Further studies on the mechanism of the central hypotensive effect of L-dopa, DL-*m*-tyrosine and L-α-methyldopa

Injection of L-dopa and DL-*m*-tyrosine after inhibition of peripheral decarboxylase produce a hypotensive response via their catabolites in the central nervous system (Henning & Rubenson, 1970a, 1970b; Rubenson, 1971). There is evidence that this action is brought about through activation of inhibitory mechanisms possibly of noradrenergic nature. With DL-*m*-tyrosine the hypotensive effect appeared to be elicited via displacement of endogenous amines, presumably noradrenaline (Rubenson, 1971).

The structurally related amino-acid L-α-methyldopa (α-MD) lowers arterial blood pressure in animals and man through mechanisms which are not yet completely understood (for review see Henning, 1969) but there is evidence that decarboxylation of α-MD within the central nervous system is necessary. Henning & Rubenson (1971) recently found that pretreatment with a dopamine-β-hydroxylase inhibitor (FLA-63) blocked the formation of α-methylnoradrenaline, further, the hypotensive response of α-MD was abolished.

The present investigation was designed to clarify further the role of direct and indirect mechanisms in the hypotensive effects seen after L-dopa, DL-*m*-tyrosine and α-MD.

Male Sprague-Dawley rats, 200–300 g, were used. Mean arterial blood pressure was recorded in conscious unrestrained animals through indwelling arterial catheters connected to Statham P 23 DC pressure transducers writing on a Grass polygraph (Henning, 1969). The blood pressure values are averages of recordings for 10 min periods immediately before the administration of the drugs, except the values of the maximal hypotensive response seen after L-dopa and DL-*m*-tyrosine which are the average of recordings 15–20 min after injections of L-dopa and 5–10 min after injection of DL-*m*-tyrosine.

Noradrenaline was determined by the method of Bertler, Carlsson & Rosengren (1958), dopamine as described by Carlsson & Lindqvist (1962). Each analysis was generally performed on pooled organs from two animals of similar weight.

The drugs used were L-3,4-dihydroxyphenylalanine (L-dopa), DL-3-hydroxyphenylalanine (DL-*m*-tyrosine), DL-α-methyl-3-hydroxyphenylalanine (DL-α-methyl-*m*-tyrosine, α-MMT), DL-α-methyl-*p*-tyrosine-methylester (H 44/68), L-α-hydrazino-α-methyl-(3,4-dihydroxyphenylpropionic acid (MK 486). All drugs were administered intraperitoneally. For doses and time intervals see Fig. 1. In blood pressure experiments, tests of significance were by analysis of variance, with two independent criteria of classification, followed by *t*-test.

Injection of MK 486 (50 mg/kg) did not influence the mean arterial blood pressure significantly within 30 min ($P > 0{\cdot}1$, see Fig. 1A). Thirty min after pretreatment with MK 486, injection of L-dopa (200 mg/kg) resulted in a significant lowering of blood pressure, the maximum effect being reached after 15–20 min ($P < 0{\cdot}001$; $n = 4$). Thirty min after the dopa injection the animals were given another injection of MK 486 (50 mg/kg) and 30 min later, when blood pressure had returned to the baseline, another injection of L-dopa (200 mg/kg) had no significant influence on the blood pressure (Fig. 1A). After both dopa injections the animals showed an aggressive behaviour and increased spontaneous motility with a maximum at about 30 min.

Responses to DL-*m*-tyrosine (400 mg/kg) given under the same conditions closely followed those for L-dopa (Fig. 1A), the blood pressure fall 5–7 min after the first

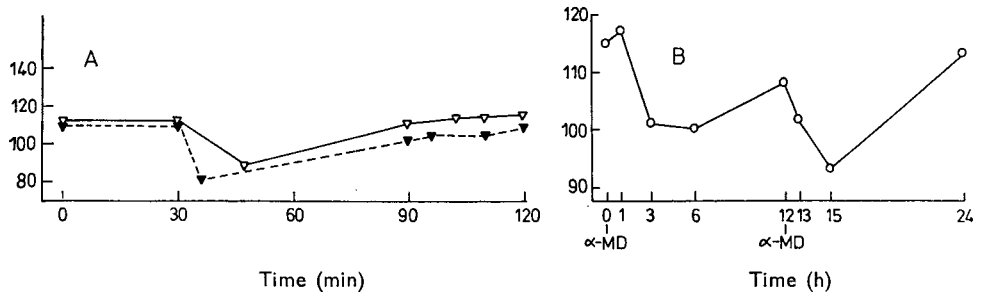

FIG. 1. Mean arterial blood pressure in conscious normotensive rats after i.p. injection of drugs as indicated. The blood pressure values represent averages of recordings during ten-minute periods before and after the drugs indicated except the values immediately after L-dopa and DL-*m*-tyrosine which represent the average of the recording 15–20 min after dopa and 5–7 min after *m*-tyrosine; s.e. were calculated by analysis of variance. Ordinate: mean arterial blood pressure (mm Hg).

A. Injections of MK 486 (50 mg/kg) at 0 and 60 min and of L-dopa (200 mg/kg) or DL-*m*-tyrosine (400 mg/kg) at 30 and 90 min: s.e. for L-dopa experiments = 3·59, 8 exp. (open symbols) and for DL-*m*-tyrosine experiments = 2·74, 6 exp. (solid symbols).

B. Injections of L-α-methyldopa (400 mg/kg) at 0 and at 12 h: s.e. = 3·73, 8 exp.

injection being significant at $P < 0{\cdot}001$ (n = 4). Both injections increased spontaneous motility.

α-MD (400 mg/kg) lowered mean arterial blood pressure significantly after 3 and 6 h ($P < 0{\cdot}001$; n = 8). Another dose given 12 h later caused a significant lowering of mean arterial blood pressure after 3 h which was not significantly different ($P > 0{\cdot}1$; *t*-test, process of pairing) from that seen 3 h after the first injection (Fig.1B).

The effect of L-dopa (200 mg/kg) on blood pressure was studied after pretreatment with α-MMT (three doses of 400 + 400 + 200 mg/kg, 27, 15 and 3 h before L-dopa) and the tyrosine hydroxylase inhibitor H 44/68 (250 mg/kg, 60 min before L-dopa) and MK 486 (100 mg/kg, 30 min before L-dopa). The α-MMT pretreatment has no significant influence on mean arterial blood pressure (Rubenson, 1971). No significant difference existed between the blood pressure values recorded (between 100–120 mm Hg), i.e., the hypotensive action of L-dopa was blocked ($P > 0{\cdot}1$).

In animals pretreated with α-MMT + H 44/68 + MK 486 as in the blood pressure experiments, brain dopamine and noradrenaline were markedly lowered [the mean values, μg/g ± s.e., with no pretreatment were: dopamine 0·69 ± 0·055 (n = 3), noradrenaline 0·38 ± 0·055 (n = 3); after treatment with α-MMT + H44/68 + MK486 these were: 0·13 ± 0·023 (n = 2), 0·01 ± 0·003 (n = 2)]. To test the possible influence of a dopa decarboxylase inhibitory effect of α-MMT (Hess, Connamacher & others, 1961; Porter, Totaro & Leiby, 1961) the accumulation of brain dopamine and noradrenaline after injection of L-dopa (200 mg/kg) was studied after α-MMT + H 44/68 + MK 486 as used in the blood pressure experiments. The values (μg/g) [dopamine 3·21 ± 0·175 (n = 3), noradrenaline 0·12 ± 0·005 (n = 3)] were compared with those obtained after MK 486 + L-dopa only [dopamine 2·90 ± 0·114 (n = 3), noradrenaline 0·36 ± 0·006 (n = 3)]. There was no decrease in formation of dopamine ($P > 0{\cdot}1$).

The rapid hypotensive response to injections of L-dopa and *m*-tyrosine after peripheral dopa decarboxylase inhibition agrees with previous findings (Henning & Rubenson, 1970a, 1970b; Rubenson, 1971). But the lack of effect of the second injection of dopa or-*m*-tyrosine might be due to a decreased availability of central monoamines displaced after the first injection of the amino-acids.

Further, the hypotensive effect of L-dopa was abolished after pretreatment with

α-MMT in combination with tyrosine hydroxylase inhibition. Similar results have been obtained with *m*-tyrosine (Rubenson, 1971). The present study has revealed that the pretreatment with α-MMT and inhibition of tyrosine hydroxylase caused a marked depletion of brain dopamine and noradrenaline. The dopa decarboxylase inhibition by α-MMT was of minor importance. Taken together these findings make it probable that endogenous stores of dopamine and noradrenaline are of great importance for the mechanism by which the hypotensive effect of L-dopa and DL-*m*-tyrosine is elicited.

Henning & Rubenson (1971) reported the hypotensive effect of α-MD to be unchanged after a combined pretreatment with α-MMT + H 44/68 + MK 486 similar to that used in the present study. However, they found the hypotensive effect was abolished after a dopamine-β-hydroxylase inhibitor. Their experiments point to the importance of a direct action of the α-MD catabolites, presumably α-methyl-noradrenaline. This view is compatible with the present results that α-MD produces the same hypotensive response after a second injection (see Fig. 1b).

In conclusion, L-dopa and DL-*m*-tyrosine cause a rapid lowering of blood pressure after dopa decarboxylase inhibition which can be blocked by central catecholamine depletion and is abolished after a second injection. On the other hand the slower developing hypotensive effect of L-α-MD remains under these conditions. L-Dopa and DL-*m*-tyrosine are rapidly decarboxylated, whereas the accumulation of α-methylated amines from α-methyldopa has a slower time course and results in a more sustained depletion of endogenous amines (for ref. see review of Muscholl, 1966).

This study was supported by the Swedish Medical Research Council (B71–14X–155–07C) and by grants from the Faculty of Medicine, University of Göteborg. For generous gift of MK 486 I am obliged to Merck Sharp & Dohme International. For skilful technical assistance I am indebted to Miss Anna Jansson and Miss Ann-Sofie Järnström.

Department of Pharmacology,
University of Göteborg,
Göteborg, Sweden.

ALLAN RUBENSON

November 9, 1970

REFERENCES

BERTLER, Å., CARLSSON, A. & ROSENGREN, E. (1958). *Acta physiol. scand.*, **44**, 273–292.
CARLSSON, A. & LINDQVIST, M. (1962). *Ibid.*, **54**, 87–94.
HENNING, M. (1969). *Ibid.*, Suppl. 322.
HENNING, M. & RUBENSON, A. (1970a). *J. Pharm. Pharmac.*, **22**, 241–243.
HENNING, M. & RUBENSON, A. (1970b). *Ibid.*, **22**, 553–560.
HENNING, M. & RUBENSON, A. (1971). *Ibid.*, **23**. In the press.
HESS, S. M., CONNAMACHER, R. H., OSAKI, M. & UDENFRIEND, S. (1961). *J. Pharmac. exp. Ther.*, **134**, 129–138.
MUSCHOLL, E. (1966). *A. Rev. Pharmac.*, **6**, 107–128.
PORTER, C. P., TOTARO, J. A. & LEIBY, C. M. (1961). *J. Pharmac. exp. Ther.*, **134**, 139–145.
RUBENSON, A. (1971). *J. Pharm. Pharmac.*, **23**. In the press.