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EFFECTS OF MIANSERIN, A NEW ANTIDEPRESSANT, ON THE *in vitro* AND *in vivo* UPTAKE OF MONOAMINES

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1 The effects of mianserin and of selected tricyclic antidepressants were compared in a number of monoamine uptake models.

2 The ability of mianserin to block the noradrenergic neurone membrane amine pump of rabbit brain stem slices was comparable to that of imipramine and amitriptyline and less than that of desipramine and nortriptyline. Both mianserin and desipramine were competitive inhibitors of noradrenaline uptake *in vitro*. The effect of mianserin on noradrenaline uptake *in vivo* was studied both peripherally and centrally. The ability of 6-hydroxydopamine to lower rat heart noradrenaline levels was found to be very sensitive to inhibition by tricyclic antidepressants. Mianserin was active in this model. However, its ability to block the 6-hydroxydopamine-induced fall in rat heart noradrenaline concentration was appreciably less than that of the tricyclics studied.

3 Mianserin, like tricyclic antidepressants, was essentially devoid of effect on dopamine uptake both *in vitro* and *in vivo*.

4 The ability of mianserin to inhibit [^{3}H]-5-hydroxytryptamine uptake by rat hypothalamic synaptosomes was appreciably less than that of the tricyclic antidepressants studied. Mianserin was essentially devoid of effect on rat brain 5-hydroxytryptamine uptake *in vivo*.

5 It is concluded that in certain situations large doses of mianserin may block noradrenaline uptake *in vivo*. However, in no way does mianserin rival tricyclic antidepressants in blocking monoamine uptake *in vivo*. The clinical efficacy of mianserin cannot be attributed to inhibition of monoamine uptake.

Introduction

The primary selection of potential antidepressants is often based on the ability of the agent to antagonize reserpine-induced hypothermia and/or its ability to block ptosis induced by drugs such as reserpine and tetrabenazine (Spencer, 1976). Activity in such models is usually attributed to a drug-induced blockade of the so-called membrane amine pump of central monoaminergic neurones. Furthermore, the clinical efficacy of compounds such as the tricyclic antidepressants is widely attributed to their ability to block central monoaminergic uptake mechanisms (Carlsson, 1976). Mianserin (Org GB94, 1, 2, 3, 4, 10, 14b-hexahydro-2-methyl-dibenzo [*c,f*]pyrazino-[1,2]-azepine monohydrochloride, Figure 1) is a clinically effective antidepressant (Itil, Polvan & Hsu, 1972; Coppen, Gupta, Montgomery, Ghose, Bailey, Burns & de Ridder, 1976). However, mianserin does not antagonize reserpine-induced hypothermia (van Riezen, 1972). Moreover, neurochemical experiments have revealed that mianserin, in contrast to the tricyclic antidepressants, increases the turnover of noradrenaline in the rat brain (Kafoe, de Ridder & Leonard, 1976). The objective of this study was to determine what effects, if any, mianserin exerts on *in vitro* and *in vivo* monoamine uptake mechanisms. Tricyclic antidepressants were included for comparative purposes. A preliminary account of some of the findings has been published in abstract form (Goodlet & Sugrue, 1974).

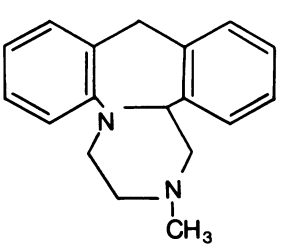

Figure 1 Structure of mianserin.

Methods

Animals used were either adult New Zealand white rabbits weighing 3.5–4.0 kg or male Wistar rats (CE/CFHB strain) weighing 200–250 grams.

In vitro *studies*

In vitro blockade of the membrane amine pump of central noradrenergic neurones was investigated by determining drug effects on the uptake of (–)-metaraminol ((–)-MA) by rabbit brain stem slices. The rabbit was killed and its brain quickly removed and immersed in ice-cold 0.9% w/v NaCl solution (saline). The brain was placed ventral side uppermost on a dissecting plate and a transverse cut was made at the level of the optic chiasma. Sagittal cuts were made on both sides at the medulla oblongata and pons to the level of the optic chiasma and a wedge of tissue comprising the hypothalamus, pons and medulla oblongata, subsequently referred to as the brain stem, was dissected free from the remainder of the brain. Ventral slices of about 0.3 mm thickness were obtained by means of a Harvard tissue slicer. Slices of 80–110 mg were placed in 5 ml of ice-cold Krebs-Ringer phosphate buffer (pH 7.4) (for composition see Sugrue & Shore, 1969) in 25 ml beakers which were then placed in a Dubnoff metabolic shaker at 37°C under an atmosphere of 95% O_2 and 5% Co_2. (–)-MA, in a volume of 0.1 ml in buffer, was added after a 15 min pre-incubation period and incubation was continued for 30 min, uptake being linear with respect to time. Following incubation, slices were removed, rinsed in ice-cold saline and superficial saline was removed by means of tissue paper. After weighing, tissues were homogenized in 0.4 N perchloric acid. The fluorometric procedure of Shore & Alpers (1964) was used to analyse the slice content of (–)-MA. Amine uptake was calculated as net uptake, concentration per ml of slice water minus medium concentration, on the basis of a tissue water content of 85%, as described elsewhere (Giachetti & Shore, 1966). Drugs under study were added in a volume of 0.1 ml at the start of the pre-incubation period.

In experiments with rabbit hypothalamic and striatal minces, brain areas were dissected out as described by Glowinski & Iversen (1966). The dissected brain areas were placed in ice-cold saline and then minced as finely as possible with iris scissors (Dorris & Shore, 1971). The minces were washed in ice-cold saline before incubation. The weight of the minces averaged 25–30 mg. The remainder of the procedure was as described above.

In vitro uptake of 5-hydroxytryptamine (5-HT) was studied with a crude synaptosomal fraction from rat hypothalamus which was dissected out as described by Glowinski & Iversen (1966). The tissue was weighed and homogenized in 19 volumes of 0.32 M sucrose with five upward and downward strokes of a motor driven teflon pestle, clearance 0.1 mm. The resultant homogenate was centrifuged at 1000 *g* for 10 minutes. The supernatant liquid was decanted and stirred to give a uniform suspension. A 0.1 ml aliquot of suspension (amount of tissue present was equivalent to 5 mg of original tissue) was added to 25 ml beakers containing 2 ml of Krebs-bicarbonate buffer (for constituents, see Coyle & Snyder, 1969) containing ascorbic acid (1.1×10^{-5} M), pargyline (1.9×10^{-5} M) and test drug. After a 10 min preincubation at 37°C under an atmosphere of 95% O_2 and 5% CO_2 in a Dubnoff metabolic shaker [^{3}H]-5-HT was added in a volume of 0.1 ml yielding a final concentration of 2.6×10^{-8}M. Incubation was continued for a further 5 min and was terminated by the addition of 5 ml of ice-cold saline and by standing the beakers in ice for 10 minutes. The homogenate was separated from the medium by filtration (Millipore 24 mm diameter, pore size 0.45 μm) under vacuum. The beakers were rinsed with 5 ml of ice-cold saline and the washings filtered. The filter was then rinsed with a further 5 ml of ice-cold saline to remove any remaining radioactivity which was not associated with the tissue. The filter disc was placed in a counting vial and 15 ml of Bray's scintillant added. After 4 h at room temperature the filter disc had disintegrated and the contents of the vial were ready for counting. Incubations were carried out in the presence of four concentrations of test drug, control at 37°C and control at 0°C. The concentration of radioactivity in the incubation media was obtained by preparing incubation vessels as described above except that 0.1 ml 0.32 M sucrose was added instead of 0.1 ml homogenate and counting 1 ml of the resultant mixture. Each incubation was performed in quadruplicate. Concentrations of 5-HT taken up were calculated by dividing d min^{-1} g^{-1} of original tissue by d min^{-1} ml^{-1} of medium and corrected for diffusion by subtracting the amount taken up at 0°C.

In vivo *studies*

In vivo blockade of peripheral noradrenaline (NA) uptake was studied by investigating the effect of drug pretreatment on the ability of 6-hydroxydopamine (6-OHDA) to lower rat heart NA content. 6-OHDA (20 mg/kg) was injected intraperitoneally and rats killed 18 h later. Compounds under study were injected intraperitoneally 30 min before 6-OHDA. In experiments investigating the effect of drug pretreatment on the ability of intracerebroventricularly (i.c.v.) administered 6-OHDA to lower rat brain NA and dopamine levels, rats were anaesthetized with halothane and 6-OHDA (either 100 or 250 μg in a volume of 20 μl) was infused for 1 min into the right ventricle of the rat brain (Noble, Wurtman & Axelrod, 1967). Rats were killed 3–5 days after intraventricular injection. Compounds under study were injected intraperitoneally 30 min before 6-OHDA. Following extraction (Neff & Costa,

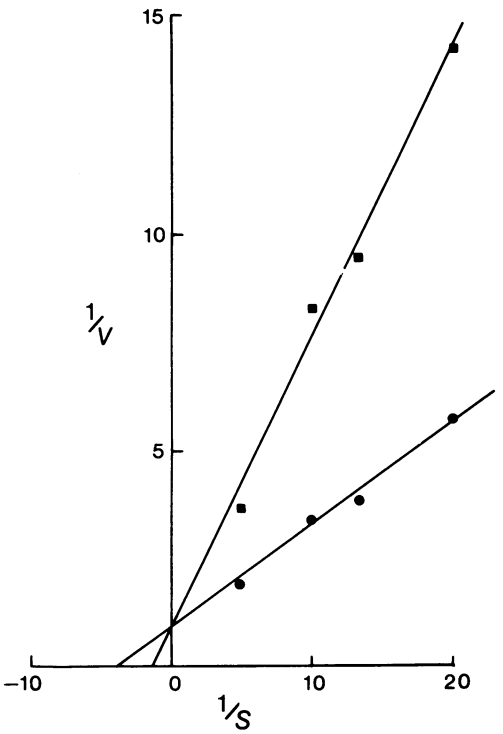

Figure 2 Lineweaver-Burk plots of (–)-metaraminol ((–)-MA) uptake by rabbit brain stem slices in the presence (■) and absence (●) of mianserin (10^{-5} M). Velocity (*V*) is taken to be net uptake of amine by slice during 30 min incubation. Substrate concentration (*S*) is μg of amine per ml of medium. Each point is the mean of at least 5 determinations. *Km* values were calculated by the method of Dixon (1953).

1966), tissue content of NA and dopamine was determined spectrophotofluorometrically (Laverty & Taylor, 1968). *In vivo* blockade of central 5-HT uptake was studied by investigating the effect of drug pretreatment on the *p*-chloroamphetamine (PCA) induced fall in rat brain 5-HT levels. Compounds were injected intraperitoneally 15 min before PCA 10 mg/kg, i.p., rats were killed 3 h after PCA injection and brain 5-HT content analysed spectrophotofluorometrically (Snyder, Axelrod & Zweig, 1965). Tissue levels of NA, dopamine and 5-HT were calculated as μg/g of wet tissue, results being corrected for 100% recovery.

The following drugs were dissolved in saline: (–)-metaraminol bitartrate, amitriptyline hydrochloride, nortriptyline hydrochloride, chlorimipramine hydrochloride, desipramine hydrochloride, *p*-chloroamphetamine hydrochloride and imipramine hydrochloride. Mianserin monohydrochloride was dissolved in distilled water. 6-Hydroxydopamine hydrobromide was freshly prepared in saline containing 5.7×10^{-3} M ascorbic acid and kept in ice. Doses refer to the free base.

Results

In vitro *studies*

Of the compounds studied for blockade of (–)-MA uptake by rabbit brain stem slices, nortriptyline and desipramine were the most potent having IC_{50} values of 2.3×10^{-7} M and 4.0×10^{-7} M respectively (Table 1). Mianserin was comparable to imipramine and amitriptyline in potency. Figure 2 shows that incubation in the presence of mianserin (10^{-5} M) did not alter the V_{max} of (–)-MA uptake but did effect a change in K_m thus indicating that mianserin is a competitive inhibitor of (–)-MA uptake. The K_ms of uptake were 1.5×10^{-6} M and 4.2×10^{-6} M for control and drug-treated respectively. Kinetic experiments with desipramine (10^{-6} M) yielded similar findings, i.e. desipramine was a competitive inhibitor of (–)-MA uptake. The ability of rabbit hypothalamic minces to accumulate (–)-MA was greatly attenuated in the presence of either desipramine or mianserin at a concentration of 10^{-5} M (Table 2). In contrast, striatal (–)-MA uptake was unaltered in the presence of either desipramine (10^{-5} M) or mianserin (10^{-5} M). The accumulation of (–)-MA by both hypothalamic and striatal minces was inhibited by (+)-amphetamine (10^{-5}M).

Of the compounds studied for inhibition of [^{3}H]-5-HT uptake by rat hypothalamic synaptosomes the descending order of potency was chlorimipramine, amitriptyline, imipramine, desipramine, nortriptyline and mianserin (Table 3). It is to be noted that mianserin is approximately ten times less potent than either desipramine or nortriptyline.

Table 1 Blockade of (–)-metaraminol (MA) uptake by rabbit brain stem slices

Drug	IC_{50}(*M*)
Nortriptyline	2.3×10^{-7}
Desipramine	4.0×10^{-7}
Imipramine	1.9×10^{-6}
Amitriptyline	2.6×10^{-6}
Mianserin	3.0×10^{-6}

Slices were incubated for 30 min in the presence of (–)-MA (3×10^{-7} M). Drugs were added at the start of the 15 min preincubation period. IC_{50} values were obtained from graphs of % inhibition of (–)-MA uptake (probability scale) plotted against log concentration of drug.

Table 2 Effects of mianserin, desipramine and (+)-amphetamine on (–)-metaraminol (MA) uptake by rabbit hypothalamic and striatal minces

	Tissue			
	Hypothalamus		*Striatum*	
	Net amine uptake (μg/ml)		*Net amine uptake (μg/ml)*	
Drug	*Control*	*Treated*	*Control*	*Treated*
Mianserin	0.46 ± 0.05	0.14 ± 0.02*	0.80 ± 0.05	0.78 ± 0.05
Desipramine	0.50 ± 0.04	0.08 ± 0.02*	0.54 ± 0.06	0.57 ± 0.07
(+)-Amphetamine	0.42 ± 0.05	0.14 ± 0.02*	0.63 ± 0.07	0.08 ± 0.04*

Tissues were incubated for 30 min in the presence of (–)-MA (6×10^{-7} M). Drugs (10^{-5} M) were added at start of 15 min pre-incubation period. Each result is the mean ± s.e. mean of 5–6 experiments.
* Differs from control, $P < 0.001$.

Table 3 Blockade of [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) uptake by rat hypothalamic synaptosomes

Drug	*IC_{50} (M)*
Chlorimipramine	7.9×10^{-9}
Amitriptyline	3.7×10^{-8}
Imipramine	7.0×10^{-8}
Desipramine	3.1×10^{-7}
Nortriptyline	3.4×10^{-7}
Mianserin	4.0×10^{-6}

Rat hypothalamic synaptosomes were incubated for 5 min in the presence of [^{3}H]-5-HT (2.6×10^{-8} M). Drugs were added at the start of the 15 min pre-incubation period. IC_{50} values were obtained from graphs of % inhibition of [^{3}H]-5-HT uptake (probability scale) plotted against log concentration of drug.

Table 4 Blockade of the ability of 6-hydroxydopamine (6-OHDA) to lower rat heart noradrenaline (NA) levels

Drug	*ID_{50} (μmol/kg)*	*95% confidence limits (μmol/kg)*
Desipramine	3.4	2.6–4.5
Imipramine	5.7	4.3–10.4
Nortriptyline	16.7	10.3–28.9
Amitriptyline	37.9	29.6–50.9
Chlorimipramine	33.6	26.7–42.8
Mianserin	94.0	77.7–112.3

Drugs were injected i.p. 30 min before 6-OHDA (20 mg/kg, i.p.) and rats were killed 18 h after 6-OHDA administration. Heart NA levels were 20.0 ± 1.5 ($n = 15$)% of control values 18 h after 6-OHDA. The concentration of NA in the hearts of vehicle-treated control was 1.056 ± 0.023 μg/g ($n = 34$). The % inhibition of NA reduction by 6-OHDA was calculated from the formula of Carlsson, Corrodi, Fuxe & Hokfelt (1969a). ID_{50} values were obtained from % block/log dose-response curves which were constructed by the method of least squares. Each log dose-response curve had at least 3 points and each point was the mean of at least 5 determinations.

In vivo *studies*

The ability of 6-OHDA (20 mg/kg, i.p.) to decrease heart NA levels was very sensitive to blockade by the tricyclics antidepressants (Table 4). ID_{50} values are defined as the concentration of drug required to produce a 50% block of the 6-OHDA induced fall of rat heart NA content. Mianserin was appreciably less potent than the tricyclics, having an intraperitoneal ID_{50} of 94.0 μmol/kg (28.2 mg/kg).

Three days after the intraventricular injection of 6-OHDA (100 μg) rat brain NA levels were 55.2 ± 3.4 ($n = 15$) percent of vehicle-treated controls. Pretreatment with desipramine, imipramine or chlorimipramine antagonized the ability of 6-OHDA (100 μg, i.c.v.) to lower rat brain NA levels (Table 5). The ability of 6-OHDA (100 μg, i.c.v.) to lower rat brain NA content was essentially unaltered by mianserin pretreatment (200 μmol/kg, i.p.). Doses greater than 200 μmol/kg were not studied since the intraperitoneal LD_{50} of mianserin in rats is 433.3 μmol/kg (van Riezen, 1972). The fall in brain dopamine content following 6-OHDA (250 μg, i.c.v.) administration was not significantly changed by the prior injection of either desipramine (112.8 μmol/kg, i.p.), chlorimipramine (190.5 μmol/kg, i.p.) or mianserin (200 μmol/kg, i.p.).

All the tricyclic antidepressants studied attenuated the ability of PCA (10 mg/kg, i.p.) to lower the concentration of 5-HT in the rat brain. This dose of PCA lowered brain 5-HT content to 44.5 ± 1.1% ($n = 12$) of vehicle control values at the time of death. On the other hand, mianserin, at intraperitoneal doses up to 200 μmol/kg, was essentially unable to block the PCA-induced fall in rat brain 5-HT content (Table 6).

Discussion

Uptake into the presynaptic neurone is considered to be the major means by which monoamines are inactivated at the neural synapse (Iversen, 1974) and tricyclic antidepressants are thought to act by virtue of their ability to block monoamine uptake. The objective of this study was to compare the effects of mianserin, a clinically effective antidepressant, on monoamine uptake mechanisms with the effects of selected tricyclic antidepressants.

Evidence for the validity of the use of (–)-MA as an index of NA uptake *in vitro* has been presented by Giachetti & Shore (1966). The observation that secondary tricyclic antidepressants (viz. desipramine and nortriptyline) are more potent than their corresponding tertiary analogues (viz. imipramine and amitriptyline) in blocking NA uptake *in vitro* is in agreement with the findings of others (Shaskan & Snyder, 1970). The ability of mianserin to inhibit rabbit brain stem (–)-MA uptake is comparable to that of imipramine and amitriptyline and is less than that of desipramine and nortriptyline. Others have also observed an inhibition of NA uptake *in vitro* by mianserin (Baumann & Maitre, 1975; Koe, 1976; Raiteri, Angelini & Bertollini, 1976). Although

Table 5 Blockade of the ability of 6-hydroxydopamine (6-OHDA) to lower rat brain noradrenaline (NA) levels

Drug	*Dose (μmol/kg)*	*% Block (mean ± s.e. mean)*	*ID_{50} (μmol/kg)*	*95% Confidence limits (μmol/kg)*
Desipramine	15.0	18.9 ± 4.3		
	30.1	41.6 ± 4.8		
	60.1	73.6 ± 5.9	34.2	29.3–41.0
Imipramine	17.9	15.0 ± 12.2		
	35.7	30.0 ± 9.5		
	71.4	73.7 ± 10.6	45.7	32.9–79.3
Chlorimipramine	47.6	24.6 ± 5.8		
	95.2	50.6 ± 5.6		
	190.5	79.7 ± 9.9	91.4	74.3–113.6
Mianserin	200.0	12.5 ± 6.3	>200.0	

Drugs were injected intraperitoneally 30 min before intraventricularly administered 6-OHDA (100 μg) and rats were killed 3 days later. Brain NA levels of vehicle-treated control rats were 0.441 ± 0.012 μg/g (n=21). For further information see legend to Table 4.

Table 6 Blockade of the ability of *p*-chloroamphetamine (PCA) to lower rat brain 5-hydroxytryptamine (5-HT) levels

Drug	*Dose (μmol/kg)*	*% Block (mean ± s.e. mean)*	*ID_{50} (μmol/kg)*	*95% Confidence limits (μmol/kg)*
Chlorimipramine	11.9	25.2 ± 4.4		
	23.8	40.3 ± 6.2		
	47.6	60.6 ± 2.1	24.4	13.6–43.8
Imipramine	26.8	30.6 ± 5.2		
	53.6	48.7 ± 11.3		
	107.1	72.2 ± 10.1	52.9	32.9–89.3
Desipramine	75.2	31.8 ± 4.3		
	112.8	54.8 ± 8.5		
	150.4	63.1 ± 3.2	108.6	90.2–130.8
Mianserin	200.0	12.2 ± 8.5	>200.0	

Drugs were injected intraperitoneally 15 min before PCA (10 mg/kg, i.p.) and rats were killed 3 h after PCA administration. Brain 5-HT levels of vehicle-treated control rats were 0.535 ± 0.012 μg/g (n=28). The % inhibition of 5-HT reduction by PCA was calculated from the formula of Carlsson, Corrodi, Fuxe & Hokfelt (1969b). For remainder of legend, see Table 4.

tricyclic antidepressants are potent inhibitors of NA uptake, a characteristic feature of this group of drugs is their lack of effect on dopamine uptake (Carlsson, Fuxe, Hamberger & Lindqvist, 1966; Dorris & Shore, 1971). In contrast to (+)-amphetamine, both mianserin and desipramine had no effect on (–)-MA uptake by rabbit striatal minces. Hence the relative inability of tricyclics to block dopamine uptake *in vitro* is mimicked by mianserin. Previous studies have shown that desipramine is a competitive inhibitor of (–)-MA uptake by rabbit heart slices (Berti & Shore, 1967). This situation also holds centrally as indicated by the observation that the uptake of (–)-MA by brain stem slices was also inhibited by desipramine in a competitive manner. Like desipramine, mianserin is also a competitive blocker of brain stem (–)-MA uptake. The ability of mianserin to inhibit 5-HT uptake *in vitro* is appreciably less than that of the tricyclics studied and agrees with findings of others (Baumann & Maitre, 1975; Koe, 1976; Raiteri *et al.*, 1976).

In vivo blockade of central 5-HT uptake was quantified by studying the effect of drug pretreatment on the ability of PCA to lower rat brain 5-HT levels. In order to achieve a reduction in brain 5-HT content, PCA is actively transported into the serotoninergic neurone by the membrane amine pump and compounds blocking the pump such as the tricyclic antidepressants prevent the lowering of brain 5-HT by PCA and related analogues (Meek, Fuxe & Carlsson, 1971). In contrast to the tricyclics studied, mianserin at doses up to 200 μmol/kg was essentially devoid of effect on the ability of PCA to lower rat brain 5-HT levels and it would appear valid to conclude that mianserin has essentially no effect on central 5-HT uptake *in vivo*.

In order to lower brain tissue levels of NA and dopamine, 6-OHDA must be taken up by the neurone membrane amine pump and consequently compounds which block the pump prevent the 6-OHDA-induced depletion of NA and dopamine (Stone, Porter, Stavorski, Ludden & Totaro, 1964; Malmfors & Sachs, 1968; Evetts & Iversen, 1970; Breese & Traylor, 1972). A characteristic feature of the tricyclic antidepressants is their lack of effect on the 6-OHDA-induced fall in rat brain dopamine content (Evetts & Iversen, 1970; Breese & Traylor, 1972) and in this respect, mianserin resembles the tricyclics. In contrast to the tricyclics investigated, mianserin is essentially devoid of effect on the ability of intracerebroventricular 6-OHDA to decrease the concentration of NA in the rat brain. This result is similar to that of Leonard (1974) who showed that mianserin has no effect on the H 77/77-induced fall in rat brain NA content. However, it should be added that von Voigtlander & Losey (1976) have recently reported that mianserin rivalled imipramine in blocking the decrease in brain NA content following the intravenous injection of 6-hydroxydopa to pargyline pretreated mice. Tricyclic antidepressants are extremely potent in preventing the 6-OHDA-induced fall in rat heart NA concentration, e.g. the ID_{50} value for desipramine is 3.4 μmol/kg. Mianserin was found to be active in this model, the ID_{50} value being 94.0 μmol/kg. However, the ability of mianserin to block the depletion of rat heart NA elicited by 6-OHDA is appreciably less than that of the tricyclics studied.

Although mianserin rivals both imipramine and amitriptyline in blocking NA uptake *in vitro*, this situation clearly does not hold *in vivo*. Perhaps in the *in vivo* situation mianserin, in contrast to the tricyclics, does not readily attain and maintain a sufficiently high concentration in plasma to compete effectively with NA for attachment to the membrane amine pump. It is of interest to note that the plasma concentration of drug in patients being treated with mianserin is appreciably less than plasma levels of drug achieved in patients on tricyclic antidepressant therapy (Coppen *et al.*, 1976). Thus inhibition of NA uptake would not be anticipated in patients being treated with clinically effective doses of mianserin. This hypothesis agrees with the recent clinical report that mianserin, in contrast to amitriptyline, has no effect on the ability of tyramine to induce both a pressor response and mydriasis (Ghose, Coppen & Turner, 1976). Hence, it is improbable that the clinical effectiveness of mianserin can be attributed to inhibition of monoamine uptake. Finally, the relative lack of effect of mianserin on monoamine uptake *in vivo* offers an explanation for the ineffectiveness of mianserin in conventional screening tests for potential antidepressant agents.

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