-dopa and a peripheral decarboxylase inhibitor significantly retarded the development of hypertension over four weeks. Treatment of SH rats with 3 daily doses (100 mg/kg each) of *p*-chlorophenylalanine resulted in a complete loss of 5-hydroxytryptamine and a slight reduction of noradrenaline in the brainstem. The blood pressure of these animals was slightly increased over that of the control SH rats. Treatment of several groups of SH rats with a monoamine oxidase inhibitor (pargyline) in various combinations with L-dopa and a peripheral decarboxylase inhibitor resulted in a wide range of noradrenaline levels in the brainstem. There appeared to be a highly significant inverse correlation between brainstem noradrenaline and blood pressure. The findings are consistent with central noradrenergic modulation of blood pressure in the rat.

In contrast to the well established mediation by noradrenaline of sympathetic vasoconstrictor activity peripherally, a role of brain noradrenaline in blood pressure control has not been proved. On the evidence of its decreased synthesis and content in the brainstem and hypothalamus of genetic (spontaneous) hypertensive rats (Yamori, Lovenberg & Sjoerdsma, 1970), we have speculated that catecholamines in the brain may participate in a central depressor system. Support for such a relation include evidence of a decreased turnover rate of noradrenaline in the brainstem of rats with doca-salt hypertension (Nakamura, Gerold & Thoenen, 1971), and the findings that

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acute blood pressure lowering effects of 3,4-L-dihydroxyphenylalanine (L-dopa) and its α-methyl-analogue (methyldopa), require the decarboxylation of these compounds to catecholamines in the central nervous system (Henning, 1969; Henning & Rubenson, 1970, 1971). Since the hypotensive effects of both these compounds are blocked by inhibitors of dopamine-β-hydroxylase, it is suggested that both act by activating central noradrenergic mechanisms.

We now report the effect of several drug regimens used to alter catecholamine and 5-hydroxytryptamine levels in the brain on blood pressure of the spontaneously hypertensive (SH) rat.

MATERIALS AND METHODS

Male SH rats of the National Institutes of Health (NIH) F_{19}–F_{23} generations and inbred Wistar/NIH rats were used (Louis, Krauss & others, 1970). The hypertensive animals were age and weight matched with their controls. Drugs used and their sources were as follows: L-dopa, Calbiochem, Inc.; DL- and L-(3,4-dihydroxyphenyl)-2-hydrazino-2-methylpropionic acid, MK 485 and MK 486, Merck and Co.; 4-bromo-3-hydroxybenzyloxyamine (NSD 1055), Smith and Nephew, Ltd.; and DL *p*-chlorophenylalanine, Aldrich Chemical Co.

Systolic blood pressure was measured in unanaesthetized rats by a tail plethysmographic method (Okamoto & Aoki, 1963) at least three times before and repeatedly after drug administration. Protocols for various experiments are given in "Results".

In experiments where brainstem noradrenaline was measured, animals were decapitated and tissues rapidly frozen on dry ice. Noradrenaline was assayed according to De Champlain, Krakoff & Axelrod (1967). Although dopa administration markedly increases dopamine and dopa levels in the brain, control experiments indicated that these catechol compounds did not influence noradrenaline analysis significantly. 5-Hydroxytryptamine was extracted according to Bogdanski, Pletscher & others (1956) and assayed by the *o*-phthaldialdehyde derivative method (Maickel, Cox & others, 1968). Student's *t*-test was used for statistical analysis of the data.

RESULTS

Studies with L-*dopa.* Three types of studies were made to ascertain the effects of increased brain levels of catecholamines after administration of L-dopa on blood pressure: 1) acute administration of L-dopa, with and without inhibition of its decarboxylation; 2) chronic administration of L-dopa along with a peripheral decarboxylase inhibitor; and 3) chronic treatment with a monoamine oxidase inhibitor alone or together with L-dopa and a peripheral decarboxylase inhibitor.

Intraperitoneal injection of L-dopa (200 mg/kg) in SH rats produced a depressor effect lasting several hours, the maximal reduction being about 50 mm Hg (Fig. 1A). Treatment with a peripheral decarboxylase inhibitor (MK 485, 4 times 100 mg/kg, i.p. at 2 h intervals) before and after L-dopa appeared to prolong slightly the depressor effect of L-dopa. Pretreatment with a decarboxylase inhibitor which also penetrates the brain (NSD 1055, 100 mg/kg, i.p.) diminished the depressor effect (Fig. 1A). We have observed similar results using the antihypertensive drug, α-methyldopa. Since an enhancement of the effect of L-dopa by MK 485 was not clearly demonstrated in the above studies, and because of an indication that in parkinsonism in man a peripheral decarboxylase inhibitor diminished the therapeutic dose of L-dopa required for central actions (Cotzias, Papavasliou & Gellene, 1969), further studies on this combination administered *via* the oral route were made. A number of single and combined doses

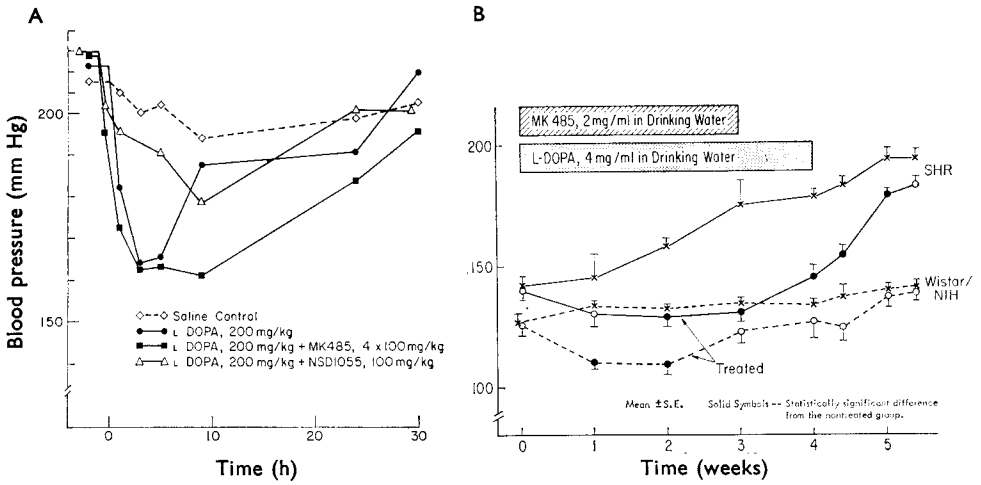

FIG. 1. A. Effects of intraperitoneal injection of 200 mg/kg L-dopa alone, and with two types of decarboxylase inhibitors, on systolic blood pressure of SH rats. Six-month-old animals with severe hypertension were used; each point is an average of blood pressure measurements in groups of 5 to 7 animals. L-dopa was administered at 0 time. NSD 1055 (both a central and peripheral decarboxylase inhibitor) was administered in a single dose of 100 mg/kg 1 h before L-dopa, while the peripheral decarboxylase inhibitor, MK 485, was administered in 4 doses of 100 mg/kg every 2 h, beginning 1 h before injection of L-dopa.

B. Effect of chronic oral administration of L-dopa and MK 485 on systolic blood pressure of hypertensive and normotensive rats. Groups of 4–5 animals, 32 days of age, were studied and each point is a mean value with brackets indicating s.e. The following symbols represent various groups: ×——× Control SHR, ×---× Control Wistar, ○——○ Treated SHR, ○---○ Treated Wistar. ● Statistically significant difference from non-treated group.

were tried with the most effective being 100 mg/kg L-dopa and 150 mg/kg MK 486 [(−)-isomer]. Neither L-dopa nor MK 486 alone had a significant effect on blood pressure or brainstem noradrenaline as measured 4 h after drug administration. Following simultaneous administration of the drugs, a significant decrease of blood pressure (−30 mm) was observed at both 2 h and 4 h, just before death. The combined treatment resulted in a marked elevation (about 50%) of brainstem noradrenaline levels while each drug alone had no effect.

When L-dopa and MK 485 were administered in the drinking water for up to 4 weeks, there appeared to be a retardation of the development of hypertension in the SH rats and a reduction in the blood pressure of normotensive animals (Fig. 1B). The blood pressure of SH rats rose slowly to that of the untreated SH rat after treatment stopped. This drug regimen was not well tolerated since the weight of treated animals by the fourth week was about 60% that of untreated animals.

Additional studies were made to confirm that these drugs administered in the drinking water were effective also in elevating brain noradrenaline. The data in Table 1 show that after 1 week of oral treatment with MK 486 and L-dopa there was a significant elevation of brain noradrenaline as well as a decrease in blood pressure. L-Dopa or MK 486 alone did not affect blood pressure. L-Dopa alone failed to alter brain noradrenaline, and treatment with MK 486, which is considered to be solely a peripheral decarboxylase inhibitor (Porter, Watson & others, 1962; Bartholini, Bales & others, 1967), resulted in a significant decrease in brainstem noradrenaline suggesting some central penetration of this drug.

It is known that monoamine oxidase inhibitors cause an elevation of amine levels both centrally and peripherally with a concomitant reduction in blood pressure,

Table 1. *Systolic blood pressure and brainstem noradrenaline level in SH rats after one week of treatment with* L-*dopa (4 mg/ml) and MK 486 (2 mg/ml) in drinking water.*

Treatment	Weight (g)	Blood pressure (mm Hg)	Brainstem noradrenaline (ng/g)
Untreated (7)	121 ± 3	165 ± 2	621 ± 14
MK 486 (4)	107 ± 6	172 ± 6	472 ± 49+
L-Dopa (4)	114 ± 11	161 ± 6	645 ± 20
L-Dopa + MK 486 (4)	93 ± 8+	148 ± 5*	744 ± 33+

Values are means (± s.e.). Significant difference from the untreated SH rats = $^{+}P < 0{\cdot}01$; $^{*}P < 0{\cdot}001$. Number of animals are indicated in parentheses. Animals (8 weeks of age) were killed one week after starting treatment.

although a causal relation between these two phenomena has not been established. It therefore seemed worthwhile to attempt to relate changes in blood pressure with changes in brainstem noradrenaline in animals treated with a monoamine oxidase inhibitor. In the current experiments seven groups of five young SH rats (32 day) were given pargyline hydrochloride for three weeks in drinking water containing 0, 0·5, or 1·5 mg/ml of drug. Some animals were given additionally either L-dopa or L-dopa with MK 485 to augment the elevation of brain noradrenaline. Blood pressures were measured routinely and some of the animals in each group were killed at one and ten days after the treatment. This protocol was followed in an attempt to obtain a wider range of brainstem noradrenaline levels than could be obtained with pargyline alone. Following these therapies an inverse relation between brainstem noradrenaline level and blood pressure was observed in these animals (Fig. 2).

Effects of p-*chlorophenylalanine.* It has been reported that combined dosage of L-dopa and a peripheral decarboxylase inhibitor markedly deplete brain 5-hydroxytryptamine (5-HT) (Bartholini, Da Prada & Pletscher, 1968; Butcher & Engel, 1969).

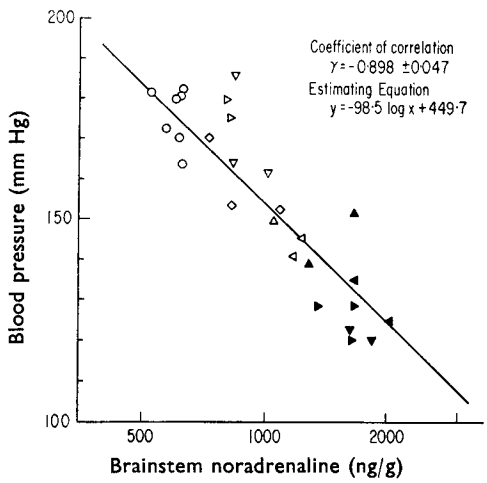

FIG. 2. Correlation between blood pressure and brainstem noradrenaline level in MAO inhibited SH rats. The circles represent individual control SH rats. The closed angular symbols represent results from SH rats treated with pargyline with or without L-dopa or MK 485 one day after treatment. The open angular symbols are data from similarly treated SH rats 10 days after the above treatment. The drugs were administered to the animals for 3 weeks in their drinking water in the following levels when present: pargyline, 0·5 or 1·5 mg/ml; L-dopa, 2 mg/ml, and MK 485, 1 mg/ml.

To ascertain whether changes in brain 5-HT levels were a factor in the present findings, the effect of the 5-HT depleting agent, *p*-chlorophenylalanine, was examined in a group of SH rats. Daily injection of 100 mg/kg, i.p. for 3 days resulted in a modest elevation of blood pressure and a decrease of 5-HT to negligible levels (Table 2). A slight decrease in brainstem noradrenaline was observed. These findings appear to eliminate 5-HT depletion as a component in the blood pressure lowering effects observed.

Table 2. *Effect of parachlorophenylalanine (PCPA, 100 mg/kg) administered i.p. once daily for 3 days on blood pressure and brainstem 5-HT and noradrenaline levels of SH rats.*

Treatment	Blood pressure mm Hg	Brainstem Noradrenaline ng/g	Brainstem 5-HT ng/g
Vehicle	162 ± 2 (14)	761 ± 12 (7)	644 ± 22 (7)
PCPA	173 ± 3 (14)	647 ± 17 (7)	Undetectable (7)

SH rats 7 weeks of age were used. Number of observations are in parentheses, values are means ± s.e. All observed differences were statistically different, P at least $<0{\cdot}01$. Drug was administered in 0·9% saline in 1% Tween 80.

DISCUSSION

To study possible central noradrenergic pathways in blood pressure regulation, it is desirable to manipulate levels of noradrenaline in brain without affecting peripheral levels. This is possible by pretreating animals with an inhibitor of aromatic L-amino-acid decarboxylase that does not penetrate the brain (hydrazino-α-methyldopa) (Porter, Watson & others, 1962; Bartholini & others, 1967) and then giving a large dose of L-dopa. This precursor of noradrenaline enters the brain and bypasses the putative rate limiting step, tyrosine hydroxylase, in noradrenaline biosynthesis. By selection of the appropriate doses of these compounds it was possible to obtain an elevation of brainstem noradrenaline (up to 50%). Using the genetic hypertensive rats a significant blood pressure reduction occurs when such treatment results in an elevation of brain noradrenaline. This occurs in both acutely and chronically treated animals. These findings are consistent with those of Henning & Rubenson (1971) who found L-dopa to be hypotensive in normal rats only if its peripheral decarboxylation was blocked. Recently it was also reported that in another model of hypertension (doca-NaCl) there appears to be a decrease in brainstem noradrenaline turnover (Nakamura & others, 1971). Treatments which cause an elevation of brainstem noradrenaline result in a somewhat more marked depressor response in SH rats than in normotensive rats. This is of interest since it was observed previously that SH rats had a lower brainstem noradrenaline than normotensive control animals of a Wistar strain and it was suggested that low brainstem concentration might be one of the factors in the pathogenesis of hypertension (Yamori & others, 1970).

The question of whether the blood pressure responses are directly related only to changes in brainstem noradrenaline is not completely resolved. The *p*-chlorophenylalanine experiment appears to eliminate changes in 5-HT content from consideration and in general only those treatment conditions which resulted in elevated brain noradrenaline were effective in reducing blood pressure. Work of Henning & Rubenson (1970, 1971) tentatively eliminated dopamine as the effective component, since simultaneous administration of a dopamine-β-hydroxylase inhibitor blocked the hypotensive

effect of L-dopa. Although the experiment using pargyline (Fig. 2) appears convincing, it must be remembered that all amines which are metabolized by monoamine oxidase become elevated during pargyline treatment and a similar relation between blood pressure and other amines could probably be construed.

Supportive evidence for central noradrenergic stimulation as a basis for blood pressure reduction comes from studies on the powerful antihypertensive drug, clonidine*. This compound, a peripheral α-adrenoceptor agonist, has recently been shown also to activate central noradrenaline receptors as well (Anden, Corrodi & others, 1970). Contrariwise, if central noradrenergic control of blood pressure exists, then anatomic ablation of specific noradrenergic fibres in the brain should result in the elevation of blood pressure in normal animals. This laboratory recently reported (Yamori, Yamabe & others, 1972) that intraventricular administration of 6-hydroxydopamine, a specific depletor of nonadrenaline and neuronal toxin, did not result in an elevation of blood pressure in normotensive or hypertensive rats. We wish to record, however, that in one of three experiments there appeared to be a significant increase in blood pressure of normotensive animals receiving 100 μg of 6-hydroxydopamine intraventricularly. The inconsistency of these results may be due to an unknown degree of stress applied to the responding animals during blood pressure measurement or to the fact that a precise placement of drug may be required to produce an effect. Recently, Williams Eichelman & Ng (to be published) reported the development of a hypertensive response after shock-induced fighting in normal rats that had been treated with 6-hydroxydopamine intracisternally; control shocked animals receiving saline intracisternally exhibit a hypotensive response. These findings are further evidence for a central noradrenergic depressor system and together with the current work tend to support the speculation that there are central noradrenergic neurons which exert a modulating effect on blood pressure.

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* 2-(2,6-Dichlorphenylamino)-2-imidazoline hydrochloride.