Br. J. Pharmac. (1984), **82**, 151–154

Effect of nonselective and selective inhibitors of monoamine oxidases A and B on pethidine toxicity in mice

R. Boden, Regina Botting, P. Coulson & Gillian Spanswick

Department of Biological Sciences, North East Surrey College of Technology, Reigate Road, Ewell, Surrey, KT17 3DS

1 The LD_{50} of pethidine was determined in mice pretreated (4 h) either with the nonselective monoamine oxidase (MAO) inhibitor, phenelzine or with clorgyline, a selective inhibitor of MAO A or deprenyl, a selective inhibitor of MAO B.

2 Phenelzine or combined clorgyline plus deprenyl pretreatments decreased pethidine LD_{50}.

3 Clorgyline or deprenyl alone did not affect pethidine toxicity.

4 Whole brain 5-hydroxytryptamine (5-HT) concentrations were measured in the pretreated mice. 5-HT levels were approximately doubled ($P < 0.001$) after phenelzine or clorgyline plus deprenyl treatment, but not after clorgyline or deprenyl given alone.

5 These results indicate that both MAO A and MAO B need to be inhibited to increase pethidine toxicity and brain 5-HT levels. They support the involvement of 5-HT in the toxic interaction between pethidine and MAO inhibitors.

Introduction

Pethidine administered to patients already receiving monoamine oxidase (MAO) inhibitors causes serious toxic effects including excitement and hyperthermia (Taylor, 1962; Goldberg, 1964). This toxic interaction can also be produced in mice (Rogers & Thornton, 1969; Jounela, 1970; Botting *et al.*, 1978) and in rabbits (Loveless & Maxwell, 1965; Penn & Rogers, 1971; Fahim *et al.*, 1972; Jounela *et al.*, 1977). Both the increased lethality of pethidine in mice and the hyperthermia in rabbits have been attributed to an increase in brain 5 hydroxytryptamine (5-HT) concentrations induced by the MAO inhibitors (Rogers & Thornton, 1969; Jounela, 1970; Gong & Rogers, 1971; Fahim *et al.*, 1972; Botting *et al.*, 1978).

Two forms of MAO are present in mammalian brain; MAO A for which the preferred substrates are 5-HT and noradrenaline (NA) and MAO B which shows selectivity for phenylethylamine (Knoll *et al.*, 1965; Hall *et al.*, 1969; Yang & Neff, 1974). Nonselective inhibitors of both types of MAO such as phenelzine, tranylcypromine or pargyline increased the toxicity of pethidine (Loveless & Maxwell, 1965; Rogers & Thornton, 1969; Jounela, 1970; Penn & Rogers, 1971; Botting *et al.*, 1978). Clorgyline, a selective inhibitor for MAO A (Hall *et al.*, 1969) and deprenyl of MAO B (Knoll *et al.*, 1965) have also been tested. The toxic pethidine hyperthermia can be seen in rabbits following pretreatment with clorgyline and deprenyl given together, but not with either MAO inhibitor given alone (Jounela *et al.*, 1977). These two selective MAO inhibitors were tested in this study for their effect on the toxicity of pethidine in mice.

Methods

Toxicity measurements

LD_{50} values for pethidine were measured following various pretreatments according to the method of Litchfield & Wilcoxon (1949). CPLF mice of either sex weighing 25–40 g were divided into groups of 10 animals. In any one test, the weight of the heaviest mouse did not differ from the weight of the lightest by more than 10 g. Between four and eight groups were used in each test. Doses of pethidine between 50 and 150 mg kg^{-1}, increasing by steps of 10 mg kg^{-1}, were given intraperitoneally to each group. Pretreatments consisted of phenelzine (50 mg kg^{-1}), clorgyline (20

and 40 mg kg^{-1}), deprenyl (20 and 40 mg kg^{-1}) or combinations of clorgyline (2.5 mg kg^{-1}) plus deprenyl (2.5 mg kg^{-1}) and clorgyline (10 mg kg^{-1}) plus deprenyl (10 mg kg^{-1}). Doses of inhibitors were administered subcutaneously 4 h before the pethidine. Ambient temperatures were between 25°C and 28°C. Percentage mortality was assessed for each group 2 h after the pethidine injection.

Brain 5-hydroxytryptamine concentrations

To compare the effects of various pretreatments on 5-HT levels, brains were removed 4 h after treatment with MAO inhibitors and immediately frozen in liquid nitrogen. 5-HT concentrations were measured by the fluorimetric method of Curzon & Green (1970).

Drugs

Pethidine hydrochloride (Roche), phenelzine hydrogen sulphate (Warner), clorgyline (May and Baker Ltd), deprenyl hydrochloride (Chinoin, Budapest) and 5-hydroxytryptamine hydrochloride (Sigma) were used.

Results

Toxicity measurements

Pethidine LD_{50} values are listed in Table 1. These show that the LD_{50} dose of pethidine was markedly reduced, by approximately 40% after pretreatment of the mice with phenelzine (50 mg kg^{-1}) and combined clorgyline plus deprenyl pretreatments (10 mg kg^{-1} of each). Pretreatment with lower doses of the selective inhibitors (2.5 mg kg^{-1} of each) caused a real but slightly smaller increase in toxicity (37%). Conversely, pretreatment with either clorgyline or deprenyl on their own did not increase mortality even if doses of 40 mg kg^{-1} were used.

Brain 5-hydroxytryptamine concentrations

The values for these are listed in Table 2. Following treatment with phenelzine (50 mg kg^{-1}) or combined clorgyline plus deprenyl (10 mg kg^{-1} of each), 5-HT concentrations increased by 103% and 90% respectively compared to saline-injected controls. After clorgyline (20 mg kg^{-1}) on the other hand, 5-HT concentrations increased by only 26% and after deprenyl (20 mg kg^{-1}) hardly at all. Only the increases produced by phenelzine or combined clorgyline plus deprenyl treatments were significantly different from saline-treated controls, ($P < 0.001$).

Discussion

The work of Rogers & Thornton (1969), Jounela (1970), and Botting *et al.* (1978) indicated that the increased toxicity of pethidine in mice after pretreatment with nonselective MAO inhibitors was due to a rise in brain 5-HT concentrations. Clorgyline has a selective inhibitory effect on MAO A, the enzyme which preferentially metabolizes 5-HT in the rat brain (Yang & Neff, 1974). It seemed likely therefore that clorgyline would also increase the toxicity of pethidine. This in fact did not happen even with high doses of clorgyline. For the toxicity of pethidine to be increased it was necessary to administer clorgyline and deprenyl together and inhibit both MAO A and MAO B. It was previously shown by Jounela *et al.*, (1977) that pethidine hyperpyrexia in rabbits occurred only when both MAO A and MAO B were inhibited by a combination of clorgyline and deprenyl administered together, and not when clorgyline was

Table 1 Lethality of pethidine after pretreatment of mice with monoamine oxidase (MAO) inhibitors

Pretreatment	(mg kg^{-1})	LD_{50} *pethidine* (mg kg^{-1})	*% change in toxicity*
Saline		127 (117–138)	
Phenelzine	50	75 (67 – 84)	+41
Clorgyline + deprenyl	10 + 10	76 (67 – 86)	+40
Clorgyline + deprenyl	2.5 + 2.5	80 (71 – 91)	+37
Clorgyline	20	125 (115 – 135)	+ 2
Clorgyline	40	122 (114 – 130)	+ 4
Deprenyl	20	132 (116 – 150)	– 4
Deprenyl	40	120 (108 – 133)	+ 6

95% confidence limits are shown in parentheses.
Pethidine was injected (i.p.) 4 h after pretreatment with MAO inhibitor (s.c.) and mortality assessed 2 h after pethidine administration. Toxicity was increased by phenelzine and by combined treatments with clorgyline plus deprenyl, but not by either selective inhibitor administered alone.

Table 2 Brain 5-hydroxytryptamine (5-HT) concentrations after treatment with monoamine oxidase (MAO) inhibitors

Treatment	(mg kg^{-1})	n	*5-HT concentration* (μg g^{-1}) (mean ± s.e.)	*% increase in 5-HT concentration*
Controls		(5)	0.59 ± 0.03	
Phenelzine	50	(6)	1.20 ± 0.04*	103
Clorgyline + deprenyl	10 + 10	(6)	1.12 ± 0.09*	90
Clorgyline	20	(6)	0.74 ± 0.07^{NS}	26
Deprenyl	20	(6)	0.61 ± 0.02^{NS}	3

The mice were killed 4 h after (s.c.) injection of MAO inhibitors. *n*, indicates number of animals. Significant differences from controls were calculated by Student's *t* test for independent means and marked: *$P < 0.001$; NS not significant ($P > 0.05$).

given alone. Another interaction which depends on increased brain 5-HT concentrations is hyperactivity produced in rats as a result of administration of a MAO inhibitor and L-tryptophan. This could only be seen after treatment with nonselective MAO inhibitors or both selective MAO inhibitors (clorgyline and deprenyl) given together (Squires & Buus Lassen, 1975; Green & Youdim, 1976). It is therefore possible that the metabolism of 5-HT can still proceed by means of MAO B if MAO A is inhibited by the selective enzyme inhibitor. Green & Youdim (1975) in fact suggested that in rat brain, MAO B can oxidize 5-HT when MAO A is completely inhibited by clorgyline. Also, Mitra & Guha (1980) showed that even in brain homogenates of untreated rats, MAO B was responsible for some of the 5-HT oxidation. This would necessitate inhibition of both MAO A and B in order to achieve the largest increase in 5-HT concentrations.

This is supported in this study by measurements of brain 5-HT concentrations after various MAO inhibitors, (Table 2). 5-HT concentrations approximately doubled after phenelzine and clorgyline plus deprenyl pretreatments. A large dose of clorgyline on the other hand only raised the 5-HT concentration moderately, and it was only slightly altered by a large dose of deprenyl. Neither of these increases was significantly different from controls. The increased toxicity of pethidine is also unlikely to be caused by a decreased breakdown by the liver. Doses of deprenyl greater than 10 mg kg^{-1} nonselectively inhibit drug metabolizing liver microsomal enzymes, (J. Knoll, personal communication) yet doses of deprenyl greater than this (without clorgyline) were without effect on pethidine lethality.

These results, therefore, show that the increased toxicity of pethidine can only be manifested when both MAO A and MAO B enzymes are inhibited and is associated with the highest 5-HT brain concentrations. It is possible that increased pethidine toxicity may be caused by an increase in the functional activity of 5-HT at the nerve endings as suggested by Rogers & Thornton (1969) and supported by the fact that methysergide prevents the increase in toxicity (Botting *et al.*, 1978). Alternatively, other brain substances as well as 5-HT, may be involved in the toxic interaction. For example, noradrenaline is a substrate for MAO A and dopamine and tryptamine are substrates for both forms of the enzyme (Yang & Neff, 1974). Pretreatment with non-selective MAO inhibitors such as tranylcypromine, increases the concentrations of these amines in mouse brains (Rogers & Thornton, 1969; Tabakoff *et al.*, 1977). The concentrations of these substances may also be greatly increased by clorgyline and deprenyl given together, but much less so when either MAO inhibitor is given alone. Estimations of the concentrations of these amines after different pretreatments would be needed to resolve this question.

Grateful thanks are due to Professor J. Knoll for the generous gift of deprenyl hydrochloride, William R. Warner for phenelzine hydrogen sulphate and May and Baker Ltd for clorgyline.

Please address correspondence to Regina Botting.

References

BOTTING, R., BOWER, S., EASON, C.T., HUTSON, P.H. & WELLS, L. (1978). Modification by monoamine oxidase inhibitors of the analgesic, hypothermic and toxic actions of morphine and pethidine in mice. *J. Pharm. Pharmac.*, **30**, 36–40.

CURZON, G. & GREEN, A.R. (1970). Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindolacetic acid in small regions of rat brain. *Br. J. Pharmac.*, **39**, 653–655.

FAHIM, I., ISMAIL, M., & OSMAN, O.H. (1972). The role of

5-hydroxytryptamine and noradrenaline in the hyperthermic reaction induced by pethidine in rabbits pretreated with pargyline. *Br. J. Pharmac.*, **46,** 416–422.

GOLDBERG, L.I. (1964). Monoamine oxidase inhibitors. *J. Am. med. Ass.*, **190,** 456–462.

GONG, S.N.C. & ROGERS, K.J. (1971). Role of brain monoamines in the fatal hyperthermia induced by pethidine or imipramine in rabbits pre-treated with pargyline. *Br. J. Pharmac.*, **42,** 646–647P.

GREEN, A.R. & YOUDIM, M.B.H. (1975). Effects of monoamine oxidase inhibition by clorgyline, deprenil or tranylcypromine on 5-hydroxy-tryptamine concentrations in rat brain and hyperactivity following subsequent tryptophan administration. *Br. J. Pharmac.*, **55,** 415–422.

GREEN, A.R. & YOUDIM, M.B.H. (1976). Use of a behavioural model to study the action of monoamine oxidase inhibition in vivo. In *Monoamine Oxidase and its Inhibition.* Ciba Foundation Symposium 39 (new series), pp. 231–245. Amsterdam: Elsevier Scientific Publishing Company.

HALL, D.W.R., LOGAN, B.W. & PARSONS, G.H. (1969). Further studies on the inhibition of monoamine oxidase by M & B 9302 (clorgyline) -I. *Biochem. Pharmac.*, **18,** 1447–1454.

JOUNELA, A.J. (1970). Influence of phenelzine on the toxicity of some analgesics in mice. *Ann. Med. exp. Fenn.*, **48,** 261–265.

JOUNELA, A.J., MATTILA, M.J. & KNOLL, J. (1977). Interaction of selective inhibitors of monoamine oxidase with pethidine in rabbits. *Biochem. Pharmac.*, **26,** 806–808.

KNOLL, J., ECSERY, Z., KELEMAN, K., NIEVEL, J., & KNOLL, B. (1965). Phenylisopropylmethylpropynylamine (E250), a new spectrum psychic energizer. *Archs int. Pharmacodyn.*, **155,** 154–164.

LITCHFIELD, J.T. & WILCOXON, F. (1949). A simplified method of evaluating dose-effect experiments. *J. Pharmac. exp. Ther.*, **96,** 99–113.

LOVELESS, A.H. & MAXWELL, D.R. (1965). A comparison of the effects of imipramine, trimipramine, and some other drugs in rabbits treated with a monoamine oxidase inhibitor. *Br. J. Pharmac. Chemother.*, **25,** 158–170.

MITRA, C. & GUHA, S.R. (1980). Serotonin oxidation by type B MAO of rat brain. *Biochem. Pharmac.*, **29,** 1213–1216.

PENN, R.G. & ROGERS, K.J. (1971). Comparison of the effects of morphine, pethidine and pentazocine in rabbits pretreated with a monoamine oxidase inhibitor. *Br. J. Pharmac.*, **42,** 485–492.

ROGERS, K.J. & THORNTON, J.A. (1969). The interaction between monoamine oxidase inhibitors and narcotic analgesics in mice. *Br. J. Pharmac.*, **36,** 470–480.

SQUIRES, R.F. & BUUS LASSEN, J. (1975). The inhibition of A and B forms of MAO in the production of a characteristic behavioural syndrome in rats after 1-tryptophan loading. *Psychopharmacologia,* **41,** 145–151.

TABAKOFF, B., MOSES, F., PHILLIPS, S.R. & BOULTON, A.A. (1977). Effects of tranylcypromine and pargyline on brain tryptamine. *Experientia,* **33,** 380–381.

TAYLOR, D.C. (1962). Alarming reaction to pethidine in patients on phenelzine. *Lancet,* **ii,** 401–402.

YANG, H.-Y.T. & NEFF, N.H. (1974). The monoamine oxidases of brain; selective inhibition with drugs and the consequences for the metabolism of the biogenic amines. *J. Pharmac. exp. Ther.*, **189,** 733–740.

(*Received September 16, 1983.*

Revised January 13, 1984.)