J. Pharm. Pharmac., 1972, **24**, 289–294 *Received November* 19, 1971

Comparative investigation of inhibitors of extracerebral dopa decarboxylase in man and rats

I. KURUMA*, G. BARTHOLINI, R. TISSOT** AND A. PLETSCHER

*Research Department, F. Hoffmann-La Roche & Co. Ltd, Basel, Switzerland; * Nippon Roche Research Centre, Kamakura, Japan ;** Psychiatric Clinic, University of Geneva, Geneva, Switzerland*

The decarboxylase inhibitor Ro 4-4602 [N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl)hydrazine] in doses of 3·4–34·0 μmol/kg (1–10 mg/kg) progressively enhances the increase of dopa and 3-*O*-methyl-dopa and diminishes the rise of phenolcarboxylic acids in human plasma induced by administration of L-dopa. A dose of 3·4 μmol/kg Ro 4-4602 is approximately equipotent to 17·0 μmol/kg MK 485 [β-(3,4-dihydroxyphenyl)-α-hydrazino-α-methyl-DL-propionic acid]. Ro 4-4602, combined with 2 mg/kg L-dopa, causes a higher increase of catecholamines than 20 mg/kg L-dopa alone in the striatum and probably in the hypothalamus of rat brain, whereas in the other brain areas the amine rise is equal after both treatments. MK 485 in doses equimolar to Ro 4-4602 has a less marked effect in all brain areas. Either inhibitor (10·2 μmol/kg) combined with 2 mg/kg L-dopa causes markedly less increase of catecholamines than 20 mg/kg L-dopa alone in the rat heart. Ro 4-4602, in small single doses, used at present in the treatment of Parkinson's syndrome, markedly inhibits the extracerebral decarboxylation of L-dopa in man and rats and is more potent than MK 485.

The increase of cerebral dopamine induced in animals by L-3,4-dihydroxyphenyl-alanine (L-dopa) is markedly enhanced by inhibitors of decarboxylase of aromatic amino-acids such as Ro 4-4602* and MK 485†. This effect was shown to be due to a preferential inhibition of the decarboxylase in extracerebral tissues as a consequence of poor penetration of the drugs into the brain. Inhibition of the decarboxylation of L-dopa in extracerebral organs including the brain capillaries (Constantinidis, Bartholini & others, 1968) raises the concentration of this amino-acid in the blood plasma and thus increases the supply of L-dopa to the brain. Since in this organ the decarboxylase remains active, major amounts of cerebral dopamine are formed, particularly in the striatum (Bartholini, Bates & others, 1967; Bartholini & Pletscher, 1968, 1969; Bartholini, Tissot & Pletscher, 1969).

From results in animals, it has been suggested that Ro 4-4602 in combination with L-dopa exerts a beneficial effect in Parkinson's syndrome (Bartholini & others, 1967; Bartholini & Pletscher, 1968). In fact, repeated administration of Ro 4-4602 enhanced the L-dopa-induced increase of this amino-acid in the blood plasma of man (Bartholini & others, 1969; Tissot, Bartholini & Pletscher, 1969a); MK 485 had an effect similar to Ro 4-4602 (Goodwin, Brodie & others, 1970). In addition, extensive clinical trials in patients with Parkinson's syndrome have shown that when Ro 4-4602 is given with L-dopa, the dose of L-dopa can be reduced 5–10 times whilst its therapeutic effect is maintained (Birkmayer & Mentasti, 1969; Birkmayer,

* [N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl)hydrazine].HCl; synthesized by Dr B. Hegedüs, Department of Chemistry, F. Hoffmann-La Roche & Co. Ltd, Basel.

† β-(3,4-Dihydroxyphenyl)-α-hydrazino-α-methyl-DL-propionic acid.

Linauer & Mentasti, 1971; Siegfried, 1971; Tissot, Gaillard & others, 1969b; Tissot & Gauthier, 1971). An L-dopa-sparing effect (reduction to 1/2–1/5 of the dose) has also been reported for MK 486, the L-isomer of MK 485 (Hsu, Bianchine & Mesiha, 1971; Calne, Reid & others, 1971).

Previous experiments in rats indicate that low and medium doses of Ro 4-4602 (up to 200 mg/kg) are more potent than equimolar amounts of MK 485 in enhancing the L-dopa-induced rise of dopamine in the brain (Bartholini & Pletscher, 1969). With very high doses (500 mg/kg) of Ro 4-4602, however, the dopamine increase is attenuated, probably because the drug then causes a partial inhibition of cerebral decarboxylase as well (Bartholini & Pletscher, 1968; Bartholini & others, 1969). In contrast, MK 485, even in high doses, does not seem to inhibit the brain enzyme (Bartholini & Pletscher, 1969; Lotti & Porter, 1970).

In earlier investigations in man (Tissot & others, 1969a), relatively high doses of Ro 4-4602 (16–24 mg/kg) were given. We now describe the action of the inhibitor in small single doses, used at present in the treatment of Parkinson's syndrome. Its effect has also been compared with that of MK 485 on the concentrations of exogenous L-dopa in the plasma of man as well as the distribution of dopamine in the rat brain.

MATERIALS AND METHODS

Hospitalized mental patients, whose illness was not in an active stage, were administered 3 mg/kg L-[2-^{14}C]3,4-dihydroxyphenylalanine (L-[^{14}C]dopa) (specific activity = 1·66 μCi/mg) by mouth. One to two weeks later, some of these patients received the same amount of L-[^{14}C]dopa by mouth $\frac{3}{4}$ h after oral doses of 3·4, 10·2 or 34·0 μmol/kg Ro 4-4602 (1·3 or 10 mg/kg of the hydrochloride). Other subjects were treated with 17·0 μmol/kg MK 485 (3·85 mg/kg), $\frac{3}{4}$ h before L-[^{14}C]dopa. The blood was collected from a cubital vein at several intervals after L-[^{14}C]dopa administration.

Male or female rats of Wistar origin (Füllinsdorf), fasted for 16 h, were administered 20 mg/kg L-[^{14}C]dopa (specific activity = 69 μCi/mg) by stomach tube. Other rats received 10·2 μmol/kg of either Ro 4-4602 (3 mg/kg of hydrochloride) or of MK 485 (2·31 mg/kg) by stomach tube followed after 30 min by 2 mg/kg L-[^{14}C]dopa by the same route. The animals were decapitated 2 h after L-[^{14}C]dopa.

The blood plasma of man as well as the heart and the various brain regions of rat were examined for their content in labelled amino-acids, catecholamines and phenolcarboxylic acids according to a chromatographic procedure using Dowex 50 × 4 as previously described (Bartholini & Pletscher, 1968). The dissection of the rat brains was made on ice.

For the calculations of significance, Student's *t*-test was used.

RESULTS

Human blood plasma

In the blood plasma of man, doses as low as 3·4 μmol/kg Ro 4-4602 (1 mg/kg of the hydrochloride) much enhanced the L-[^{14}C]dopa-induced increase in the fraction of labelled amino-acids and diminished that of [^{14}C]phenolcarboxylic acids. These effects were progressively more pronounced with rising doses of the inhibitor (Fig. 1).

The fraction of labelled amino-acids consisted of [^{14}C]dopa and [^{14}C]*O*-methyldopa. The concentration of both amino-acids in the plasma was enhanced by Ro 4-4602, that of [^{14}C]dopa reaching its maximum after $\frac{1}{2}$ h and then progressively declining.

In contrast, [^{14}C]*O*-methyldopa rose more slowly, attaining a plateau between 2 and 4 h (Figs 2 and 3). No radioactivity was detected in the amine fraction. Seventeen μmol/kg MK 485 (3·85 mg/kg) did not significantly differ from 3·4 μmol/kg Ro 4-4602 ($P > 0{\cdot}05$) in the enhancement of the L-[^{14}C]dopa-induced increase of [^{14}C]dopa and [^{14}C]*O*-methyldopa in plasma. Thus, the maximal rise and the time course of the concentrations of the amino-acids were similar with both inhibitors in the doses mentioned (Figs 2 and 3).

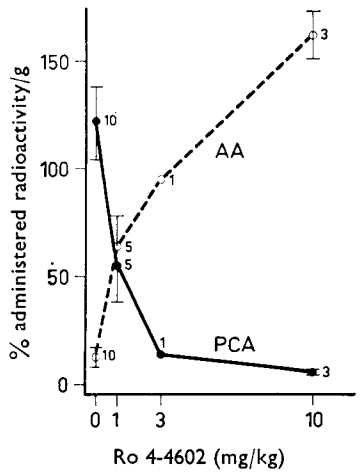

FIG. 1. Effect of different doses of Ro 4-4602 on the L-[^{14}C] dopa-induced increase of labelled amino-acids (AA) and phenolcarboxylic acids (PCA) in the blood plasma of man. L-[^{14}C] Dopa (3 mg/kg) was administered by mouth alone or 3/4 h after various doses of Ro 4-4602 orally. Blood was collected 1 h after L-[^{14}C]dopa administration. The values are expressed in % of the administered radioactivity per g weight and represent averages with s.e. of experiments with different individuals. The number of experiments is indicated by the small figures. The values corresponding to point 0 on the abscissa have been obtained from patients treated with L-[^{14}C]dopa alone. The AA and PCA values after 1 and 10 mg/kg Ro 4-4602 plus L-dopa are significantly different from those after L-dopa alone ($P < 0{\cdot}01$).

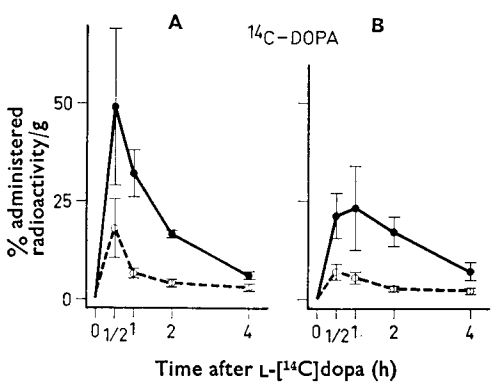

FIG. 2. Effect of (A) Ro 4-4602 and (B) MK 485 on the L-[^{14}C]dopa-induced increase of labelled dopa in the blood plasma of man. Two groups of 4 individuals each received L-[^{14}C]dopa (3 mg/kg) by mouth and one to two weeks later the same groups were given the same amount of L-[^{14}C]dopa after oral pretreatment with either 3·4 μmol/kg Ro 4-4602 (1 mg/kg of the hydrochloride) or 17 μmol/kg MK 485 (3·85 mg/kg). The blood was collected at different times after L-[^{14}C]dopa administration. The values are expressed in % of the administered radioactivity per g weight and represent averages with s.e. of 4 individuals. Values obtained with Ro 4-4602 and MK 485 are not significantly different ($P > 0{\cdot}05$). ○— —○ L-[^{14}C]dopa ●——● Inhibitor + L-[^{14}C] dopa.

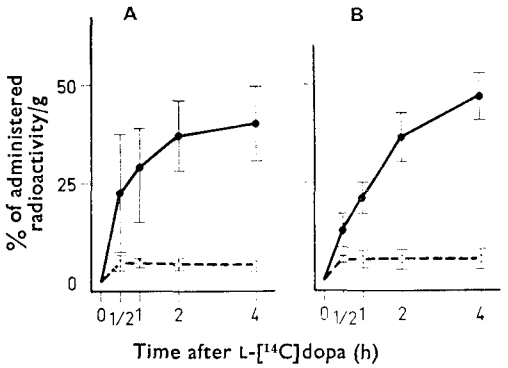

FIG. 3. Effect of Ro 4-4602 and MK 485 on the L-[^{14}C]dopa-induced increase of labelled 3-*O*-methyldopa in the blood plasma of man. For key see Fig. 2.

Brain and heart of rats

Figs 4 and 5 show that in the striatum and the hypothalamus of rats, doses as low as 2 mg/kg L-[^{14}C]dopa in combination with 10·2 μmol/kg Ro 4-4602 (3 mg/kg) caused a more marked increase of labelled catecholamines (about 130 and 120% respectively) than 20 mg/kg L-dopa alone (100%). In the other brain regions, the [^{14}C]catecholamine rise due to the combination (96–103%) was not significantly different from that induced by the 10 times higher doses of [^{14}C]dopa alone ($P > 0{\cdot}05$). Two mg/kg [^{14}C]dopa combined with 10·2 μmol/kg MK 485 (2·31 mg/kg) increased in all the brain regions the L-[^{14}C]catecholamines less than 20 mg/kg of L-[^{14}C]dopa alone. Thereby, the percentage rise of [^{14}C]catecholamines due to the combination (related to that induced by 20 mg/kg L-[^{14}C]dopa alone) was the same (about 50%) in all brain areas including the striatum and the hypothalamus. In the heart of rats, 2 mg/kg L-[^{14}C]dopa combined with 10·2 μmol/kg of either Ro 4-4602 or MK 485 was much less effective than 20 mg/kg L-[^{14}C]dopa in increasing catecholamines. The

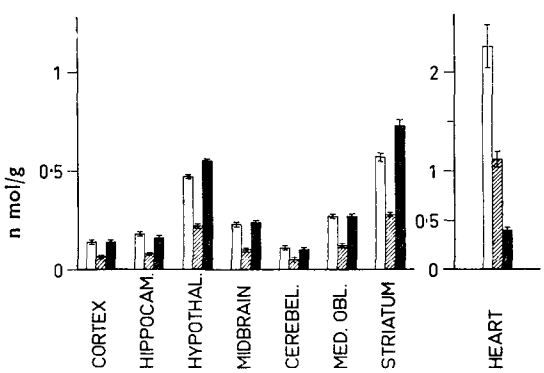

FIG. 4. Effect of Ro 4-4602 and MK 485 on the L-[^{14}C] dopa-induced increase of labelled catecholamines in various brain parts as well as in the heart of rats. White bars: 20 mg/kg L-[^{14}C]dopa by stomach tube. Striped bars: 2 mg/kg L-[^{14}C]dopa 30 min after 10·2 μmol/kg MK 485 (2·31 mg/kg), both by stomach tube. Black bars: 2 mg/kg L-[^{14}C]dopa 30 min after 10·2 μmol/kg Ro 4-4602 (3 mg/kg of the hydrochloride), both by stomach tube. The animals were killed 2 h after L-[^{14}C]dopa. Averages with s.e. of 2–3 experiments each made with a pool of brain parts from 6 rats or of 3 hearts. In the brain, the difference between black and white bars is significant ($P<0{\cdot}01$) only in the striatum; that between black and striped bars as well as between white and striped bars is significant ($P<0{\cdot}01$) for each region. In the heart, the difference between each bar is significant ($P<0{\cdot}01$).

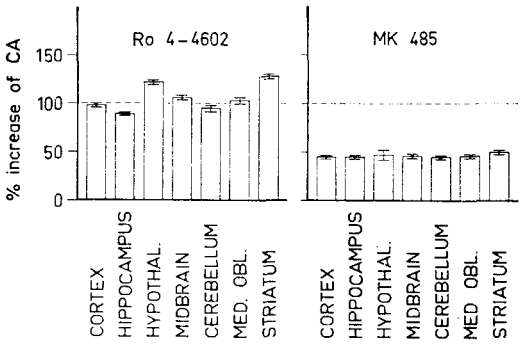

FIG. 5. Percentage increase of labelled catecholamines in various brain areas of rat after oral administration of 2 mg/kg L-[^{14}C]dopa combined with 10·2 μmol of either Ro 4-4602 or MK 485. The percentage calculation was based on the absolute values used for Fig. 4. The values obtained after the combinations of 2 mg/kg L-dopa plus inhibitor were expressed in % of those obtained after oral administration of 20 mg/kg L-[^{14}C]dopa alone (= 100%). The values of Fig. 5 represent averages with s.e. of 2–3 individual percentages. Each individual percentage was calculated from experiments made on the same day. In those with Ro 4-4602, only the values of the striatum and hypothalamus are significantly higher than 100% ($P<0.01$). With MK 485, all values are significantly below 100% ($P<0.01$), but not significantly different ($P>0.05$) from each other.

combination with Ro 4-4602 induced a rise in the [^{14}C]amines significantly less than that seen with MK 485 ($P < 0.01$).

DISCUSSION

As previously shown, Ro 4-4602 enhances the L-dopa-induced rise in the fraction of amino-acids (L-dopa and 3-*O*-methyldopa) in the blood plasma of man (Bartholini & Pletscher, 1968) and diminishes that of phenolcarboxylic acids (mainly homovanillic acid and 3,4-dihydroxyphenylacetic acid) (Bartholini & Pletscher, 1968; Bartholini & others, 1969; Tissot & others, 1969a). This effect of Ro 4-4602 is probably due to inhibition of the decarboxylation of L-dopa in extracerebral tissues (see above). The present results demonstrate that Ro 4-4602 in a dose range as low as 3·4–34·0 μmol/kg (1–10 mg/kg) is an effective inhibitor of decarboxylase in man. MK 485 has a similar action to Ro 4-4602 (Goodwin & others, 1970); but doses several times higher than those of Ro 4-4602 are needed to obtain the same enhancement of the plasma concentrations of either dopa or 3-*O*-methyldopa. These results confirm earlier findings with the two inhibitors in the whole brain of rats (Bartholini & Pletscher, 1969).

In animals, the L-[^{14}C]dopa-induced [^{14}C]catecholamine increase is more marked in the striatum and hypothalamus [mainly dopamine and noradrenaline (Bartholini & Pletscher, 1968)] than in other brain regions (Pletscher & Gey, 1962; Pletscher, Bartholini & others, 1970) (Fig. 4). According to the present results in rats, Ro 4-4602 further enhances this difference. Thus, the [^{14}C]catecholamine rise due to 10·2 μmol/kg Ro 4-4602 plus 2 mg/kg L-[^{14}C]dopa does not significantly differ ($P > 0.05$) in most brain areas from that due to 20 mg/kg L-[^{14}C]dopa alone. In the striatum and the hypothalamus, however, the increase of the amines is more marked ($128 \pm 1\%$ and $121 \pm 1\%$ respectively) after the combination than after L-dopa alone (100%) (Fig. 5). This enhanced electivity of the [^{14}C]catecholamine rise is possibly due to a preferential decarboxylase inhibition in the brain capillaries of these areas. In fact, it has been demonstrated by a histofluorimetric method that Ro 4-4602 inhibits the decarboxylation of L-dopa more markedly in the capillaries of the striatum and the hypothalamus than in those of other brain regions like the

cortex (Constantinidis & others, 1970). It remains to be elucidated whether the use of Ro 4-4602 reduces the appearance of central side effects of L-dopa in Parkinson's syndrome as claimed by some authors (Birkmayer & Mentasti, 1969; Birkmayer & others, 1971; Tissot & others, 1969b; Tissot & Gauthier, 1971; Siegfried, 1971).

The combination of L-dopa with a dose of MK 485 equimolar to that of Ro 4-4602 causes in all brain regions of rats a less marked rise of catecholamines than the high dose of L-dopa alone. This is probably due to a relatively weak inhibition of extracerebral decarboxylase by MK 485 in the dose used. Experiments with the heart, in which the combination of L-dopa with Ro 4-4602 increases the catecholamine content less markedly than that with MK 485, confirm this view. A relatively weak effect of 10·2 μmol/kg MK 485 on extracerebral decarboxylase is also indicated by the finding that the percentage of catecholamine increase induced by the combination of this inhibitor with L-dopa is the same in all the brain regions. This may be due to the fact that the decarboxylase of the brain capillaries has not been inhibited to a marked extent. It should, however, be pointed out that the mentioned differences in potency alone are not sufficient to establish the clinical usefulness of the two inhibitors, since the present data give no information on possible differences in efficacy and specificity. On the other hand, the results in animals together with those in man may be helpful in establishing the dosage schedule of the two drugs in Parkinson's syndrome.

In conclusion, these results provide experimental evidence that the use of the decarboxylase inhibitor Ro 4-4602 in therapeutic doses allows a considerable reduction of the L-dopa dose in Parkinson's syndrome. Ro 4-4602 seems to be more potent than MK 485. Furthermore, the combination of L-dopa with Ro 4-4602 may cause a more selective increase of catecholamines in the striatum and hypothalamus than L-dopa alone.

REFERENCES

BARTHOLINI, G., BATES, H. M., BURKARD, W. P. & PLETSCHER, A. (1967). *Nature, Lond.*, **215**, 852–853.

BARTHOLINI, G. & PLETSCHER, A. (1968). *J. Pharmac. exp. Ther.*, **161**, 14–20.

BARTHOLINI, G. & PLETSCHER, A. (1969). *J. Pharm. Pharmac.*, **21**, 323–324.

BARTHOLINI, G., TISSOT, R. & PLETSCHER, A. (1969). In *Third Symposium on Parkinson's disease*, pp. 192–195. Editors: Gillingham, F. J. & Donaldson, I. M. L. Edinburgh/London: Livingstone.

BIRKMAYER, W. & MENTASTI, M. (1969). *Wien. klin. Wschr.*, **81**, 677.

BIRKMAYER, W., LINAUER, W. & MENTASTI, M. (1971). In *IV Symposium Bel-Air: Monoamines et noyaux gris centraux*, pp. 435–442. Editor: Ajuriaguerra de J. Genève/Paris: Georg/Masson.

CALNE, D. B., REID, J. L., VAKIL, S. D., RAO, S., PETRIE, A., PALLIS, C. A., GAWLER, J., THOMAS, P. K. & HILSON, A. (1971). *Br. med. J.*, **3**, 729–732.

CONSTANTINIDIS, J., BARTHOLINI, G., GEISSBÜHLER, F. & TISSOT, R. (1970). *Experientia*, **26**, 381–383.

GOODWIN, F. K., BRODIE, H. K. H., MURPHY, D. L. & BURMEY, W. E. (1970). *Lancet*, **1**, 908–911.

HSU, T. H., BIANCHINE, J. R. & MESIHA, F. S. (1971). *Clin. Res.*, **19**, 350.

LOTTI, V. J. & PORTER, C. C. (1970). *J. Pharmac. exp. Ther.*, **172**, 406–415.

PLETSCHER, A. & GEY, K. F. (1962). *Experientia*, **18**, 512–513.

PLETSCHER, A., BARTHOLINI, G., GEY, K. F. & JENNI, A. (1970). *Schweiz. med. Wschr.*, **100**, 797–804.

SIEGFRIED, J. (1971). In *IV Symposium Bel-Air: Monoamines et noyaux gris centraux*, pp. 443–449. Editor: Ajuriaguerra de J. Genève/Paris: Georg/Masson.

TISSOT, R., BARTHOLINI, G. & PLETSCHER, A. (1969a). *Arch. Neurol.*, **20**, 187–190.

TISSOT, R., GAILLARD, J. M., GUGGISBERG, M., GAUTHIER, G. & AJURIAGUERRA DE, J. (1969b). *Presse méd.*, **77**, 619–622.

TISSOT, R. & GAUTHIER, G. (1971). In *IV Symposium Bel-Air: Monoamines et noyaux gris centraux*, pp. 455–464. Editor: Ajuriaguerra de, J. Genève/Paris: Georg/Masson.