J. Pharm. Pharmacol. 1981, 33: 639–643
Received January 15, 1981

0022–3573/81/100639–05 $02.50/0

Some biochemical aspects of the potential benefit of associating MD780515 with tricyclic antidepressants

P. DOSTERT, M. STROLIN BENEDETTI* AND N. SONTAG

Centre de Recherche Delalande, 10, rue des Carrières–F-92500 Rueil-Malmaison, France

In the rat, suitable oral doses of tricyclic antidepressants (amitriptyline 20 mg kg^{-1}, imipramine, desipramine 2·5 mg kg^{-1}) are able to antagonize the increase of cardiac levels of intravenous tyramine after a pharmacologically active dose (3·5 mg kg^{-1} orally) of a reversible and specific type A MAO inhibitor, MD780515 (3-[4-(3-cyanophenylmethoxy)phenyl]-5-(methoxymethyl)-2-oxazolidinone). MD780515, in oral doses up to 35 mg kg^{-1}, does not alter the liver microsomal drug metabolizing enzymes in the rat. Therefore, when given with tricyclic antidepressants, it should not interfere with their metabolism.

The combination of MAO inhibitors (MAOIs) and tricyclic antidepressants (TCAs) seems to be one of the most potent treatments for resistant cases of depression. However, statements persist in medical texts concerning severe morbidity and even lethality of the combination of TCAs with MAOIs, although several authors have recently shown that the association is much safer than anticipated, and that adverse effects could be due to mishandling of the drugs (Schuckit et al 1971; Spiker & Pugh 1976; Ananth & Luchins 1977; Berger & Barchas 1977; Ponto et al 1977; Goldberg & Thornton 1978; Quitkin et al; Kline & Cooper 1980; White et al 1980). Clinicians in favour of this combination have suggested that it would be useful to have pharmacological and biochemical bases to strengthen the rationale of such a therapeutic approach, and to support the hypothesis that such a combination if properly used might even be safer than MAOIs by themselves, particularly towards the so-called 'cheese-reaction' (Pare 1976).

The major biochemical property of TCAs is their inhibition of the uptake of biogenic amines both at central and peripheral nerve endings (Iversen 1975; Slotkin & Bareis 1980), although they are not devoid of effect on the vesicle membrane pump system (Iversen 1975). With tyramine, which competes for the neuronal uptake site with noradrenaline (Iversen 1975), whether TCAs only inhibit its uptake into the neuron (Gessa et al 1966; Brodie et al 1968) or also into the intraneuronal vesicle (Baldessarini 1975; Steinberg & Smith 1970) has not been unequivocally demonstrated. In the heart, the effect of such drugs on tyramine seems to occur at the neuronal uptake sites (Steinberg & Smith 1971). Whatever the precise mechanism of the interaction of TCAs with tyramine, it has been unequivocally shown that TCAs such as desipramine, amitriptyline and its metabolite nortriptyline decrease tyramine sensitivity, as the dose of tyramine required to elevate the systolic blood pressure of volunteers by a specific amount needs to be substantially increased after treatment with TCAs (Åström 1970; Ghose et al 1976). This observation has led some workers to use the decreased tyramine sensitivity produced by TCAs as an index of their pharmacological activity, and as an alternative technique to assess their biological activity when facilities to measure their plasma concentrations are limited (Mulgirigama et al 1977; Ghose 1980).

The effect of MAOI is to increase the levels of endogenous and exogenous amines, the extent of such an increase depending on several factors, mainly the type of MAOIs used (A, B or mixed) and the specificity of each amine towards the enzyme.

Among the possible benefits of an association of MAOIs and TCAs, we have investigated whether suitable doses of TCAs could antagonize the increase of cardiac levels of tyramine following a pharmacologically active dose of a MAOI. Amitriptyline, imipramine and desipramine were chosen, but chlorimipramine was discarded for the reasons stated by Pare (1976, 1979). Trimipramine was not considered, even though it is recommended in combination with MAOIs for the treatment of resistant depression (Pare 1979), as it is a very weak inhibitor of noradrenaline uptake in the heart compared with the three TCAs chosen (Møller Nielsen 1980). MD780515, 3-[4-(3-cyanophenylmethoxy)phenyl]-5-(methoxymethyl)-2-oxazolidinone, a new reversible and specific type A MAOI devoid of inhibiting properties on biogenic amine uptake was used (Strolin Benedetti et al 1979; Kan & Strolin Benedetti 1981).

* Correspondence.

One of the interactions between MAOIs and TCAs is pharmacokinetic in origin and involves inhibition of hepatic microsomal mixed function oxidase activity both in animals (Sjöqvist 1965; Clark 1967; Kato et al 1969; Eade & Renton 1970; Clark & Thompson 1972; Valerino et al 1978) and in man (Smith et al 1980). A possible action of MD780515 on hepatic drug metabolizing enzymes has therefore been investigated by studying its effect on the metabolism of amphetamine (Dring et al 1966; Ellison et al 1966); the urinary elimination of unchanged amphetamine was measured after MAOI administration. Amphetamine was chosen because Rand & Trinker (1968) ascribed the potentiation of the cardiovascular actions of this agent by several MAOIs to impairment of the liver microsomal enzymes that normally metabolize amphetamine. Their interpretation was further supported by the work of O'Dea & Rand (1969) who found a potentiation of amphetamine toxicity by the most commonly used MAOIs. A possible action of MD780515 on hepatic drug metabolizing enzymes has also been investigated by studying the effect of the compound on the levels of hepatic cytochrome P-450 in the rat, since a reduction of the content of this enzyme system was demonstrated in rats pretreated with pargyline (Valerino et al 1978).

MATERIALS AND METHODS

Male Sprague Dawley rats (Charles River, CD, France), 150–250 g were fasted for 15 h before the experiment.

Tyramine uptake

Groups of 5 rats received one of the following treatments: (i) tyramine (i.v.), (ii) TCA (oral) and 1 h later tyramine (i.v.), (iii) MD780515 (oral) and 30 min later tyramine (i.v.), (iv) TCA (oral), 30 min later MD780515 (oral) and 30 min after MD780515, tyramine (i.v.). In each experiment, 15 min after tyramine injection the animals were decapitated, the heart rapidly removed, washed to eliminate blood, weighed and dissolved in 2 ml Soluene 350 (Packard), at 55–60 °C. Radioactivity was measured by liquid scintillation counting using 15 ml Dimilume 30 (Packard)/HCl 0·5 M (v/v, 9:1). The composition of heart radioactivity was not analysed. In fact, according to Musacchio et al (1965), the radioactivity in rat heart after i.v. tyramine is almost completely present as amines and at the time of killing in the present study (15 min) the tyramine has been almost completely transformed to octopamine.

[Side chain -2-^{14}C] Tyramine hydrochloride was supplied by Amersham (specific activity: 50 mCi mmol^{-1}, aqueous solution) and cold tyramine hydrochloride by Sigma. Amitryptyline hydrochloride (Dr Bonapace and Co., Milan) was administered in aqueous solution at the doses of 10, 20, 30 and 40 mg kg^{-1}, imipramine hydrochloride (Dr Bonapace and Co., Milan) at the doses of 2·5, 5 and 10 mg kg^{-1}, and desipramine hydrochloride (Dr Bonapace and Co., Milan) at 1, 2·5 and 5 mg kg^{-1}. MD780515 was administered at 3·5 mg kg^{-1} as a suspension in aqueous solution of methylcellulose (0·5%). The doses of TCAs have been selected from the ED50 values in the antagonism of reserpine-induced ptosis in the rat (amitriptyline 40·5 mg, imipramine 4·5 mg, desipramine 2 mg kg^{-1}) (Jalfre & Bucher, personal communication). The dose of MD780515 has been chosen taking into account the ED50 value in the 5-HTP potentiation test in the rat (Jalfre et al 1980) and the dose giving a marked increase of the biogenic amines in the rat brain (Strolin Benedetti et al 1979). In most experiments, [^{14}C]tyramine was injected at the dose of 10 μg kg^{-1} in 0·9% NaCl (saline). In an additional experiment with imipramine (2·5, 5, 10 mg kg^{-1}) and MD780515 (3·5 mg kg^{-1}) a higher dose of tyramine (150 μg kg^{-1}) was used. Doses of TCAs and tyramine are expressed as the amount of base administered.

Urinary elimination of unchanged amphetamine

A group of 5 animals received orally an aqueous solution of methylcellulose (0·5%) alone or a suspension of MD780515 (3·5 and 35 mg kg^{-1}) and 1 h later [^{14}C](+)amphetamine sulphate (labelled in the β-position, specific activity: 27 mCi mmol^{-1}, C.E.A.) at the dose of 3 mg kg^{-1} i.p. of the base. Animals were kept in individual metabolic cages and urine collected over 24 h. Urinary pH was measured. Unchanged amphetamine was extracted with toluene (10 ml) from urine (4 ml) in basic conditions (1 ml NaOH 1 M). No metabolite was extracted by this procedure. The extraction efficiency of amphetamine under these conditions was 99·6 (1·2)% (mean with s.d. n = 10). An aliquot of the organic phase was deposited on thin layer plates (Silica gel Merck F_{254}) and chromatographed in the system n-butanol–acetic acid–water (50:25:25, v/v/v); another aliquot was used to determine the radioactivity by liquid scintillation counting (10 ml Unisolve 1, Koch-Light Laboratories, Ltd).

Cytochrome P-450 determination

Rats received MD780515 (35 mg kg^{-1} day^{-1} orally for 3 days) as a suspension in aqueous solution of

methylcellulose (0·5%) and were killed 24 h after the last dose. Control rats received the aqueous solution according to the same protocol. The liver was removed, microsomes prepared and cytochrome P-450 levels determined according to Mazel (1971). Microsomal protein content was determined by the method of Lowry et al (1951).

RESULTS AND DISCUSSION

Tyramine uptake

As shown in Table 1, MD780515 (3·5 mg kg^{-1}) increased the radioactivity in the heart by 124% compared with the control animals given [^{14}C]tyramine alone. Amitriptyline at the dose of 20 mg kg^{-1} inhibited significantly the uptake of tyramine and substantially antagonized the effect of MD780515.

Table 1. Effect of MD780515 (3·5 mg kg^{-1} orally), amitriptyline (AMI) and their association on the cardiac radioactivity levels after injection of [^{14}C]tyramine (Tyr, 10 μg kg^{-1} i.v.) in the rat.

Treatment (dose mg kg^{-1})	% injected Tyr g^{-1} cardiac tissue (mean with s.d.)
Tyr	1·32 s.d. 0·13 (controls)
AMI (10) + Tyr	1·12 s.d. 0·18
AMI (20) + Tyr	0·75 s.d. 0·07*
AMI (40) + Tyr	0·71 s.d. 0·08*
Tyr	1·38 s.d. 0·13 (controls)
MD780515 + Tyr	3·09 s.d. 0·26*
AMI (10) + MD780515 + Tyr	3·15 s.d. 0·21*
AMI (20) + MD780515 + Tyr	1·74 s.d. 0·26
AMI (40) + MD780515 + Tyr	1·13 s.d. 0·23

As the variances were homogeneous according to the Bartlett test, the results were analysed using the Dunnett test.

* Significantly different from controls at the level $P \leq 0·05$.

Imipramine at 2·5 mg kg^{-1} and above, inhibited significantly the uptake of tyramine (Table 2). At 2·5 mg kg^{-1} imipramine given with MD780515 cancelled the effect of the latter and at 10 mg kg^{-1}, was able to decrease significantly the amount of amine present in the heart.

As shown in Table 3, desipramine significantly inhibited the uptake of tyramine at all three doses studied, but it did not cancel the effect of MD780515 at the lowest dose. At 2·5 mg kg^{-1} desipramine given with MD780515 was able to antagonize the effect of the MAOI and at 5 mg kg^{-1} it significantly reduced the amount of the amine present in the heart. From these results, it appears that imipramine and amitriptyline associated with MD780515 are able to reduce the cardiac radioactivity levels to values not significantly different from the controls at doses equal to about half their ED50 in the antagonism of reserpine-induced ptosis, whereas desipramine antagonizes the effect of MD780515 at its ED50 value in the same test. The additional experiment with imipramine and MD780515 using 150 μg kg^{-1} of tyramine was carried out to check the effect of the association of both drugs at a dose of amine possibly able to produce a pressor response in the rat.

Table 2. Effect of MD780515 (3·5 mg kg^{-1} orally), imipramine (IMI) and their association on the cardiac radioactivity levels after injection of [^{14}C]tyramine (Tyr, 10 μg kg^{-1} i.v.) in the rat.

Treatment (dose mg kg^{-1})	% injected Tyr g^{-1} cardiac tissue (mean with s.d.)
Tyr	1·50 s.d. 0·20 (controls)
IMI (2·5) + Tyr	1·11 s.d. 0·14*
IMI (5) + Tyr	0·61 s.d. 0·14*
IMI (10) + Tyr	0·58 s.d. 0·11*
Tyr	1·33 s.d. 0·10 (controls)
MD780515 +Tyr	3·17 s.d. 0·41*
IMI (2·5) + MD780515 + Tyr	1·57 s.d. 0·48
IMI (5) + MD780515 + Tyr	1·02 s.d. 0·21
IMI (10) + MD780515 + Tyr	0·63 s.d. 0·09*

* See footnote to Table 1.

Table 3. Effect of MD780515 (3·5 mg kg^{-1} orally), desipramine (DMI) and their association on the cardiac radioactivity levels after injection of [^{14}C]tyramine (Tyr, 10 μg kg^{-1} i.v.) in the rat.

Treatment (dose mg kg^{-1})	% injected Tyr g^{-1} cardiac tissue (mean with s.d.)
Tyr	1·40 s.d. 0·23 (controls)
DMI (1) + Tyr	0·99 s.d. 0·16*
DMI (2·5) + Tyr	0·75 s.d. 0·07*
DMI (5) + Tyr	0·49 s.d. 0·08*
Tyr	1·20 s.d. 0·14 (controls)
MD780515 + Tyr	2·87 s.d. 0·17*
DMI (1) + MD780515 + Tyr	2·32 s.d. 0·54*
DMI (2·5) + MD780515 + Tyr	1·26 s.d. 0·21
DMI (5) + MD780515 + Tyr	0·84 s.d. 0·09*

* See footnote to Table 1.

Rattray & Fennessy (1973) have shown that tyramine at a dose of 1 mg kg^{-1} i.v. in the anaesthetized rat produces a marked rise in blood pressure. Recently, Pourrias et al (personal communication) have obtained a threshold effect on blood pressure after injection of 150 μg kg^{-1} in conscious rats. Imipramine (2·5 to 10 mg kg^{-1}) does not inhibit the uptake of tyramine (150 μg kg^{-1} i.v.) (Table 4). These results are similar to those obtained by Brodie et al (1968) with desipramine. MD780515 signifi-

cantly increases the amount of radioactivity in the heart of rats treated with this dose of tyramine. However, in rats administered imipramine, MD780515 treatment no longer produces a significant increase in cardiac radioactivity.

Table 4. Effect of MD780515 (3·5 mg kg^{-1} orally), imipramine (IMI) and their association on the cardiac radioactivity levels after injection of [^{14}C]tyramine (Tyr, 150 μg kg^{-1} i.v.) in the rat.

Treatment (dose mg kg^{-1})	% injected Tyr g^{-1} cardiac tissue (mean with s.d.)
Tyr	0·68 s.d. 0·05 (controls)
IMI (2·5) + Tyr	0·68 s.d. 0·24
IMI (5) + Tyr	0·70 s.d. 0·18
IMI(10) + Tyr	0·69 s.d. 0·13
Tyr	0·69 s.d. 0·11 (controls)
MD780515 + Tyr	0·87 s.d. 0·09*
IMI (2·5) + MD780515 + Tyr	0·74 s.d. 0·07
IMI (5) + MD780515 + Tyr	0·79 s.d. 0·14
IMI (10) + MD780515 + Tyr	0·76 s.d. 0·12

* See footnote to Table 1.

TCAs are competitive inhibitors of the noradrenaline uptake at the central and peripheral nerve endings (Iversen 1975; Møller Nielsen 1980) even if, according to Horn et al (1971), a non-competitive inhibition is seen in the striatum. Tyramine acts as an alternative substrate for the noradrenaline neuronal uptake system (Iversen 1975). It seems therefore reasonable that there might be some competitive effect between imipramine and tyramine at the postganglionic sympathetic innervation of the heart, explaining at least partially why imipramine, at the doses tested, is not effective in antagonizing the uptake of tyramine when the dose of the latter is increased 15 times. Moreover, there is evidence for an extraneuronal exogenous amine uptake (Iversen 1975) on which tricyclic antidepressants like imipramine and desipramine are only weakly active. Since extraneuronal uptake has a low affinity for amines, its importance should be greater with higher doses of tyramine. Although imipramine itself does not alter the accumulation of radioactivity in the heart after a high dose of tyramine, it antagonizes the effects of MD780515 on cardiac radioactivity (Table 4). The reason for this effect is not known, but may suggest that while most of the radioactivity after 150 μg kg^{-1} tyramine accumulates in the heart via the extraneuronal imipramine-insensitive uptake, MAO inhibition might preferentially lead to an increase in intraneuronal radioactivity (via the imipramine-sensitive uptake system).

Urinary elimination of unchanged amphetamine and cytochrome P-450 determination

MD780515 does not modify the amount of unchanged amphetamine eliminated in urine: 20·80 (2·17)% (mean with s.d.) of the administered radioactivity in controls compared with 18·66 (4·03)% and 20·29 (4·72)% in rats treated with 3·5 and 35 mg kg^{-1} of MD780515 respectively. Therefore MD780515 should not inhibit the liver microsomal drug metabolizing enzymes. Further evidence that MD780515 has no effect on this enzyme system was obtained by its lack of effect on cytochrome P-450: 0·74 (0·06) nmol mg^{-1} rat liver microsomal protein (mean and s.d.) in controls compared with 0·81 (0·05) in rats treated with MD780515. Another MAOI which does not alter the metabolism of amphetamine is clorgyline (Simpson 1978). Tricyclic antidepressants are mainly metabolized by the cytochrome P-450-dependent mono-oxygenases. Thus in the case of imipramine, metabolic pathways such as *N*-demethylation, hydroxylation and *N*-oxide formation have been described (Hathway 1970). Therefore it is reasonable to expect that MD780515 administered in association with tricyclic antidepressants should not interfere with their metabolism. For this reason, one could conceivably start a treatment with tricyclic drugs immediately after stopping MD780515 administration. Finally, by their property of inhibiting the uptake of endogenous and exogenous amines, tricyclic antidepressants might protect against possible, but short lasting, pressor effects of tyramine after MD780515 administration (Ilett et al 1980).

Acknowledgements

The authors would like to thank Dr P. E. Keane for useful discussions, and Mrs M. Farny for careful preparation of the manuscript. Mr J. P. Defaux provided skilful technical assistance, and Mr B. Laquais gave advice on statistical analysis of the data.

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