Br. J. Pharmac. (1981), **73**, 65–74

TYRAMINE ANTAGONISTIC PROPERTIES OF AGN 1135, AN IRREVERSIBLE INHIBITOR OF MONOAMINE OXIDASE TYPE B

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1 The effects of the irreversible monoamine oxidase (MAO) inhibitors, AGN 1133, AGN 1135 and (−)-deprenyl, on tyramine and noradrenaline responses and uptake of [^{3}H]-metaraminol were investigated in the isolated vas deferens of the rat. Uptake of [^{3}H]-metaraminol and [^{3}H]-octopamine was compared in mouse vas deferens. The modification of tyramine and noradrenaline-induced pressor responses by AGN 1133 and AGN 1135 was examined in anaesthetized rats and cats.

2 AGN 1133 (7.5×10^{-6}M) greatly potentiated responses to tyramine in the rat isolated vas deferens. Both AGN 1135 and (−)-deprenyl inhibited tyramine responses selectively at concentrations above 10^{-5}M (which caused almost complete inhibition of MAO types A and B) but tyramine responses were potentiated on washing out the inhibitors.

3 AGN 1135 (10^{-4}M) and (−)-deprenyl (10^{-5}M) inhibited [^{3}H]-metaraminol uptake by about 20% in rat and mouse vas deferens; neither inhibitor affected [^{3}H]-octopamine uptake in mouse vas deferens. Desmethylimipramine (10^{-6}M) inhibited amine uptake by more than 70%.

4 AGN 1133 (1.5 mg/kg) potentiated pressor responses to tyramine in rats and cats whereas AGN 1135 (1.5 mg/kg) did not.

5 AGN 1135 possesses tyramine antagonistic activity which is qualitatively similar to that of (−)-deprenyl but which cannot satisfactorily be explained by inhibition of neuronal or granular amine uptake.

Introduction

The discovery in recent years that monoamine oxidase (MAO) consists of multiple forms which differ in their substrate affinities and can be selectively inhibited by drugs (Squires, 1972; Youdim, 1972; Houslay, Tipton & Youdim, 1976) has led to the renewal of interest in the therapeutic value of MAO inhibitors (Birkmayer, Riederer, Ambrozi & Youdim, 1977; Fuller, 1978). Most studies point to the existence of two distinct forms of the enzyme (see Fowler, Callingham, Mantle & Tipton, 1978, for review). The enzyme form which is selectively inhibited by clorgyline has been termed MAO-A (Johnston, 1968) and shows greatest affinity for 5-hydroxytryptamine (5-HT) as substrate, whereas (−)-deprenyl selectively inhibits the enzyme form (MAO-B) which shows highest affinity for the substrates β-phenylethylamine (PEA) and benzylamine (Knoll & Magyar, 1972; Yang & Neff, 1973). Tyramine and dopamine are substrates for both types of the enzyme (Johnston, 1968). Inhibition of MAO is accompanied by potentiation of the effects of indirectly acting sympathomimetic amines, such as tyramine. This effect is the basis of the well-known 'cheese reaction' which has limited the clinical use of the MAO inhibitors (Blackwell, Marley, Price & Taylor, 1967; Stockley, 1973; Youdim, 1977).

The selective inhibitor of MAO-B, (−)-deprenyl, does not potentiate the pressor action of tyramine in man at the low daily dose of 10 mg (Elsworth, Glover, Reynolds, Sandler, Lees, Phuapradit, Shaw, Stern, & Kumar, 1978). Deprenyl has also been shown to inhibit the action of tyramine in isolated nictitating membrane, vas deferens and perfused arterial strips (Knoll, 1978). The mechanism of this effect has largely been attributed by Knoll (1978) to inhibition of neuronal uptake of tyramine; only a slight degree of inhibition of [^{3}H]-noradrenaline uptake in mouse cerebral cortex slices was produced, however, by a concentration of the (−)-isomer of deprenyl (5×10^{-6}M) which inhibits tyramine-induced contractions of isolated tissue preparations (Knoll, Vizi & Magyar, 1972). Indeed, the (+)-isomer of deprenyl is more potent as an inhibitor of [^{3}H]-noradrenaline uptake than the (−)-isomer, although, the latter is more potent in antagonizing tyramine effects (Knoll & Magyar, 1972). In addition to these actions, deprenyl also possesses intrinsic amphetamine-like activity (Green & Youdim, 1975; Simpson, 1978a). We have further investigated the tyramine-antagonistic property of (−)-deprenyl by measuring uptake of ^{3}H-labelled amines, and interaction with the tyramine contractile mechanism, in rat vas deferens. In addi-

0007–1188/81/050065–010 $01.00

tion, we have studied the modification of tyramine effects by two other propargylamine derivative MAO inhibitors based on the indane nucleus. One of these compounds, *N*-methyl-*N*-propynyl-l-indanamine (AGN 1133) was originally described by Huebner, Donoghue, Plummer & Furness (1966) and subsequently by Knoll, Ecsery, Magyar & Satory (1978) who referred to it as J 508. This compound is a potent inhibitor of MAO types A and B and does not antagonize the pharmacological effects of tyramine. The other compound, AGN 1135, is the *N*-desmethyl derivative of AGN 1133, which produces selective inhibition of MAO type B similar to that produced by deprenyl (Kalir, Sabbagh & Youdim, 1980) and has now been found to possess a similar tyramine antagonistic effect.

Methods

Contractions of rat isolated vas deferens

Rats were killed by a blow on the head, the vasa deferentia removed rapidly and suspended in 2 ml organ baths containing Krebs solution gassed with 95% O_2 and 5% CO_2 at 37°C. The Krebs solution had the following composition (mM): NaCl 118, KCl 4.7, $MgCl_2$ 2.5, Na_2HPO_4 1.2, $NaHCO_3$ 25 and glucose 11. Contractile responses were measured with Statham UC2 isometric transducers coupled to a Beckman Dynograph recorder. Resting tension was adjusted to 0.5 g. The tissues were allowed to equilibrate for 60 min before determination of approximately 50% maximal responses to noradrenaline and tyramine. The appropriate dose of the two agents was then added alternately to the bath at 5 min intervals. Bath fluid was changed after the peak of the contractile response. Three to 4 control responses to each agent were determined followed by 3 to 4 responses in the presence of the inhibitor (added immediately after each change of the bath fluid). Three to four responses were then obtained in the absence of the inhibitor. This type of experimental protocol was used in order to avoid tachyphylaxis to tyramine which occurred when the larger doses of tyramine needed to construct dose-effect curves were repeatedly administered. It was also found that alternating tyramine and noradrenaline doses resulted in a tyramine response which was stable throughout the course of a control experiment (untreated with inhibitor). Such control experiments were obtained from one of a pair of vasa deferentia, the other being treated with inhibitor. Ratios of mean responses during and after MAO inhibitor application to mean control (pretreatment) responses were calculated and compared with response ratios for comparable periods in inhibitor-untreated experiments. In some experiments electrical field stimulation was carried out with twin 1 ms duration square wave pulses (2 Hz) passed between 0.1 mm diameter platinum electrodes on either side of the tissue (supramaximal voltage).

Uptake of ^{3}H-amines by vas deferens

Rat and mouse vasa deferentia were removed from animals killed as above, placed in Krebs solution at room temperature, blotted and weighed. The tissues were then incubated (37°C) in Krebs solution gassed with 95% O_2 and 5% CO_2 for 15 min followed by 20 min incubation in the presence of the inhibitor. Subsequently, the labelled amines (10^{-7} M) were added, and incubation continued for a further 5 min. Uptake was stopped by transferring the tissues rapidly to a large volume (100 ml) of Krebs solution at room temperature. The organs were washed in this solution for 10 min before solubilization in Soluene 100 (Packard) and quantitation of total ^{3}H content by liquid scintillation counting in a Packard model B2450 counter with automatic external standardisation.

Measurement of blood pressure in anaesthetized animals

Male albino rats (250–300 g) were anaesthetized with urethane (150 mg/kg i.p.) and systemic blood pressure measured from a carotid artery using a Statham P 23 Db transducer coupled to a Beckham Dynograph or Brush-Gould recorder. Drugs were administered intravenously via a jugular vein. Rectal temperature was measured with a mercury thermometer and maintained at 37°C by warming the animal as necessary. The increases in mean blood pressure produced by submaximal doses of tyramine and noradrenaline following intravenous injection of the inhibitors was expressed as the ratio to control responses obtained before injection of the inhibitors.

Cats of either sex were anaesthetized with 2% halothane in nitrous oxide/oxygen (8:2, vol:vol) followed by intravenous injection of chloralose (80 mg/kg). Blood pressure was measured from a femoral artery. Modification of pressor responses to intravenously injected tyramine and noradrenaline by intravenous injection of the inhibitors was examined.

Determination of monoamine oxidase activity

Rat vasa deferentia were homogenized in 0.32 M sucrose (1 ml sucrose per vas deferens) and the homogenate centrifuged at 500 *g* for 10 min. The MAO activity of the supernatant was determined in the presence of increasing concentrations of AGN 1133, AGN 1135 and (−)-deprenyl by incubation with [^{14}C]-5-HT (1 mM) or [^{14}C]-PEA (20 μM) and separation of the deaminated metabolites on small ion-exchange columns as described by Tipton & Youdim (1976).

Drugs

Drugs were made up in physiological saline (0.15 M) which, in the case of noradrenaline, contained 0.001 M ascorbic acid. Doses of noradrenaline refer to the base, and of tyramine, to the hydrochloride. AGN 1135 and AGN 1133 were obtained from Aspro Nicholas, (−)-noradrenaline acid tartrate from Bayer, and (−)-deprenyl was a gift from Prof. J. Knoll (Semmelweiss University of Medicine, Budapest, Hungary). All other chemicals were obtained from Sigma. [^{14}C]-5-HT and [^{3}H]-tyramine were obtained from Amersham Radiochemical Centre; [^{14}C]-PEA, DL[^{3}H]-metaraminol and DL[^{3}H]-octopamine were from New England Nuclear.

Results

Effects of AGN 1133, AGN 1135 and (−)-deprenyl on tyramine and noradrenaline responses in rat vas deferens

Addition of AGN 1133 (7.5×10^{-6} M) to the organ bath produced a rapidly developing potentiation of responses to tyramine but not to noradrenaline (Figures 1 and 2). A similar degree of potentiation was seen both in the presence of the inhibitor, and after it had been washed from the bath. In the presence of AGN 1135 (10^{-5} M), responses to tyramine were not significantly altered, although on washing out the inhibitor some potentiation was seen. When the concentration of AGN 1135 was increased to 7.5×10^{-5} M, tyramine responses were significantly reduced, but on washing out the inhibitor, a marked potentiation occurred (Figures 1, 2 and 3). Responses to noradrenaline were slightly enhanced by AGN 1135 at a concentration of 10^{-5} M but increasing the inhibitor concentration to 7.5×10^{-5} M resulted in some antagonism of noradrenaline as well as tyramine responses. However, responses to tyramine were inhibited to a greater extent than those to noradrenaline in the presence of the higher concentration of AGN 1135. No inhibition of the response to transmural stimulation was noticed with AGN 1135 (10^{-4} M).

(−)-Deprenyl (10^{-5} M) produced inhibition of responses to tyramine but not to noradrenaline. However, on washing out the inhibitor, as with AGN 1135, responses to tyramine were potentiated. Tyramine responses were depressed further when the concentration of (−)-deprenyl was increased to 3×10^{-5} M but potentiation of the tyramine response was even greater when deprenyl was washed from the bath. Noradrenaline responses were also potentiated following treatment with both concentrations of deprenyl (Figures 1, 2 and 3).

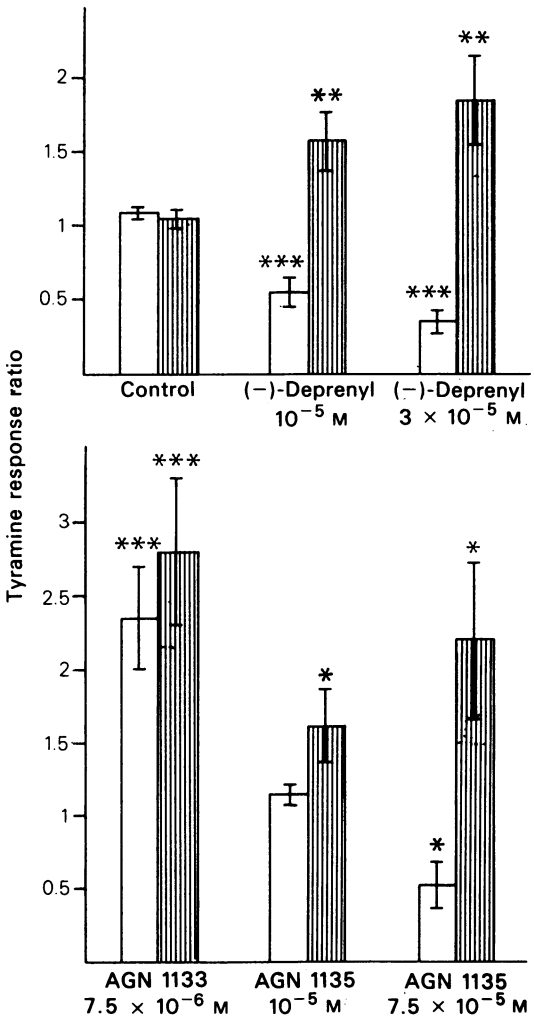

Figure 1 Modification of contractile response to tyramine in isolated vas deferens of the rat by AGN 1133, AGN 1135 and (−)-deprenyl. Mean results shown for $n=4$ to 6 experiments; vertical lines indicate s.e. mean. Open columns indicate change in response in presence of inhibitors, shaded columns represent change in response following washout of inhibitor from organ bath. Responses in inhibitor-treated periods were compared with responses before addition of inhibitor to obtain response ratio. Controls were not treated with inhibitors. Concentrations of inhibitors refer to final concentration in bath fluid. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ by t test analysis.

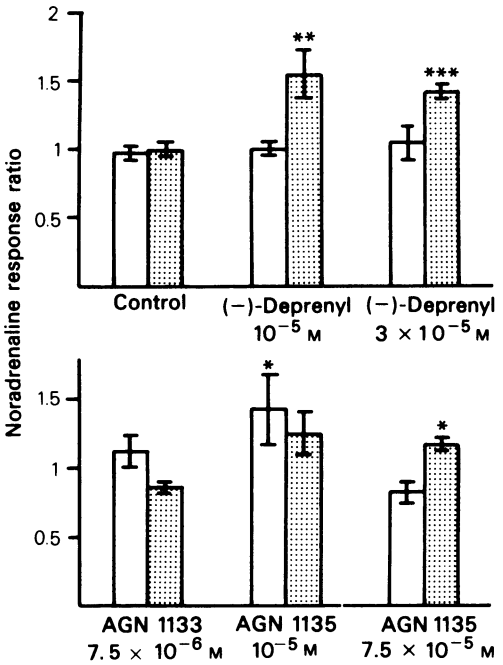

Figure 2 Modification of contractile response to noradrenaline in rat isolated rat vas deferens by AGN 1133, AGN 1135 and (−)-deprenyl. For explanation see legend to Figure 1.

Uptake of ^{3}H-amines

Uptake of [^{3}H]-metaraminol was investigated in the rat vas deferens, and both [^{3}H]-metaraminol and [^{3}H]-octopamine uptake was studied in mouse vas deferens. In initial experiments it was found that [^{3}H]-metaraminol uptake was linear with time over the period under study in both rat and mouse organs. Uptake was also highly temperature-dependent, being only 10–20% at 0°C of the value at 37°C (Figure 4). Desmethylimipramine (DMI) reduced uptake of [^{3}H]-metaraminol to 28% of control values in rat vas deferens at a concentration of 10^{-6} M. This concentration of DMI also caused marked inhibition of the contractile response to tyramine. In the presence of (−)-deprenyl, 1×10^{-5} M, uptake of [^{3}H]-metaraminol was 81% of control ($P<0.01$) and increase of (−)-deprenyl concentration to 3×10^{-5} M resulted in only a slight further reduction of uptake, to 73% of control values ($P<0.01$). These concentrations of (−)-deprenyl caused considerable reduction of the contractile response to tyramine when responses were compared to pretreatment values. The inhibition of tyramine contractions was even greater when responses in the presence of the drug were compared to those following its removal from the organ bath (Figure 1). AGN 1135 at 1×10^{-5} M had no effect on uptake and at 1×10^{-4} M produced only a small degree

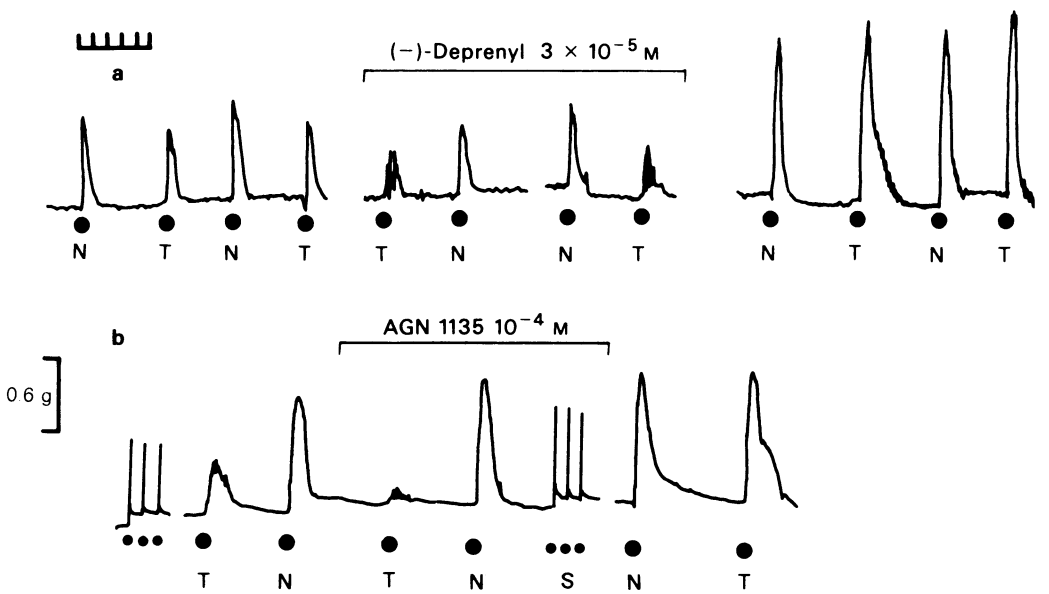

Figure 3 Portions of tracings from two representative experiments showing isometric contractions of rat vas deferens in response to tyramine (T) and noradrenaline (N), before, during and after treatment with (−)-deprenyl (a) and AGN 1135 (b). Doses of noradrenaline used were 0.5 μg (a) and 2 μg (b), and of tyramine were 5 μg (a) and 10 μg (b) in 2 ml organ bath. S = responses to electrical field stimulation (2 pulses of 1 ms duration at 2 Hz and supramaximal voltage). Time trace = 1 min. Concentrations of inhibitors shown are final concentrations in bath fluid.

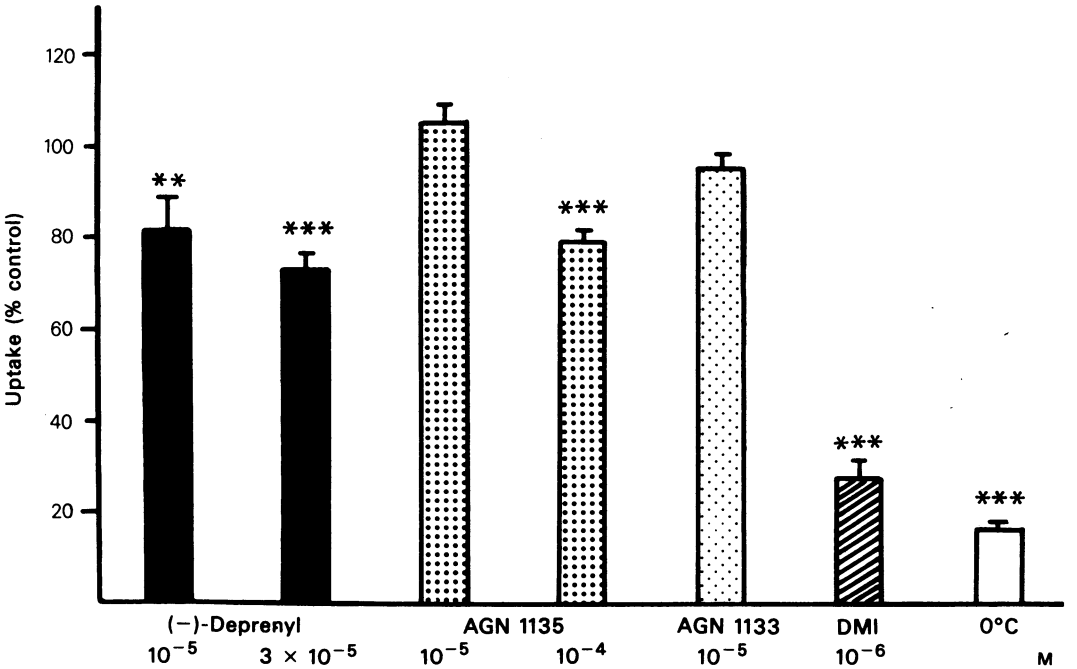

Figure 4 Uptake of [^{3}H]-metaraminol by rat isolated vas deferens. Uptake in presence of inhibitors expressed as percentage of control uptake in untreated vasa deferentia. Vertical lines indicate s.e. mean DMI = desmethylimipramine. $^{**}P<0.01$; $^{***}P<0.001$ by t test analysis.

of inhibition of [^{3}H]-metaraminol uptake (to 79% of control values); AGN 1133 (1×10^{-5}M) produced no significant alteration in uptake of this amine.

The uptake of [^{3}H]-metaraminol and [^{3}H]-octopamine were compared in the mouse vas deferens (Figure 5). The reduction in [^{3}H]-metaraminol uptake produced by (−)-deprenyl (1×10^{-5}M) and AGN 1135 (1×10^{-4}M) in the mouse organ was of similar magnitude to that in the rat. However, the uptake of [^{3}H]-octopamine was not significantly reduced by these concentrations of the two agents. DMI (1×10^{-6}M) reduced uptake of both [^{3}H]-metaraminol and [^{3}H]-octopamine to a similar extent, whereas reserpine (1×10^{-6}M) caused a much greater reduction in [^{3}H]-octopamine than in [^{3}H]-metaraminol uptake.

Effects of pretreatment with AGN 1133 and AGN 1135 on pressor responses to tyramine and noradrenaline

The dose of inhibitors selected for this experiment was 1.5 mg/kg. Studies from this department (Kalir *et al.*, 1981) have shown that this dose of AGN 1133 produces a more than 80% inhibition of PEA and 5-HT metabolism in brain and liver. However, the same dose of AGN 1135 caused 85% inhibition of PEA metabolism and only 20% inhibition of 5-HT metabolism.

Soon after injection of AGN 1133 in rats anaesthetized with urethane pressor responses to tyramine were seen to be potentiated, both in height and duration (Figure 6). The actual degree of potentiation was greater than that shown in the Figure, since only maximum increase in blood pressure was measured and tyramine responses were potentiated in duration as well as in maximum effect obtained. Neither tyramine nor noradrenaline responses were altered significantly by this dose of AGN 1135. The dose of tyramine used (25 μg/rat) caused an increase of 19.1±2.2 (s.e. mean) mmHg in mean blood pressure, while noradrenaline (50 ng/rat) produced an increase of 24.5±3.8 mmHg in blood pressure in the control (pre-drug) period.

In anaesthetized cats, similar results were obtained. At 1.5 mg/kg, AGN 1133 caused potentiation of tyramine responses whereas the same dose of AGN 1135 did not (Figure 7).

Effects of AGN 1133, AGN 1135, (−)-deprenyl and clorgyline on monoamine oxidase activity of rat vas deferens homogenates

Concentrations of AGN 1133 above 1×10^{-6} M caused complete inhibition of both PEA and 5-HT oxidation. At 1×10^{-6}M, AGN 1135 produced almost complete inhibition of PEA but not of 5-HT metabolism, whereas at concentrations of 1×10^{-5} M and above,

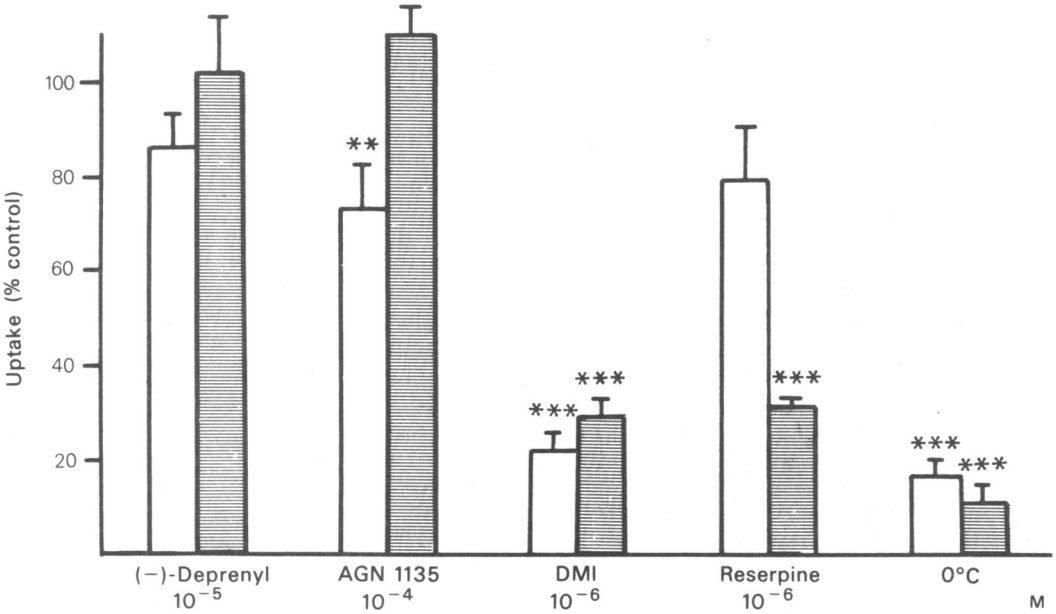

Figure 5 Uptake of [^{3}H]-metaraminol (open columns) and [^{3}H] octopamine (filled columns) by rat isolated vas deferens. Uptake in presence of inhibitors expressed as percentage of control uptake in untreated vasa deferentia. Vertical lines indicate s.e. mean. DMI = desmethylimipramine. *P<0.05; **P<0.01; ***P<0.01 by t test analysis.

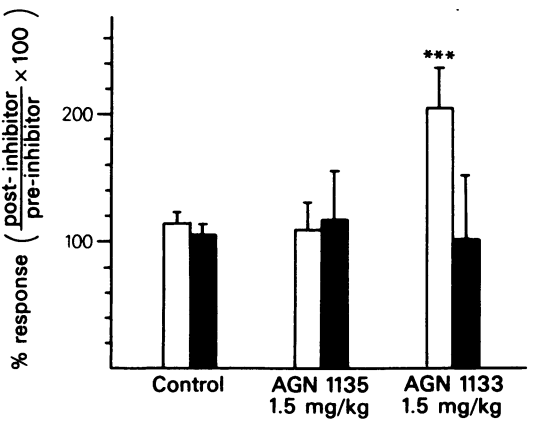

Figure 6 Modification of tyramine and noradrenaline pressor responses in rats anaesthetized with urethane by AGN 1135 and AGN 1133. Open columns = change in tyramine (50 μg/rat) response; filled columns = change in noradrenaline (50 ng/rat) response as percentage of responses before injection of inhibitors. Control results from comparable time periods of non-inhibitor treated experiments. Vertical lines represent s.e. mean. ***P<0.001.

oxidation of both substrates was inhibited by more than 85%. (−)-Deprenyl showed a similar spectrum of inhibitory activity against PEA and 5-HT deamination to that of AGN 1135. At a concentration of 1×10^{-6} M, selectivity for PEA oxidation was optimal, whereas concentrations of 1×10^{-6} M and above of (−)-deprenyl caused almost complete inhibition of both PEA and 5-HT deamination (Figure 8).

Discussion

Inhibitors of MAO can potentiate the actions of tyramine and other indirectly acting sympathomimetic amines by several possible mechanisms: (a) by increasing the noradrenaline content in the tyramine-releasable pool; (b) by reducing intraneuronal deamination of tyramine; (c) by reducing hepatic and extraneuronal metabolism of tyramine; and (d) the MAO inhibitors may have intrinsic sympathomimetic effects which may have a synergistic action with the noradrenaline release produced by the indirectly-acting amines.

Intraneuronal MAO is thought to play a role in maintaining cytoplasmic noradrenaline concentration at a low level (Kopin, 1964), and evidence exists that this MAO may be mainly of type A (Neff & Goridis, 1972; Coquil, Goridis, Mack & Neff, 1973). Doses of clorgyline that selectively inhibit MAO type A in the rat caused an increase in noradrenaline levels

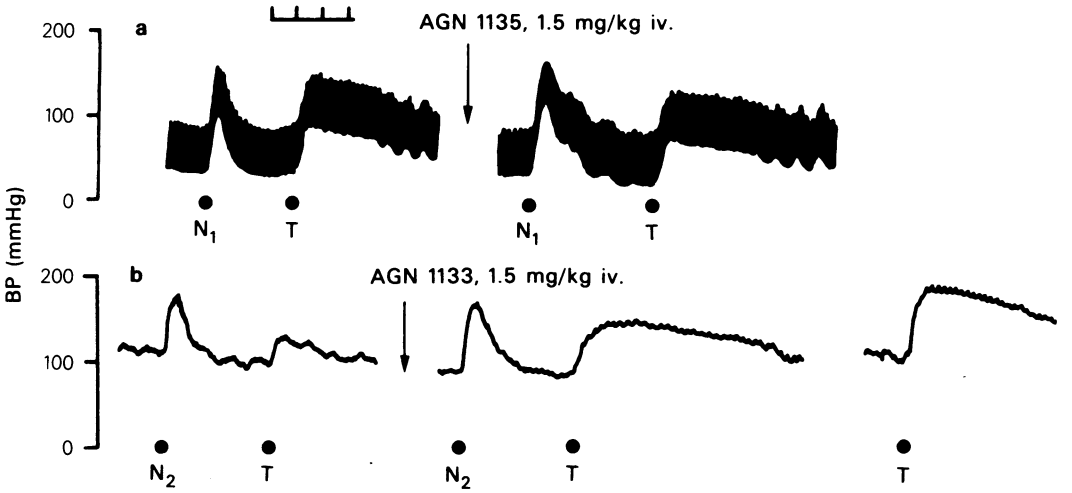

Figure 7 Blood pressure responses of two cats anaesthetized with chloralose (a and b) to tyramine and noradrenaline. Upper trace, pulsatile pressure; lower trace, electronically damped mean pressure. $N_1 = 0.5\ \mu g$, $N_2 = 1\ \mu g$ noradrenaline. T = 50 μg tyramine. In (a) second part of trace was recorded 30 min after injection of AGN 1135. In (b) middle part of trace was recorded 5 min after injection of AGN 1133, and right-hand part of trace was recorded 100 min later. Time trace = 1 min intervals.

in heart and mesenteric artery and potentiated the cardiovascular effects of amphetamine (Simpson, 1978b); thus inhibition of metabolism of the indirectly acting sympathomimetic amines is not the only factor involved in potentiation of their actions, since amphetamine is not a substrate for MAO. More evidence is required for a definitive conclusion that intraneuronal MAO is mainly or solely of type A; however, if this is so, it offers one explanation for the lack of potentiation of the actions of tyramine by agents such as (−)-deprenyl which selectively inhibit MAO type B. Both intraneuronal and extraneuronal metabolism of tyramine can continue in the presence of selective inhibition of MAO-B, since tyramine is a substrate for both forms of the enzyme. With all MAO inhibitors so far described, selective inhibition is dose-dependent. Thus (−)-deprenyl inhibits MAO type A at concentrations 20 to 250 times greater than those which inhibit MAO type B (Fuller, 1978). Concentrations of AGN 1135 and (−)-deprenyl above 10^{-5}M caused almost complete inhibition of MAO types A and B and also resulted in potentiation of contractile effects of tyramine in the vas deferens, following wash-out of the inhibitors from the bath. However, in addition, these two inhibitors produced a reversible antagonism of the contractile effect of tyramine. The tyramine-antagonistic effect of (−)-deprenyl was described originally by Knoll and coworkers (Knoll, Vizi & Somogyi, 1968; Knoll & Magyar, 1972; Knoll, 1978). Potentiation of the tyramine-induced contraction appears to be dependent on a certain minimum contact time with the inhibitor, which may explain why this effect was not observed by Knoll and coworkers.

Knoll has explained the tyramine antagonistic property of (−)-deprenyl on the basis of (a) blockade of neuronal uptake of tyramine; i.e. a cocaine-like action (Knoll, 1978), and (b) inhibition of noradrenaline release from storage sites (Knoll & Magyar, 1972). The latter suggestion was made on the basis of experiments in which [^{3}H]-noradrenaline was found to accumulate in subcellular fractions of rat heart tissue following treatment of the animal with a large dose of (−)-deprenyl (25 mg/kg). This observation is difficult to reconcile with the amphetamine-like action of (−)-deprenyl (Simpson, 1978a) which would have been expected to enhance noradrenaline release at the high dose used. In order to test the first proposal, we originally used [^{3}H]-tyramine to measure amine uptake in the rat vas deferens (Finberg, Sabbagh & Youdim, 1979). (−)-Deprenyl (10^{-5}M) produced only a 15% reduction in uptake whereas AGN 1135 (10^{-4}M) had no effect; DMI (10^{-6}M) produced a 50% inhibition of uptake. In the present work, uptake of [^{3}H]-metaraminol was studied, since this amine is not a substrate for MAO, and appears to be concentrated within the sympathetic neurone largely by neuronal membrane high affinity uptake (Carlsson & Waldeck, 1965). The relatively minor degree of inhibition of [^{3}H]-metaraminol uptake produced by (−)-deprenyl and AGN 1135 does not appear to be adequate to explain the profound inhibition of the tyramine response by these agents.

Whereas metaraminol is accumulated within the

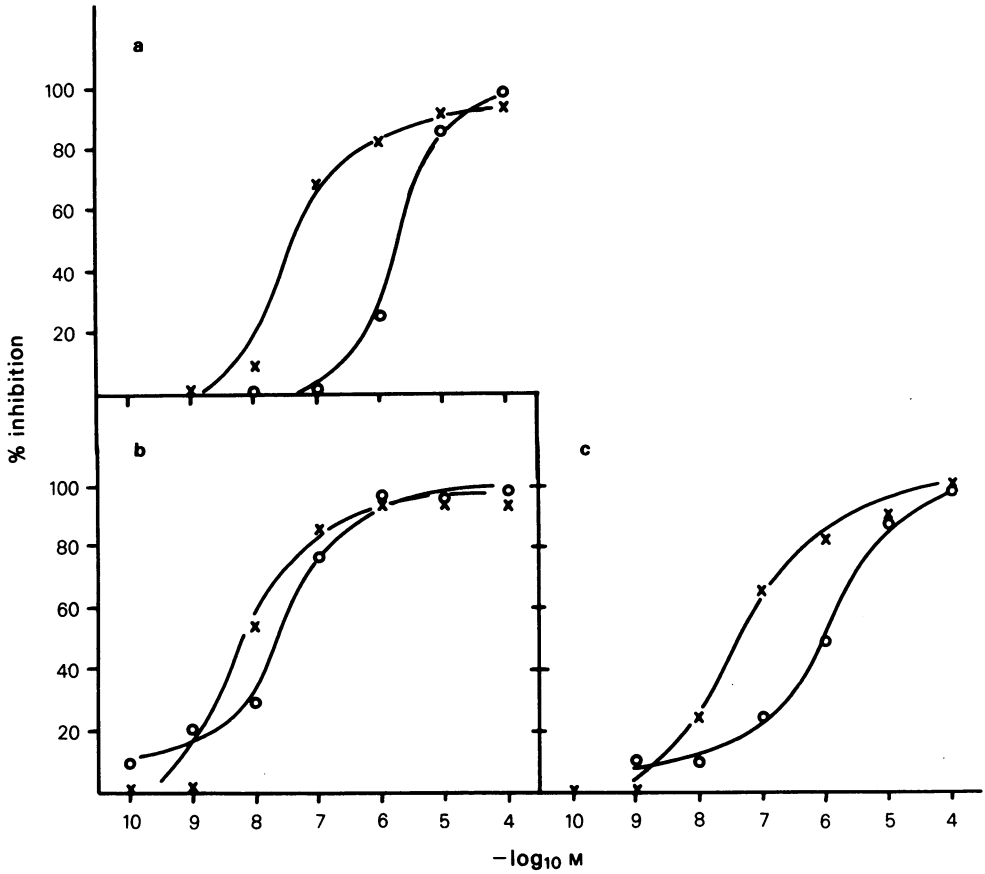

Figure 8 Inhibition of monoamine oxidase activity in homogenates of rat vas deferens by (−)-deprenyl (a), AGN 1133 (b) and AGN 1135 (c). Mean results from two experimental runs. (×) β-Phenylethylamine as substrate; (○) 5-hydroxytryptamine as substrate.

neurone by a largely reserpine-independent process, octopamine is a substrate for the vesicular membrane amine pump (Giachetti & Shore, 1966). The differential action of reserpine in inhibiting uptake of [^{3}H]-octopamine far more than that of [^{3}H]-metaraminol was apparent in the present experiments (Figure 5). Neither (−)-deprenyl, nor AGN 1135 caused any significant reduction in [^{3}H]-octopamine uptake, showing that prevention of amine uptake into vesicles by a reserpine-like mechanism was also not an important factor in blockade of the effects of the indirectly acting sympathomimetic amines. (−)-Deprenyl and AGN 1135 may exert their effects by inhibiting noradrenaline release from the vesicles as suggested by Knoll & Magyar (1972). Alternatively, their site of action may be inhibition of active efflux of noradrenaline from the nerve ending following its release into the cytoplasm by tyramine. Evidence in favour of a role of active efflux in mediation of the responses to phenylethylamines had recently been reviewed by Trendelenburg (1979). Efflux of labelled transmitter in response to administration of phenylethylamines such as amphetamine and β-phenylethylamine has been shown to be decreased following treatment with cocaine (Paton, 1973; Raiteri, del Carmine & Bertolini, 1977). Drugs such as (−)-deprenyl and AGN 1135, therefore, could antagonize the effect of tyramine by inhibiting active efflux of neurotransmitter without necessarily reducing neuronal uptake of tyramine.

An additional observation pointing to the absence of a marked effect of AGN 1135 and (−)-deprenyl on neuronal membrane amine uptake is the lack of potentiation of noradrenaline responses in the presence of tyramine antagonism. However, noradrenaline responses were potentiated following wash out of (−)-deprenyl. Both AGN 1135 and (−)-deprenyl possess α-adrenoceptor blocking activity in high doses, and blockade of α-receptors may have masked potentiation of noradrenaline in presence of

the inhibitors. Although MAO does not play a major role in inactivation of exogenous noradrenaline (Kopin, 1964) inhibition of MAO results in a slowly developing potentiation of responses to noradrenaline and other directly acting sympathomimetic amines which are substrates for MAO (Trendelenburg, Draskoczy & Graefe, 1972). One explanation put forward to explain this phenomenon is that intraneuronal cytoplasmic levels of noradrenaline increase following MAO inhibition resulting in reduction of active uptake and enhanced action of exogenous noradrenaline on postsynaptic receptors. Under the conditions of the uptake experiments, inhibition of MAO by AGN 1133 was not accompanied by reduction of [^{3}H]-metaraminol uptake, so the small reductions in uptake produced by (−)-deprenyl and AGN 1135 appear to indicate a cocaine-like effect of these compounds. Potentiation of noradrenaline responses by MAO inhibitors is a time-dependent phenomenon (Trendelenburg *et al.*, 1972) which may explain why this effect was not consistently observed with the inhibitors used in this study. (−)-Deprenyl has been shown to inhibit noradrenaline uptake in rat heart (Simpson, 1978a) although a dose of 10 mg/kg inhibited uptake by only about 45%. Similarly, a weak inhibitory effect on noradrenaline uptake in rat brain synaptosomes was observed by Braestrup, Andersen & Randrup (1975).

Since the inhibitory effects on uptake of (−)-deprenyl are only apparent at doses far above those that cause complete inhibition of MAO type B (1.0 mg/kg in the rat, see Green & Youdim, 1975), such an action does not apparently play a role in the non-potentiation of tyramine responses at selective MAO-B inhibitory clinical doses of (−)-deprenyl. In addition, since selective inhibition of MAO-B by AGN 1135 also did not result in potentiation of tyramine pressor responses, it may be that complete inhibition of MAO-B is not accompanied by potentiation of indirectly acting amines.

(−)-Deprenyl has been shown to possess amphetamine-like actions on rat locomotor activity (Green & Youdim, 1975) and cat cardiovascular system (Simpson, 1978a) in higher doses than those that selectively inhibit MAO type B. Deprenyl is metabolized to amphetamine and methamphetamine in man (Reynolds, Elsworth, Blau, Sandler, Lees & Stern, 1978), although it is not clear whether deprenyl itself or its metabolites are responsible for this effect. However, AGN 1135 does not possess such intrinsic sympathomimetic activity (Finberg *et al.*, 1979). Further work is being carried out at present to determine whether the spectrum of MAO inhibitory activity and tyramine-antagonistic effect shown by AGN 1135 will render it suitable for clinical evaluation.

This work was supported by grants from the Ronald Lawrence Neuropsychopharmacology Fund (Technion), and the Israel National Council for Research and Development. The technical assistance of Mrs Ilana Spanir is gratefully acknowledged. Some of these results are to be included in an M.Sc. thesis by M. Tenne.

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(Received June 13, 1980)