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Modification of blood pressure and nictitating membrane response to sympathetic amines by selective monoamine oxidase inhibitors, types A and B, in the cat

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1 The selective monoamine oxidase (MAO) inhibitors clorgyline, selegiline and AGN 1135 did not cause a change in responses of the cat nictitating membrane to preganglionic sympathetic nerve stimulation at 5 Hz.

2 Both selective MAO-A and MAO-B inhibitors markedly potentiated nictitating membrane contractions in response to β-phenylethylamine (PEA). However, the responses to tyramine were unchanged.

3 The pressor responses to tyramine were potentiated by the selective MAO-A inhibitor clorgyline ($2\,mg\,kg^{-1}$) but not by selegiline ($1.0\,mg\,kg^{-1}$) and AGN 1135 ($1.5\,mg\,kg^{-1}$), selective MAO-B inhibitors.

4 At the doses used selegiline and AGN 1135 caused a near total selective inhibition of liver and brain MAO-B, while clorgyline inhibited MAO-A only in the brain.

5 AGN 1135, like selegiline, could be a useful drug in potentiating the action of L-DOPA in Parkinson's disease.

Introduction

The major limitation to the clinical use of monoamine oxidase (MAO) inhibitors is their ability to potentiate the actions of tyramine on the cardiovascular system ('cheese effect') (see Youdim & Finberg 1982, for review). Tyramine is a substrate for both type A and type B MAO and so inhibition of either of these enzyme subtypes might be expected to reduce its metabolism and lead to potentiation of cardiovascular and other effects. Pressor responses to tyramine in man, however, are potentiated by selective MAO type A inhibition with clorgyline (Lader *et al.*, 1970), but not during selective MAO type B inhibition with selegiline (Birkmayer *et al.*, 1977; Elsworth *et al.*, 1978). This observation may be related to the fact that neuronal MAO is largely of type A (Jarrot, 1971; Coquil *et al.*, 1973). The effects of oral tyramine would also be affected more by MAO type A than by type B inhibition, since MAO in the gastrointestinal tract is mainly of the A subtype (Youdim, 1977; Garcha *et al.*, 1979). Knoll (1978) has shown that selegiline can block neuronal uptake of tyramine and interfere with tyramine-induced noradrenaline release, although these effects of selegiline were seen only at very high concentrations. Glover *et al.* (1983) have demonstrated that relatively low concentrations of selegiline (1 μM) can antagonize tyramine-induced noradrenaline release from rat cerebral cortex slices.

An important question to answer, therefore, is whether tyramine potentiation is a property of MAO type A but not type B inhibitors in general, or whether selegiline has a specific, tyramine-antagonistic property. We (Finberg *et al.*, 1980; Kalir *et al.*, 1981; Finberg *et al.*, 1981a,b) have previously described some pharmacological properties of AGN 1135 (N-2-propynyl-1-indanamine hydrochloride). This compound is an irreversible MAO type B inhibitor with a selectivity in the rat *in vivo*, similar to that of selegiline. At doses selective for MAO-B inhibition, neither AGN 1135 nor selegiline potentiated tyramine responses in the rat vas deferens *in vitro* but higher concentrations of both inhibitors caused an initial inhibition of tyramine responses followed by potentiation when the inhibitor was washed out of the organ bath (Finberg *et al.*, 1981a). AGN 1135 was also devoid of the sympathomimetic effects seen following selegiline administration (Finberg *et al.*, 1981b). The present study was

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undertaken in order to investigate modification of the effects in the cat of the indirectly acting amines tyramine and β-phenylethylamine (PEA) by the two selective MAO type B inhibitors, AGN 1135 and selegiline in comparison with that produced by clorgyline. Some of these data were presented in a preliminary form at a symposium on Monoamine Oxidase, Hakone, Japan (Finberg, 1982).

Methods

Anaesthetized cats

Cats of either sex weighing 2.5 to 3.5 kg were anaesthetized with 4% halothane in oxygen/nitrous oxide (15/85 parts by volume) followed by intravenous α-chloralose (80 mg kg^{-1}). Rectal temperature was maintained at 37 ± 1°C by an electrical heating pad coupled to a thermostatic temperature controller. Contractions of the right nictitating membrane were recorded by means of an isometric Statham UC 2 transducer coupled to a Beckman physiological recorder. The nictitating membrane was given a resting tension of 1.0g. The right superior cervical nerve was stimulated preganglionically using 1.0 ms pulses at maximal voltage, and 2, 5 and 10 Hz for 30 s. Drugs were administered intravenously via a femoral vein catheter. Blood pressure was recorded with a Statham P23Db transducer connected to a femoral artery and coupled to the Beckman recorder. Control blood pressure and nictitating membrane responses to two submaximal intravenous doses of noradrenaline, tyramine and PEA were obtained, as well as control nictitating membrane responses to preganglionic nerve stimulation, following which the inhibitors were injected intravenously, and the same doses of the amines repeated, starting 30 min after injection of the inhibitor. Peak blood pressure and nictitating membrane responses after inhibitor injection were expressed as a percentage of control responses in the same animal and compared to values obtained with control animals, in which normal saline was injected instead of MAO inhibitor. At the end of the experiment, animals were killed by air embolism, portions of liver and the entire brain were removed and stored at − 20°C for subsequent determination of MAO.

Determination of monoamine oxidase activity

This was carried out by the general method of Tipton & Youdim (1976) in which tissue homogenates are incubated with ^{14}C-labelled 5-hydroxytryptamine (5-HT) (MAO type A activity) and PEA (MAO type B activity), following which deaminated metabolites are separated on small Amberlite columns. Brain MAO activity was determined on portions of a homogenate prepared from half of the brain (divided by a midline longitudinal incision).

Drugs

AGN 1135 and AGN 1133 were obtained from Aspro Nicholas Research Ltd. (Australia). Selegiline ((−)-deprenyl) was donated by Professor J. Knoll (Semmelweiss University, Budapest, Hungary); clorgyline was a gift from May and Baker, Ltd., and the remaining chemicals were obtained from Sigma Ltd. Phenylethylamine, β-[-ethyl-1-^{14}C] and hydroxytryptamine, [5-1,2-^{3}H]-creatinine sulphate were supplied by New England Nuclear Ltd.

Results

Response of nititating membrane to monoamine oxidase inhibition

None of the MAO inhibitor drugs caused any significant change in responses of cat nictitating mem-

Table 1 Modification of nictitating membrane responses by MAO inhibitors in anaesthetized cats

	Control ($n = 5$)	*Clorgyline* 2 mg kg^{-1} ($n = 5$)	*Selegiline* 0.5 mg kg^{-1} ($n = 6$)	*AGN 1135* 1.5 mg kg^{-1} ($n = 9$)
Noradrenaline (0.4 μg kg^{-1})	0.81 ± 0.29	0.92 ± 0.08	1.45 ± 0.37	1.16 ± 0.15
Tyramine (40 μg kg^{-1})	1.28 ± 0.11	1.69 ± 0.39	1.99 ± 0.40	1.64 ± 0.13
Phenylethylamine (50 μg kg^{-1})	0.95 ± 0.13	3.59 ± 0.73 $P<0.01$	5.5 ± 1.25 $P<0.01$	4.4 ± 1.26 $P<0.01$
SNS (5 Hz)	1.05 ± 0.05	1.22 ± 0.13	1.11 ± 0.09	1.11 ± 0.16

Data shown are response ratios, i.e. peak contractile response after MAO inhibitor treatment divided by peak contractile response before injection of MAO inhibitor. Response ratios shown ± s.e. and significance of difference from ratios in control animals as determined using Wilcoxon rank sum test. Values without P level are not significantly different from controls.

Table 2 Modification of pressor responses to sympathomimetic amines by MAO inhibitors in anaesthetized cats

Sympathomimetic amine and dose ($\mu g\,kg^{-1}$)	*Control* ($n = 6$)	*Clorgyline* $2\,mg\,kg^{-1}$ ($n = 5$)	*Selegiline* $1.0\,mg\,kg^{-1}$ ($n = 6$)	*AGN 1135* $1.5\,mg\,kg^{-1}$ ($n = 9$)
Noradrenaline 0.2	1.08 ± 0.08	1.1 ± 0.11	1.09 ± 0.19	1.23 ± 0.19
0.4	1.15 ± 0.12	1.25 ± 0.11	0.93 ± 0.05	1.23 ± 0.22
Tyramine 20	1.32 ± 0.18	1.60 ± 0.15	1.33 ± 0.33	1.25 ± 0.42
40	1.13 ± 0.07	1.71 ± 0.1 $P<0.05$	0.98 ± 0.18	1.39 ± 0.33
Phenylethylamine 25	1.07 ± 0.10	2.00 ± 0.16 $P<0.05$	1.36 ± 0.17	1.42 ± 0.23
50	0.99 ± 0.06	1.70 ± 0.32 $P<0.05$	1.17 ± 0.14	1.19 ± 0.12

Data shown are response ratios i.e. peak pressor response to amine after MAO inhibitor treatment divided by peak pressor response before injection of MAO inhibitor. Response ratios shown < s.e. and significance of difference from ratios in control animals (no treated with MAO inhibitor) determined by Wilcoxon rank sum test.

brane to preganglionic sympathetic nerve stimulation at 2, 5, 10 Hz. The results at 5 Hz are shown in Table 1. Clorgyline, selegiline and AGN 1135 all caused powerful potentiation of nictitating membrane contractions in response to PEA, but none of the inhibitors potentiated nictitating membrane responses to tyramine or noradrenaline (Table 1).

Pressor responses to sympathomimetic amines in anaesthetized cats

Clorgyline ($2\,mg\,kg^{-1}$), selegiline ($1.0\,mg\,kg^{-1}$) and AGN 1135 ($1.5\,mg\,kg^{-1}$) did not significantly affect pressor responses to noradrenaline but pressor responses to tyramine (high dose, $40\,\mu g\,kg^{-1}$) and PEA

Table 3 MAO types A and B activity in brain and liver of cats following treatment with selective inhibitors

		MAO type A (5-HT substrate) Brain	Liver	*MAO type B* (PEA substrate) Brain	Liver
Controls ($n = 8$)	mean	9.9	32.8	5.2	13.6
	s.e.mean	1.5	4.4	1.5	1.7
Selegiline $0.5\,mg\,kg^{-1}$ ($n = 4$)	mean	9.6	21.8	0.24	2.00
	s.e.mean	1.75	4.9	0.045 $P<0.01$	0.59 $P<0.001$
AGN 1135 $1.5\,mg\,kg^{-1}$ ($n = 4$)	mean	15.1	39.0	0.21	0.91
	s.e.mean	4.2	6.5	0.065 $P<0.05$	0.08 $P<0.001$
Clorgyline $2\,mg\,kg^{-1}$ ($n = 4$)	mean	3.5	22.6	5.5	10.7
	s.e.mnea	1.01 $P<0.01$	2.83	1.53	1.43

Enzyme activities expressed as $nmol\,h^{-1}\,mg^{-1}$ protein.
P = probability of difference from control values using Student's t test.

were potentiated by clorgyline. Neither selegiline nor AGN 1135 caused significant modification of the pressor responses to noradrenaline, tyramine and PEA when the inhibitors were injected intravenously in low doses (1.0 and 1.5 mg kg^{-1} respectively), see Table 2. When the dose of AGN 1135 was increased to 5 mg kg^{-1}, pressor responses to tyramine (40 μg kg^{-1}) were potentiated in 3 out of 4 cat experiments (data not shown). Increasing the dose of selegiline to 2 mg kg^{-1} (3 cat experiments) caused an inhibition of tyramine (40 μg kg^{-1}) pressor responses in two cats and no change in the third (data not shown).

Monoamine oxidase type A and type B activities

The activity of MAO type B in both brain and liver was decreased to about 10–15% of control values by the doses of selegiline (0.5 mg kg^{-1}) and AGN 1135 (1.5 mg kg^{-1}) used (Table 3). However, clorgyline (2 mg kg^{-1}) was almost without effect on this enzyme activity. In contrast, the activity of MAO type A in the liver and brain was unaffected by selegiline and AGN 1135. Hepatic MAO type A activity was reduced but not significantly by clorgyline, whereas brain MAO type A activity was significantly decreased to 35% of control values (Table 3).

Discussion

The present results show that AGN 1135 is a selective inhibitor of MAO type B in the cat at a dose of 1.5 mg kg^{-1} by intravenous injection. This was demonstrated by a greater than 95% inhibition of MAO activity towards the B-substrate, PEA in liver and brain, without inhibition of MAO activity using 5-HT as substrate. AGN 1135 also caused significant potentiation of nictitating membrane responses to PEA without changing the response of the nictitating membrane or blood pressure to tyramine. AGN 1135, therefore, closely resembles selegiline, in that tyramine responses are not potentiated at a dose that selectively inhibits MAO type B. However, increasing the dose of AGN 1135 to a level (5 mg kg^{-1}) which in the rat produces substantial inhibition of MAO type A (Finberg *et al.*, 1980) resulted in potentiation of tyramine pressor responses, whereas increasing the dose of selegiline produced profound sympathomimetic effects on the nictitating membrane (Finberg *et al.*, 1981b) but an inhibition of tyramine pressor responses. The inhibition of tyramine pressor responses by large doses of selegiline was originally described by Knoll & Magyar (1972) and sympathomimetic responses to selegiline were originally reported by Simpson (1978). We have previously shown that the nictitating membrane contracture produced by selegiline in the cat is an indirect effect, since it is absent in the chronically denervated organ (Finberg *et al.*, 1981b).

The present work extends our original observations (Finberg *et al.*, 1980) that sympathomimetic effects are absent following AGN 1135 administration. This drug produced neither contracture of the nictitating membrane nor change in blood pressure in the cat at doses up to 5 mg kg^{-1} (present study) and no chronotropic or inotropic effects in the rat at the same dose level (Finberg *et al.*, 1980).

The biochemical determinations showed that both selegiline and AGN 1135 were effective in causing inhibition of MAO type B in cat brain and liver. A surprising result, however, was that clorgyline was ineffective in reducing liver MAO type A activity, although this drug did produce significant inhibition of brain MAO A activity. This observation may be related to the fact that cat liver MAO is almost all of the type B variety (Squires, 1972), so that in this tissue, 5-HT may be deaminated by the type B enzyme, if the concentration used (1.0 mM) approaches the K_m for MAO type B (Green & Youdim, 1977; Youdim, 1983). In rat brain, a K_m value of about 2 mM has been calculated for deamination of 5-HT by MAO type B (Fowler & Tipton, 1982). Since MAO type B inhibition had little effect on the deamination of 5-HT by the cat liver *in vitro*, it appears that 5-HT can serve as a substrate for both forms of the enzyme in this tissue, like that of the brain (Green & Youdim, 1975).

The results of these experiments provide further evidence that the most important factor in potentiation of tyramine effects by an MAO inhibitor is inhibition of the neuronal MAO. Since hepatic MAO is mainly type B in the cat, the injection of an MAO type B inhibitor should markedly reduce systemic tyramine metabolism; however, neither AGN 1135 (in a dose selective for MAO B inhibition) nor selegiline caused significant potentiation of tyramine effects, while clorgyline potentiated not only the pressor effects of tyramine but also those of the MAO-B substrate, PEA. These results are similar to those reported by Sandler *et al.* (1978) in the pig, an animal in which most tissue MAO is of the B type. Thus although inhibition of extraneuronal tyramine metabolism would be expected to potentiate effects of the amine by elevating circulating levels, this effect is not seen, since the major pathway of tyramine distribution is the high-affinity neuronal uptake system.

Since K_m values for high affinity uptake of tyramine are in the range of 1.0 μM, while K_m values for low affinity (extraneuronal) uptake of tyramine in rat tissues are of the order of 100 μM (Ross, 1975) it is obvious that high affinity uptake is the preferred route. The route of administration of tyramine may also play an important role in determining its degree of potentiation by MAO inhibitors. Following oral administration, tyramine would be exposed in greatest

concentration to metabolism by hepatic MAO, whereas after intravenous administration, neuronal uptake may be a more important pathway.

Nictitating membrane responses to tyramine were not potentiated significantly by clorgyline, although in individual experiments definite potentiation was seen. This lack of consistent potentiation may be related to the fact that brain MAO type A activity was inhibited by only 65% at the dose of clorgyline used (2 mg kg^{-1}), although this dose level is adequate to cause more than 90% inhibition of brain and liver MAO type A in the rat (Waldmeier *et al.*, 1981). This explanation is based on the fact that PEA is not a substrate for neuronal amine uptake and thus would not be selectively concentrated in nerves and exposed primarily to MAO type A as is tyramine. The effects of PEA are potentiated by clorgyline, since this amine, being an indirectly acting sympathomimetic, releases noradrenaline into the nerve cell cytoplasm, where it is partially deaminated by MAO type A before leaving the neurone.

In conclusion, both selegiline and AGN 1135 produced almost complete inhibition of MAO type B activity in brain and liver without potentiating the pressor and smooth muscle effects of tyramine. Thus AGN 1135 potentially could be a useful drug like selegiline (Birkmayer *et al.*, 1977; Lees *et al.*, 1977) for potentiating the anti-Parkinson action of L-DOPA.

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References

BIRKMAYER, W., RIEDERER, P., AMBROZI, L. & YOUDIM, M.B.H. (1977). Implications of combined treatment with Madopar and l-deprenil in Parkinson's disease. *Lancet*, **i**, 439–444.

COQUIL, J.F., GORIDIS, C., MACK, G. & NEFF, N.H. (1973). Monoamine oxidase in rat arteries: evidence for different forms and selective localization. *Br. J. Pharmac.*, **48**, 590–599.

ELSWORTH, J.D., GLOVER, V., REYNOLDS, G.P., SANDLER, M., LEES, A.J., PHUAPRADIT, P., SHAW, K.M., STERN, G.M. & KUMAR, P. (1978). Deprenyl administration in man: a selective monamine oxidase inhibitor without the cheese effect. *Psychopharmacologia* (Berlin), **57**, 33–38.

FINBERG, J.P.M. (1982). Effect of selective inhibition of MAO types A and B on peripheral sympathetic function. In *Monoamine Oxidase – Basic and Clinical Frontiers*. ed. Kamijo, K., Usdin, E. & Nagatsu, T. pp. 174–182. Amsterdam: Excerpta Medica.

FINBERG, J.P.M., SABBAGH, A. & YOUDIM, M.B.H. (1980). Pharmacology of selective propargyl "suicide" inhibitors of monoamine oxidase. In *Enzymes and Neurotransmitters in Mental Disease*. ed. Usdin, E., Sourkes, T. & Youdim, M.B.H. pp. 205–219. Chichester: Wiley.

FINBERG, J.P.M., TENNE, M. & YOUDIM, M.B.H. (1981a). Tyramine antagonistic properties of AGN 1135, an irreversible inhibitor of monoamine oxidase type B. *Br. J. Pharmac.*, **73**, 65–74.

FINBERG, J.P.M., TENNE, M. & YOUDIM, M.B.H. (1981b). Selective irreversible propargyl derivative inhibitors of monoamine oxidase (MAO) without the cheese effect. In *Monoamine Oxidase Inhibitors – The State of the Art*, ed. Youdim, M.B.H. & Paykel, E.S. pp. 31–44. Chichester: Wiley.

FOWLER, C.J. & TIPTON, K.F. (1981). Concentration dependence of the oxidation of tyramine by the two forms of rat liver mitochondrial monoamine oxidase. *Biochem. Pharmac.*, **30**, 322a–3332.

FOWLER, C.J. & TIPTON, K.F. (1982). Deamination of 5-hydroxytryptamine by both forms of monoamine oxidase in the rat brain. *J. Neurochem.*, **38**, 733–736.

GARCHA, G., IMRIE, P.R., MARLEY, E. & RHOMAS, D.V. (1979). Effects of monoamine oxidase inhibitor (MAOI) pretreatment on the fate of intraduodenally instilled [^{14}C]-tyramine. *Br. J. Pharmac.*, **67**, 454–455P.

GLOVER, V., PYCOCK, C.J. & SANDLER, M. (1983). Tyramine-induced noradrenaline release from rat brain slices: prevention by (−)-deprenyl. *Br. J. Pharmac.*, **80**, 141–148.

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

JARROTT, B. (1971). Occurrence and properties of monoamine oxidase in adrenergic neurons. *J. Neurochem.*, **k18**, 7–16.

KALIR, A., SABBAGH, A. & YOUDIM, M.B.H. (1981). Selective acetylenic "suicide" and reversible inhibitors of monoamine oxidase types A and B. *Br. J. Pharmac.*, **73**, 55–64.

KNOLL, J. (1978). The possible mechanism of action of (−)-deprenyl in Parkinson's disease. *J. Neural. Transmiss.*, **43**, 177–198.

KNOLL, J. & MAGYAR, K. (1972). Some puzzling effects of monoamine oxidase inhibitors. *Adv. Biochem. Psychopharmac.*, **5**, 393–408.

LADER, M.H., SAKALIS, G. & TANSELLA, M. (1970). Interaction between sympathomimetic amines and a new monoamine oxidase inhibitor. *Psychopharmacologia* (Berlin), **18**, 118–123.

LEES, A.J., KOHOUT, L.J., SHAW, K.M. STERN, G.M., ELSWORTH, J.D., SANDLER, M. & YOUDIM, M.B.H. (1977). Deprenyl in Parkinson's disease. *Lancet*, **i**, 791–796.

ROSS, S.B. (1976). Structural requirements for uptake into catecholamine neurons. In *The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines*. ed. Paton, D.M. pp. 67–93. New York: Raven Press.

SANDLER, M., GLOVER, V., ASHFORD, A. & STERN, G.M. (1978). Absence of "cheese effect" during deprenyl therapy; some recent studies. *J. Neural. Transmiss.*, **43**, 209–215.

SIMPSON, L.L. (1978). Evidence that deprenyl, a type B

monoamine oxidase inhibitor, is an indirectly acting sympathomimetic amine. *Biochem. Pharmac.*, **27**, 1591–1595.

SQUIRES, R.F. (1972). Multiple forms of monoamine oxidase in intact mitochondria as characterized by selective inhibitors and thermal stability: a comparison of eight mammalian species. *Adv. Biochem. Psychopharmac.*, **5**, 355–370.

TIPTON, K.F. & YOUDIM, M.B.H. (1976). Assay of monoamine oxidase. In *Monoamine Oxidase and its Inhibition*. ed. Wolstenholme, G.E.W. & Knight, J. Ciba Foundation Symposium No. 39. pp. 393–403. Amsterdam: Elsevier.

WALDMEIER, P.C., FELNER, A.E. & MAITRE, L. (1981). Long term effects of selective MAO inhibitors on MAO activity and amine metabolism. In *Monoamine Oxidase Inhibitors – The State of the Art*. ed. Youdim. M.B.H. & Paykel, E.S. pp. 87–102. Chichester: Wiley.

YOUDIM, M.B.H. (1977). Tyramine and psychiatric disorders. In *Neuroregulators and Psychiatric Disorders*. ed. Usdin, E., Hamburg, D. & Barchas, J.D. pp. 57–67. Amsterdam: Oxford University Press.

YOUDIM, M.B.H. (1983). *In vivo* noradrenaline is a substrate for monoamine oxidase A and B. *Br. J. Pharmac.*, **79**, 477–480.

YOUDIM, M.B.H. & FINBERG, J.P.M. (1982). Monoamine oxidase inhibitor anti-depressants. In *Psychopharmacology*, I. ed. Grahame-Smith, D., Hippius, H. & Winokur, G. pp. 38–78. Amsterdam: Excerpta Medica.

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