EFFECT OF CAROXAZONE, A NEW ANTIDEPRESSANT DRUG, ON MONOAMINE OXIDASES IN HEALTHY VOLUNTEERS

A. MORETTI¹, C. CACCIA¹, A. MARTINI¹, L. BONOLLO¹, A. AMICO¹, R. SEGA², V. NICOLELLA¹, & F. B. NICOLIS¹

¹ Farmitalia-Carlo Erba Research Institute Nerviano and Milan, Italy and

² Institute of Patologia Medica V^a, L. Sacco Hospital, University of Milan, Milan, Italy.

- 1 Caroxazone is a new antidepressant drug with a reversible inhibitory effect on monoamine oxidases (MAO) as previously shown experimentally in animals.
- 2 The effect of caroxazone on MAO was explored in healthy volunteers and compared with that of tranylcypromine and imipramine. Daily urinary excretion of tryptamine and MAO activity in platelets were assayed at various times during and after treatment and the differences from basal values were statistically analysed. In addition, caroxazone plasma levels were determined.
- 3 Caroxazone administered orally at doses of 300 or 600 mg/day for 12 days induced a significant, dose-dependent increase in urinary tryptamine excretion. Tranylcypromine (20 mg/day for 8 days) was even more active in this respect; imipramine (50 mg/day for 12 days) was completely inactive.
- 4 MAO activity in platelets was not affected by caroxazone or imipramine, but was completely inhibited by tranylcypromine.
- 5 Mean steady-state plasma levels of caroxazone were about 6 µg/ml and 11-12 µg/ml with the dose of 300 mg/day and 600 mg/day respectively.
- 6 It can be concluded that caroxazone is a MAO inhibitor in man too, at a clinically effective dose such as 600 mg/day. The fact that its MAO inhibition is not apparent in platelet preparation may be explained by its reversibility.
- 7 Tranylcypromine confirmed its potent irreversible MAO inhibitory effect, while imipramine lacked any effect at the tested dose.

Introduction

Caroxazone, 2-oxo-2H-1,3-benzoxazine-3(4H)-acetamide, is a new drug which has strong antiserpine activity in a number of animal species (Suchowsky & Pegrassi, 1969) and potentiates the effects of noradrenaline and indolealkylamines (Suchowsky, Pegrassi, Moretti & Bonsignori, 1969). In man caroxazone showed antidepressant activity in double-blind studies versus placebo (Conti, Bonollo, Martini, Fornaro, Guerrini, Romerio, Scillieri, Soverini, Stramba-Badiale & Cassano, 1980) and reference drugs (Cecchini, Petri, Ardito, Bareggi & Torriti, 1978; Pellegrini, Broglia & Colombo, 1981; Vescovini, 1974).

Its mechanism of action is based on the inhibition of monoamine-oxidases (monoamine: oxygen oxidoreductase, deaminating; E.C. 1.4.3.4., MAO) which has been demonstrated in rat brain both *in vitro* and *in vivo* (Moretti, Caccia, Calderini, Menozzi and Amico, in preparation). However, caroxazone differs from all other clinically used MAO inhibitors in that its action is reversible. Caroxazone has no inhibitory effect on amine uptake in rat brain and heart (Moretti *et al.*, in preparation).

These findings prompted the present study in which the effects of caroxazone on MAO in man (healthy volunteers) were assessed on the basis of biochemical parameters. MAO activity was assayed directly in an easily accessible tissue such as platelets and indirectly by measuring urinary excretion of tryptamine. These two procedures appear the most suitable to assess drug-induced inhibition of MAO in man, and are reported to correlate well with each other (Robinson, Lovenberg, Keiser & Sjoerdsma, 1968).

The effect of caroxazone was compared in double-blind conditions with that of a known MAO inhibitor, tranylcypromine, and a tricyclic antidepressant drug, imipramine, for which MAO inhibition has also been reported by some workers.

Methods

Volunteers

Twenty-three healthy males (medical students or

young doctors) aged 21–29 years and weighing 56–95 kg volunteered for this study. All subjects were given a written memorandum with instructions concerning dietary restrictions throughout the trial (to avoid any tyramine-containing food and alcoholic beverages). No other drugs were allowed.

Treatments

Subjects were assigned at random to one of the following oral treatments:

- caroxazone, 100 mg three times daily (08.00 h, 13.00 h and 20.00 h) for 12 days (7 subjects);
- caroxazone, 200 mg three times daily (08.00 h, 13.00 h and 20.00 h) for 12 days (7 subjects);
- imipramine, 25 mg twice daily (08.00 h and 20.00 h) for 12 days (6 subjects);
- tranylcypromine, 10 mg twice daily (08.00 h and 20.00 h) for 8 days (3 subjects).

Biochemical methods

MAO activity in platelets: venous blood samples were taken from each subject at 10.00 h on two different days of the week before treatment (basal values) and on various days during (therefore 2 h after the morning administration) and after treatment as specified under **Results**. Blood was collected in polypropylene centrifuge tubes containing 3.8% sodium citrate, centrifuged at 300 g for 30 min and platelet rich plasma (PRP) was removed and centrifuged at 2000 g for 20 min.

Platelet MAO was solubilized with Triton X-100 and dissolved in enough H₂O to give a protein concentration of 1–2 mg/ml (Lowry, Rosebrough, Farr & Randall, 1951).

MAO activity was assayed radiometrically as described by Robinson *et al.* (1968) except for the substrate which was β -ethyl-1-[¹⁴C]-phenylethylamine hydrochloride, ([¹⁴C]PEA) (NEN Chemicals GmbH, Dreieich, West Germany) sp. act. 9.86 mCi/mmol, final concentration 3.3×10^{-7} M. At the end of 30 min incubation the reaction was stopped by addition of 0.15 ml of 60% perchloric acid. The reaction product was then extracted into 3 ml toluene and radioactivity of 2 ml of the toluene phase was counted in a Packard Tri-Carb model 3375 liquid scintillation counter with 85% efficiency. Blank values were obtained by blocking the reaction immediately before adding the substrate. MAO activity was expressed as pmol of reaction product per mg protein per hour.

Urinary excretion of tryptamine: 24 h urines were collected on three different days of the week before treatment (basal values) and on various days during and after treatment as specified under **Results**. Tryptamine was measured by the method of Sjoerdsma, Oates, Zaltman & Udenfriend (1959) and expressed as μ g/g creatinine.

Determination of caroxazone plasma levels

Caroxazone was assayed in platelet-free plasma by high pressure liquid chromatography (HPLC) after repeated extractions with ethyl acetate.

Statistical evaluation

Non-parametric Wilcoxon's test was used to analyse the differences within treatments. Three-way analysis of variance (treatments, subjects and times) was applied for comparison between treatments. Due to the small number of subjects in the tranylcypromine group, these data were not statistically analysed.

Results

All subjects treated with either caroxazone or imipramine concluded the trial and both drugs were well tolerated. Two out of the three subjects who received tranylcypromine experienced side effects and one of them had to discontinue the treatment. Therefore this subject was not taken into consideration in the evaluation of the results.

Effect of treatments on platelet MAO activity

Figure 1 shows that neither caroxazone at 300 or 600 mg/day nor imipramine at 50 mg/day induced any

Figure 1 Effect of oral caroxazone (○ 300 mg/day $n = 7$, ● 600 mg/day $n = 7$), imipramine (△ 50 mg/day $n = 6$) or tranylcypromine (▲ 20 mg/day $n = 2$) on platelet MAO activity with [¹⁴C]PEA in healthy volunteers. Statistical analysis: Wilcoxon's test (basal values v each of the days of treatment or post-treatment with caroxazone or imipramine): $P \leq 0.05$.

Data of tranylcypromine treated subjects were not analysed.

significant change in platelet MAO activity during treatment as compared to the basal values (Wilcoxon's test). The curves for caroxazone and imipramine were practically superimposable. The same was true for the post-treatment period except for a slight, though significant, decrease in MAO activity 4 days after the end of imipramine treatment. The lack of effect on platelet MAO activity of caroxazone and imipramine was statistically confirmed by analysis of variance with three factors (treatments, times and subjects). Tranylcypromine administration resulted in immediate and almost complete inhibition of MAO activity which persisted during the entire treatment period. In the two subjects studied, recovery was still not complete ten days after the end of treatment. Although no statistical analysis was performed on this very small sample, there is no doubt about the extremely high and persistent MAO inhibitory effect of tranylcypromine.

Figure 2 Effect of oral caroxazone (○ 300 mg/day $n = 7$, ● 600 mg/day $n = 7$), imipramine (△ 50 mg/day $n = 6$), or tranylcypromine (▲ 20 mg/day $n = 2$) on daily urinary excretion of tryptamine in healthy volunteers. Statistical analysis: see Figure 1.

Effect of treatments on daily urinary excretion of tryptamine

Figure 2 shows that caroxazone increased urinary tryptamine excretion. With 300 mg/day this increase was between 345% on day 2 of treatment and 518% on day 9. With 600 mg/day the effect was even more pronounced, being between 360% after 2 days and 676% after 4 days with values of 570–580% on subsequent days. All these effects are statistically significant (Wilcoxon's test). With both caroxazone doses this parameter returned to normal values within

6 days from the end of treatment. Analysis of variance shows that 600 mg/day was significantly more effective than 300 mg/day.

In contrast to caroxazone, imipramine did not induce any significant change in tryptamine excretion. Tranylcypromine was again the most active drug: tryptamine rose 70% after 2 days, 650–700% after 4–6 days and remained at this value on the first post-treatment day. After that, urinary tryptamine fell rapidly and basal values were attained around 8 days after the end of treatment.

Figure 3 Mean caroxazone plasma levels in healthy volunteers treated with 300 mg/day (○ $n = 7$) or 600 mg/day (● $n = 7$). Blood samples were taken 2 h after caroxazone morning dose.

Caroxazone plasma levels

Figure 3 shows that a steady-state was reached by the 4th day of treatment and levels were dose-related. After the end of treatment, caroxazone disappeared with a $T_{1/2}$ of about 1 day.

Discussion

The significant, dose-related increase in tryptamine urinary excretion found in the present study confirms in man the MAO inhibitory effect of caroxazone already described in animals (Moretti *et al.*, in preparation). Although the increase in tryptamine urinary excretion reflects MAO inhibition in the whole body, it is conceivable that it could also take place in brain where tryptamine is usually absent and accumulates after treatment with MAOIs. Moreover, it has been repeatedly shown in rats that the level of other monamines in brain is also increased by caroxazone administration (Moretti *et al.*, in preparation; Ponzio, Curcio, Brunello & Algeri, personal communication), as well as by all other MAOIs tested in

animals (Green & Youdim, 1975; Maitre, Delini-Stula & Waldmeier 1976; Pletscher, Gey & Zeller, 1960; Sharman, 1976; Valzelli & Garattini, 1968; Yang & Neff, 1974) and in man (Bevan Jones, Parte, Nicholson, Price & Stacey, 1972; Maclean, Nicholson, Pare & Stacey, 1965).

However there is a difference between caroxazone and all other clinically-used MAOIs, namely the apparent lack of effect on platelet MAO. This finding can now be explained by a peculiar property of this drug, i.e. its reversible effect on MAO clearly demonstrated in experimental studies in animals (Moretti, Caccia, Martini, Calderini & Amico, 1978) and further supported by the close correlation between drug levels in plasma and the time-course and dose-dependence of urinary tryptamine excretion found in the present trial. This reversibility would result in caroxazone being removed from platelets during their preparation, so that it would be no longer present when MAO activity is assayed.

This hypothesis is further validated by the following findings:

1. *in vitro*, caroxazone inhibits human platelet MAO with an I.C.₅₀ of 2.7×10^{-5} M (Moretti *et al.*, unpublished observations), a concentration comparable to that observed in the plasma of caroxazone-treated subjects;
2. inhibition of human platelet MAO has been demonstrated by assaying this enzyme in PRP of caroxazone-treated patients where the drug is certainly present. In fact, the oral administration of caroxazone (800 mg/day for 11 days) induces highly significant 60% inhibition of MAO activity in PRP (mean of 5 patients) (Martini, Cignoli, Bonollo, Moretti, Caccia, Amico & Scillieri, in preparation).

References

- BEVAN JONES, A.B., PARE, C.M.B., NICHOLSON, W.J., PRICE, K. & STACEY, R.S. (1972). Brain amine concentrations after monoamineoxidase inhibitor administration. *Br. med. J.*, **1**, 17–19.
- BRUINVELS, J. (1972). Inhibition of the biosynthesis of 5-hydroxytryptamine in rat brain by imipramine. *Eur. J. Pharmac.*, **20**, 231–237.
- CECCHINI, S., PETRI, P., ARDITO, R., BAREGGI, S.R. & TORRITI, A. (1978). A comparative double-blind trial of the new antidepressant caroxazone and amitriptyline. *J. int. med. Res.*, **6**, 388–394.
- CONTI, L., BONOLLO, L., MARTINI, A., FORNARO, P., GUERRINI, A., ROMERIO, C., SCILLIERI, E., SOVERINI, S., STRAMBA-BADIALE, M. & CASSANO, G.B. (1980). Double-blind comparative study of the therapeutic activity and safety of caroxazone (F.I. 6654) and placebo in depressed in-patients. *Curr. Ther. Res.*, **27**, 458–473.
- GILLER, E., JATLOW, P., BIALOS, D., HARKNESS, L. & DOCHERTY, J.P. (1980). Platelet MAO and amitriptyline treatment. *Psych. Res.*, **2**, 259–265.
- GREEN, A.R. & YODIM, M.B.H. (1975). Effects of monoamineoxidase inhibition by clorgyline, deprenyl or tranylcypromine on 5-hydroxytryptamine concentrations in rat brain and hyperactivity following subsequent tryptophan administration. *Br. J. Pharmac.*, **55**, 415–422.
- HALARIS, A.E., LOVELL, R.A. & FREEDMAN, D.X. (1973). Effect of chlorimipramine on the metabolism of 5-hydroxytryptamine in the rat brain. *Biochem. Pharmac.*, **22**, 2200–2202.
- HONECKER, H. & HILL, R. (1977). Does long term treatment with amitriptyline alter the monoamine oxidase of rat brain? *Pharmakopsychiat.*, **10**, 32–35.
- KIVALO, E., RINNE, U.K. & KARINKANTA, H. (1961). The effect of imipramine on the 5-hydroxytryptamine

In contrast, the observation that tranylcypromine is highly effective not only on tryptamine excretion but also on platelet MAO can be explained by its irreversible and therefore long-lasting inhibition of the enzyme.

Finally, the present work clearly shows that imipramine administered at the dose of 50 mg/day has no MAO inhibitory effect. MAO inhibition by tricyclic antidepressants has been reported *in vitro* (see Roth, 1976) but the contribution of such mechanism in the *in vivo* action of these drugs at pharmacological doses is still controversial (Bruinvels, 1972; Giller, Jatlow, Bialos, Harkness & Docherty, 1980; Halaris, Lovell & Freedman, 1973; Honecker & Hill, 1977; Kivalo, Rinne & Karinkanta, 1961; Meek & Werdinius, 1970; Roth, 1976; Schildkraut, Winokur & Applegate, 1970; Von Voigtlander & Losey, 1976). To our knowledge, the only work that reports MAO inhibition in man by tricyclic antidepressants (Sullivan, Zung, Stanfield & Cavenar, 1978) is hardly comparable with the present one because of differences in subjects, dose, parameters analysed and substrate used for platelet MAO.

Acknowledgements

Statistical analyses were performed by the Farmitalia-Carlo Erba Unit of Biometry (Head: Dr V. Mandelli).

The Authors wish to thank also Mr Pietro D'Orazio, Mr Giuseppe Marchi, Mr Carmelo Zappulla and Mrs Luigia Frontini for their precious technical assistance.

Reprint requests should be addressed to Prof. A. Moretti, Biochemical Pharmacology, Farmitalia-Carlo Erba Research Centre, 20014 Nerviano (Milan), Italy.

- content and monoamine oxidase activity of the rat brain and on the excretion of 5-hydroxyindoleacetic acid. *J. Neurochem.*, **8**, 105–108.
- LOWRY, O.H., ROSENBERG, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.
- MACLEAN, R., NICHOLSON, W.J., PARE, C.M.B. & STACEY, R.S. (1965). Effect of monoamine oxidase inhibitors on the concentrations of 5-hydroxytryptamine in the human brain. *Lancet*, **ii**, 205–208.
- MAITRE, L., DELINI-STULA, A. & WALDMEIER, P.C. (1976). Relations between the degree of monoamine oxidase inhibition and some psychopharmacological responses to monoamine oxidase inhibitors in rats. In *Monoamine oxidase and its inhibition*, Ciba Foundation Symposium 39 (new series), pp. 247–267. Amsterdam, Oxford and New York: Elsevier, Excerpta Medica and North Holland.
- MEEK, J. & WERDINIUS, B. (1970). Hydroxytryptamine turnover decreased by the antidepressant drug chlorimipramine. *J. Pharm. Pharmac.*, **22**, 141–143.
- MORETTI, A., CACCIA, C., MARTINI, A., CALDERINI, G. & AMICO, A. (1978). The mechanism of action of caroxazone. Communication at the *Seventh International Congress of Pharmacology*, Paris, July 16–21.
- PELLEGRINI, V., BROGLIA, S. & COLOMBO, R. (1981). A double-blind comparative trial with caroxazone and amitriptyline in depressed in-patients. *Int. Pharmacopsych.* (in press).
- PLETSCHER, A., GEY, K.F. & ZELLER, P. (1960). Monoaminoxidase-Hemmer. In *Fortschritte der Arzneimittelforschung*, Vol. 2, ed. Jucker, E., pp. 417–590. Basel und Stuttgart: Birkhauser Verlag.
- ROBINSON, D.S., LOVENBERG, W., KEISER, H. & SJOERDSMA, A. (1968). Effects of drugs on human blood platelet and plasma amine oxidase activity *in vitro* and *in vivo*. *Biochem. Pharmac.*, **17**, 109–119.
- ROTH, J.A. (1976). Multiple forms of monoamine oxidase and their interaction with tricyclic psychomimetic drugs. *Gen. Pharmac.*, **7**, 381–386.
- SHARMAN, D.F. (1976). Can the intra- and extra-neuronal metabolism of catecholamines be distinguished in the mammalian central nervous system? In *Monoamine oxidase and its inhibition*, Ciba Foundation Symposium 39 (new series), pp. 203–216. Amsterdam, Oxford and New York: Elsevier, Excerpta Medica and North Holland.
- SCHILDKRAUT, J.J., WINOKUR, A. & APPLIGATE, C.W. (1970). Norepinephrine turnover and metabolism in rat brain and long-term administration of imipramine. *Science*, **168**, 867–869.
- SJOERDSMA, A., OATES, J.A., ZALTMAN, P. & UDEN-FRIEND, S. (1959). Identification and assay of urinary tryptamine: application as an index of monoamine oxidase inhibition in man. *J. Pharmac. exp. Ther.*, **126**, 217–222.
- SUCHOWSKY, G.K. & PEGRASSI, L. (1969). The pharmacology of 4H-3-methylcarboxamide-1,3-benzoxazine-2-one (F.I.6 654), a compound acting on the central nervous system. *Arzneim.-Forsch. (Drug Res.)*, **19**, 643–648.
- SUCHOWSKY, G.K., PEGRASSI, L., MORETTI, A. & BONSIGNORI, A. (1969). The effect of 4H-3-methylcarboxamide-1,3-benzoxazine-2-one (F.I. 6654) on monoamine oxidase and cerebral 5-HT. *Arch. int. Pharmacodyn.*, **182**, 332–340.
- SULLIVAN, J.L., ZUNG, W.W.K., STANFIELD, C.N. & CAVENAR, Jr., J.O. (1978). Clinical correlates of tricyclic antidepressant mediated inhibition of platelet monoamine oxidase. *Biol. Psych.*, **13**, 399–407.
- VALZELLI, L. & GARATTINI, S. (1968). Biogenic amines in discrete brain areas after treatment with monoamine oxidase inhibitors. *J. Neurochem.*, **15**, 259–261.
- VESCOVINI, L. (1974). Studio clinico 'double-blind' sull'attività di un nuovo composto antidepressivo: il caroxazone (F.I. 6654). *Riv. sper. Freniat.*, **98**, 151–165.
- VON VOIGTLANDER, P.F. & LOSEY, E.G. (1976). Inhibition of phenylethylamine metabolism *in vivo*. Effect of antidepressants. *Biochem. Pharmac.*, **25**, 217–218.
- YANG, H.Y.T. & NEFF, N.H. (1974). The monoamine oxidase of brain: selective inhibition with drugs and the consequences for the metabolism of the biogenic amines. *J. Pharmac. exp. Ther.*, **189**, 733–740.

(Received June 26, 1980)