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Comparison of the monoamine oxidase inhibiting properties of two reversible and selective monoamine oxidase-A inhibitors moclobemide and toloxatone, and assessment of their effect on psychometric performance in healthy subjects

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1 The effects of two reversible, predominantly monoamine oxidase-A (MAO-A) inhibitors, moclobemide (150 mg three times daily) and toloxatone (400–200–400 mg day^{-1}) on monoamine metabolites and psychometric performance were compared in a double-blind placebo controlled crossover study in 12 healthy subjects.

2 After 7 days of moclobemide/toloxatone/placebo administration subjects were hospitalized for 24 h on day 8. Blood samples were drawn every 2 h for determination of plasma noradrenaline (NA), 3,4-dihydroxyphenylglycol (DHPG), homovanillic acid (HVA) and 5-hydroxyindolacetic acid (5-HIAA). Urine was collected for measurements of normetanephrine and 3-methoxytyramine excretion. Psychometric performance (short- and long-term memory, critical flicker fusion frequency, choice reaction time) and subjective feelings were assessed before each drug intake (in the morning, at noon, in the evening).

3 Compared with placebo, both reversible monoamine oxidase inhibitors decreased the plasma concentration of DHPG and HVA. The overall fall in DHPG (AUC from 0 to 24 h) was 44% during moclobemide and 12% during toloxatone ($P < 0.001$) and the overall decrease in HVA was 38% and 20% ($P < 0.005$) on moclobemide and toloxatone, respectively.

4 Before the next drug intake, MAO-A inhibition, as judged by the decrease of plasma DHPG concentration, was significantly different from placebo with moclobemide but not with toloxatone.

5 Moclobemide, but not toloxatone, exerted a moderate, but significant inhibition of the deamination of 5-hydroxytryptamine (5-HT) as judged by the fall in plasma 5-HIAA concentration. Neither drug influenced plasma NA concentration.

6 A significant rise in urinary excretion of normetanephrine was observed on moclobemide and to a lesser extent on toloxatone. The urinary excretion of 3-methoxytyramine was significantly raised by moclobemide but not by toloxatone.

7 Neither moclobemide nor toloxatone altered memory function, vigilance, subjective feelings or sleep characteristics of the subjects.

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Introduction

Monoamine oxidase (MAO) is present in two forms: MAO-A and MAO-B. They differ in substrate specificity: MAO-A preferentially deaminates noradrenaline, adrenaline, normetanephrine, serotonin, tyramine and dopamine and it is selectively inhibited by clorgyline; MAO-B metabolises phenylethylamine, benzylamine, tyramine and dopamine and its selective inhibitor is selegiline. Most of the classical monoamine oxidase inhibitors (MAOIs) in clinical use inhibit both forms of MAO, and in an irreversible manner.

MAOIs were widely used as antidepressants until enthusiasm waned because of life-threatening hypertensive crises set off by ingestion of tyramine-containing foods while patients were taking MAOIs.

In the last few years interest in MAOIs has again increased since their efficacy is no longer doubted and it seems that the frequency and gravity of their adverse effects especially the 'cheese reaction' have been overestimated (Bass & Kervin, 1989; Sullivan & Shulmann, 1984). Moreover, recent research has been directed to develop new MAOIs which block the MAO in a selective and reversible manner and with a shorter duration of action.

Toloxatone is the only reversible and selective MAO-A inhibitor available for clinical use. It has been marketed in France since 1985.

Moclobemide is a new, reversible (Waldmeier, 1985) MAO-A selective (Koulu *et al.*, 1989; Wiesel *et al.*, 1985) MAOI with few adverse effects and clear antidepressant properties (Casacchia *et al.*, 1984; Dajas *et al.*, 1984; Larsen *et al.*, 1984; Stabl *et al.*, 1989). Moclobemide penetrates well the brain, maximal concentrations of moclobemide in CSF are reached 2 h after oral administration and high CSF/plasma ratio (0.5) can be obtained (Lauy *et al.*, 1990). Non-selective MAOIs interact with tyramine to raise blood pressure but this interaction is negligible with moclobemide (Berlin *et al.*, 1989; Korn *et al.*, 1988).

The present study was carried out in order to assess and compare the effect of 1 week administration of moclobemide and toloxatone on biochemical parameters that are considered to reflect monoamine metabolism. We therefore evaluated the kinetics of MAO inhibition at steady state, during administration of therapeutic doses of both MAOIs, throughout a period of 24 h. In addition, we investigated the effect of these two reversible and predominantly MAO-A inhibiting agents on vigilance, memory functions, subjective feelings and subjective characteristics of sleep in healthy subjects.

Methods

Subjects

Twelve healthy male volunteers were selected for the study. Their age ranged from 20 to 29 years (mean 23.9), their weight from 58 to 77 kg (mean 67.7) and height from 168 to 186 cm (mean 178). Subjects had no previous history of cardiovascular, pulmonary, psychiatric, or other diseases, and had been free of medication for at least 1 month. Every subject had a normal 12 lead ECG and normal results following clinical, biochemical and haematological examinations before the study. All of the subjects had normal results on three psychological examinations (Minnesota Multiphasic Personality Inventory, Eysenck Personality Inventory and Cattell anxiety test). Approval was obtained from the Ethics Committee of the Pitié-Salpêtrière Hospital and each subject gave written informed consent before the study.

Dietary restrictions Subjects were asked not to eat tyramine-containing foods during the 5 weeks of the study. A dietary card was given to each subject and the importance of avoiding tyramine-containing foodstuffs and other drugs than study medications was emphasized. Alcohol consumption was prohibited during the three treatment periods. Subjects were asked not to drink caffeine containing beverages, and not to eat chocolate during the 24 h prior to and during study days. During hospitalization (day 8) meals were standardized and only water and milk were authorized as beverages.

Study design and dosage regimen

This was a double-blind crossover study. Drugs were administered according to a balanced Latin square design. Each treatment period was separated by 1 week of wash out. Treatments were given throughout 8 days three times per day. Subjects took two capsules in the morning,

one capsule at noon and two capsules in the evening 10 min after meals. The dose of moclobemide was 150 mg three times daily, the dose of toloxatone was 1000 mg day^{-1} (400 mg in the morning, 200 mg at noon and 400 mg in the evening). As the capsules of toloxatone are 200 mg, one capsule of placebo was given with the morning and evening doses of moclobemide, respectively. All medications were administered in identical nonidentifiable capsules. Treatments were supplied by Produits Roche (France).

The dose of toloxatone administered in this study (1000 mg day^{-1}) is the highest dose used in therapeutics. The dose of moclobemide (450 mg day^{-1}) is the mean daily dose used in most clinical studies (Stabl *et al.*, 1989).

Study outline

Subjects began to take their treatment at home. On day 3 they underwent a clinical examination and they reported the adverse effects (if any) observed. On day 8 in the morning subjects arrived after an overnight fast at the department. A polyethylene cannula was inserted into a forearm vein. The contralateral arm was used for blood pressure and heart rate measurements. The first blood sample and blood pressure and heart rate recording were taken at least 20 min after the insertion of the cannula, the subjects being in the supine position. During this time, the subjects filled a questionnaire concerning the subjective characteristics of sleep. The subjects took their standardised breakfast after the first blood sample was drawn. The first psychometric assessment (T0) started after the breakfast. At the end of this assessment, subjects were asked to void their bladders and took the morning dose of the treatment with 150 ml of tap water. The next psychometric assessment (T1) started 30 min before the ingestion of the next dose of treatment, and the third (T2) before the ingestion of the evening dose. Treatments were administered 6 hourly. Blood samples were drawn every 2 h throughout the day (0, 2, 4, 6, 8, 10, 12, 14, 16 h) and the next day in the morning before the subjects were discharged from the department (24 h). At this time a 12 lead ECG was performed and blood was drawn for routine biochemistry and blood cell count. Urine was collected from 0 to 12 and from 12 to 24 h.

Assessments

Chemical determinations Blood for chemical determinations was collected into silicone coated tubes containing 0.5 ml of 0.129 M buffered sodium citrate. Blood samples were immediately centrifuged at 4° C and the plasma frozen. The plasma samples were stored at −70° C.

Assay of 5-HIAA, HVA and NA in plasma Plasma (1 ml) was mixed with 20 µl internal standard (containing 10 ng 5-hydroxyindole-2-carboxylic acid and 400 pg 3,4-dihydroxybenzyl-amine), 20 µl cystein solution and 1.0 ml 0.2 M ammonium acetate buffer pH 6.5. The mixture was passed through a polypropylene column (inner diameter 5 mm), filled with Bio-Rex 70, sodium form, filling height 2.5 cm, and then the column rinsed with 2 ml demineralized water. The column effluate and rinsing solution were combined and passed through a second polypropylene column. The column was rinsed with 3×5 ml demineralized water, 1 ml methanol (50% in demineralized water) and 2 ml absolute methanol. 5-HIAA and HVA were eluted with 8 ml 4 M formic acid/methanol solution. 10 µl cystein solution (20 g l^{-1}) was added to the formic acid/methanol eluate and 2 ml of this evaporated to dryness at 40° C in a rotary evaporator. The residue was dissolved using 0.5 ml of the mobile phase and an aliquot injected onto the h.p.l.c. system. The mobile phase consisted of 1.4 g NaOH, 5.25 g citric acid, 110 mg 1-octane-sulphonic acid (sodium salt) and 100 ml methanol l^{-1}, pH was 4.5. Flow rate was 0.4 ml min^{-1} and the amperometric detector was set at +0.80 V *vs* an Ag/AgCl reference electrode. This method was developed and validated in our laboratory (H-M. Thiede) and normal plasma values agree well with those reported by others (Ortiz *et al.*, 1988; Sagara *et al.*, 1988). Intra- and interassay variance was below 10%.

Noradrenaline, retained from plasma by the first cation exchanger column, was eluted from the column with 5 ml 2 M NaCl solution after three times washings with 5 ml demineralized water. 50 µl sodium disulphite solution (50 g l^{-1}), 1.0 ml 2 M Tris acetate buffer pH 8.6 and 20 mg acid-washed aluminium oxide were added to the column eluate. This mixture was stirred for 15 min, the supernatant was removed, and the aluminium oxide was washed three times with demineralized water. After the last washing noradrenaline was eluted from the aluminium oxide by adding 150 µl 0.1 M perchloric acid. 90 µl of the perchloric acid extract was injected onto the h.p.l.c. system. Intra- and interassay variance was below 5%. The mobile phase used in this system was the same as described for HVA and 5-HIAA. Sample cleanup was made according to Jackman *et al.* (1984).

Assay of DHPG in plasma To 1 ml of plasma were added 50 µl internal standard (containing 2 ng α-methyl-noradrenaline), 1.0 ml demineralized water, 50 µl sodium disulphite (10 g l^{-1}), 20 mg acid-washed aluminium oxide and 250 µl 2 M Tris acetate buffer pH 8.6. Upon addition of Tris, the plasma samples were mixed for 15 min. After the aluminium oxide was allowed to settle and the supernatant was discarded. The aluminium oxide was then washed with three 8 ml portions of demineralized water. DHPG was eluted from aluminium oxide with 250 µl of mobile phase which consisted of 9.66 µg sodium dihydrogenphosphate × H20, 10 ml methanol, 100 mg 1-octane-sulphonic acid (sodium salt), 37 mg EDTA and 8.5 ml 2 M phosphoric acid l^{-1}. Up to 100 µl was injected onto the h.p.l.c. system. Flow was 0.8 ml min^{-1}. For detection an ESA coulometric detector (ESA Inc., Bedford, MA) was used (Coulochem Model 5100 A) with a guard cell 5020 and a high sensitivity analytical cell 5011. The guard cell potential was +0.50 V, the working electrode potentials were +0.35 V and −0.35 V, respectively. The sample cleanup was done according to a modified version of Eriksson & Persson (1987). Intra- and interassay variance was about 5%.

Assay of total normetanephrine and 3-methoxytyramine in urine Urine (0.1 ml) was mixed with 0.1 ml internal standard solution (containing 20 ng 3-hydroxy-4-methoxybenzylamine) and 0.2 ml 0.2 M perchloric acid. The acid diluted urine samples were then hydrolysed in glass stoppered tubes in a water bath at 95° C for 30 min. After hydrolysis samples were pH adjusted to pH 6.5 with ammonium hydroxide and passed through a cation exchanger column. The column was rinsed with demineralized water, 4 ml methanol and *O*-methylated catecholamines were eluted with 2.5 ml 2 M ammonium hydroxide in methanol. The eluate was evaporated to dryness at 45° C in a rotary evaporator and the residue dissolved with mobile phase as described by Shoup & Kissinger (1977). An aliquot was injected onto the h.p.l.c. system. Inter- and intraassay variance was below 5%.

Heart rate and blood pressure measurements Heart rate (HR) and blood pressure (BP) were recorded in supine position after 15 min of bedrest, and in standing position after 2 min of standing by an oscillometric automated BP and HR recorder (Dinamap 1846, Critikon, Tampa, FL). HR and BP were measured before ingestion of the morning dose (T0) and 2, 4, 6, 8, 10, 12 and 24 h thereafter.

Psychometric evaluations a) Critical flicker fusion frequency (Hindmarch, 1980) was measured using the Leeds Psychomotor Tester (ZAK GmbH, D-8346 Simbach/Inn, FRG). Threshold frequency was taken as the mean of three ascending and three descending readings. All measurements were carried out at a viewing distance of 1 m in a room with constant subdued artificial lighting, after allowing sufficient time for subjects to adapt to the light.

b) Choice reaction time was also measured by the Leeds Psychomotor Tester. Total reaction time, recognition time and motor reaction time were determined. The measure given in milliseconds was the mean of 50 stimulus presentations.

c) The Digit Symbol Substitution Test is a subtest of the Wechsler Adult Intelligence Schedule involving coding skills. Parallel forms of the test were used in order to control any memorizing or learning which could interfere with the performance assessment (Hindmarch, 1980). The score was the number of items completed correctly in 3 min.

d) The coupled word test uses paired-associate words to evaluate short-term memory. Twelve pairs of words were read to the subjects. Recall was checked immediately after presentation.

e) The picture test was used as a measure of long-term memory. A set of 20 simple pictures was used. Free recall and recognition were assessed after 30 min delay.

f) Visual analogue rating scales (VAS) were used to assess subjective feelings. The subjects were asked to rate their current feelings by marking the appropriate place on a 100 mm line which had a central area corresponding to a normal state. These VAS contained the following items: anxious, tired, happy, relaxed, drowsy, dizzy, clumsy, alert, energetic, sad and depressed.

Sleep characteristics were evaluated by a structured questionnaire.

Adverse effects were recorded by free interviews on day 3 and day 8.

Statistical analysis

Analysis of the plasma concentrations and urinary excretion For each variable the area under the plasma concentration-time curve (AUC) was determined by trapezoidal rule from *t*0 to the last concentrations measured (*t*24 h). In addition, plasma concentrations just before drug intake (*t*0, *t*6 h, *t*12 h and *t*24 h) and minimal plasma concentrations between the first and second, and second and third drug intake were analysed. The statistical analysis was performed using an ANOVA for repeated measurements with

factors subject, time, period and treatment.

Urinary parameters were also tested by analysis of variance using as factors subject, period and treatment.

If a significant treatment effect occurred, treatments were compared by the Tukey test (Winer, 1971; Zar, 1984).

Analysis of psychometric results An ANOVA was performed on the T0 values with factors subject, period and treatment. Assessments of T1 and T2 were tested by an ANOVA including factors subject, time, period, treatment. The ANOVA was followed by the Tukey test if indicated.

Nominal scale data were tested by the McNemar's test (Zar, 1984).

The statistical analyses were performed by the statistical package BMDP (BMDP, Statistical Software, Inc., Los Angeles, California). Data are presented as mean ± s.e. mean. A P value < 0.05 was considered significant.

Results

Adverse effects

Drop out From the initially included 12 subjects one subject was excluded from the study after the first treatment period which was found to be with toloxatone. This subject had electrocardiographical changes (T wave inversion in leads II, III, avF and V4–6) without subjective symptoms. No abnormalities were found concerning the plasma adrenaline, NA, DHPG, HVA and 5-HIAA concentrations. Since a toxic effect of the drug could not be excluded, the subject was replaced. The results of this subject were not included in the analysis of data.

Subjective adverse effects Table 1 shows adverse effects reported on day 8. There was no difference in adverse effects reported on day 3 and day 8. More subjects reported one or more symptoms during active treatment (toloxatone *vs* placebo $P < 0.05$, moclobemide *vs* placebo $P < 0.025$) but there was no difference with regard to adverse effects between toloxatone and moclobemide.

Heart rate and blood pressure No modification of systolic and diastolic BP was found. No subject had orthostatic hypotension and there was no statistically significant difference between supine and standing BP. A significant treatment effect occurred for supine and standing heart rate (Figure 1) but further analysis could not differentiate between the treatments. Mean standing heart rate seemed to be lower on moclobemide than on toloxatone or placebo but this difference was statistically not significant (Tukey test: q = 1.503, NS).

Plasma concentrations of DHPG, HVA, 5-HIAA and noradrenaline (Table 2 and Figure 2) Both reversible MAOIs significantly decreased plasma DHGP concentration as judged on AUC. The overall fall was 44% on moclobemide and 12% on toloxatone ($P < 0.001$), thus the decrease in plasma DHPG concentration was 3.5 fold greater during moclobemide than during toloxatone administration. The maximal decrease in plasma DHPG concentration was 60% with moclobemide and 29% with toloxatone.

Table 1 Adverse effects reported on day 8. Numbers indicate the number of subjects having reported the symptom.

Adverse effects	*Moclobemide*	*Toloxatone*	*Placebo*
Excitation	0	2	0
Tiredness	0	2	0
Drowsiness	2	1	0
Diarrhoea	1	1	0
Memory problems	0	1	0
Dizziness	1	0	0
Epigastric pain	2	0	0
Difficulty in sleeping	1	1	0
Blurred vision	1	0	0
Awakening in the night	1	0	0
Sensation of a heavy head	0	1	0
Abdominal pain	0	1	0
Insomnia	0	1	0
Slowing down	0	1	0
Total	9	12	0

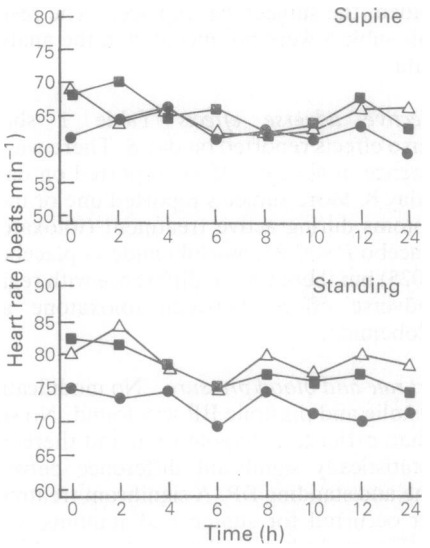

Figure 1 Supine and standing heart rate after administration of moclobemide (●), toloxatone (△) or placebo (■) for 1 week (active treatment *vs* placebo $P = 0.025$ for supine and $P = 0.031$ for standing heart rate).

Before the next drug intake, plasma DHPG concentrations were not different from placebo while subjects were taking toloxatone. In contrast to toloxatone, moclobemide produced a constant decrease in plasma DHPG: plasma DHPG concentrations before the next drug intake, even 12 h after the evening dose, were significantly lower on moclobemide than on placebo or toloxatone.

Areas under the plasma HVA concentration-time curves were significantly smaller during treatment with the two MAOIs. Compared with placebo, AUCs were 38% smaller on moclobemide and 20% smaller on toloxatone. The difference between the two active treatments was significant ($P < 0.005$). Nadir plasma HVA concentrations on toloxatone were not different from those found on placebo. Moclobemide, compared with placebo, significantly reduced (−41%) the nadir plasma HVA level. Plasma HVA concentrations before drug intake were significantly lower on moclobemide than on placebo. At $t24$ h a significant treatment effect ($P = 0.0035$) occurred accompanied by a period × treatment interaction ($P = 0.0024$) which invalidated the analysis of this time point.

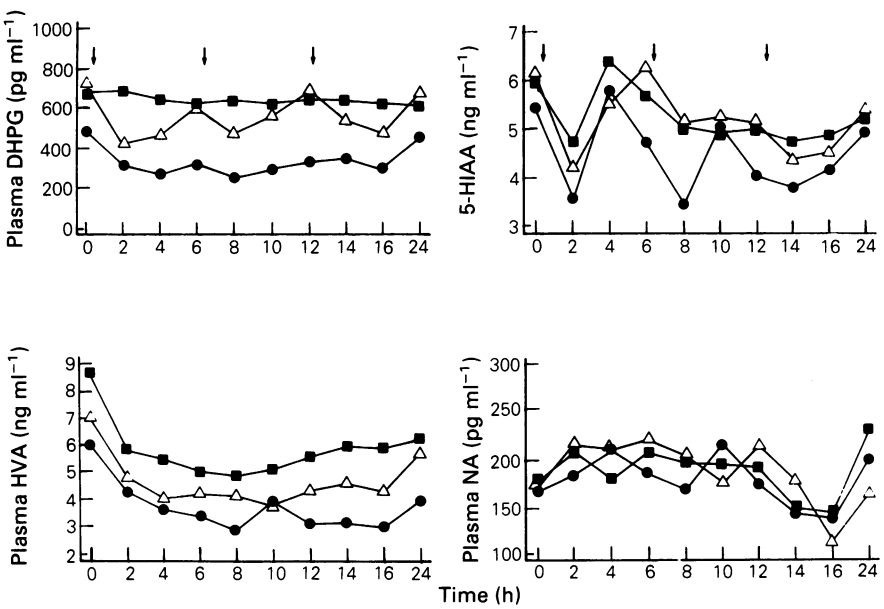

Figure 2 Mean plasma DHPG, HVA, 5-HIAA and noradrenaline concentrations on day 8 after administration of moclobemide (450 mg day^{-1}, ●), toloxatone (1000 mg day^{-1}, △) and placebo (■) for 1 week. (Arrows indicate drug intake. S.e. means have been omitted for clarity.)

Table 2 Mean (± s.e. mean) area under curve (AUC), minimal plasma concentration and plasma concentration just before drug intake (* $P < 0.001$, ** $P < 0.005$, *** $P < 0.01$, moclobemide (M) *vs* toloxatone (T), a: $P < 0.001$, b: $P < 0.005$, c: $P < 0.025$, d: $P < 0.05$ active treatment *vs* placebo (P), #: treatment effect: $P = 0.0035$ and period × treatment interaction $P = 0.0024$).

		AUC (×*h*)	*Minimal plasma concentration* *After 1st drug intake*	*After 2nd drug intake*	t0	t6h	t12h	t24h
DHPG (pg ml^{-1})	P	15040 (778)	586 (33)	591 (28)	674 (42)	614 (42)	633 (32)	602 (20)
	M	8378 (925)*[d]	242 (13)[a]	233 (16)*[a]	487 (18)*[a]	321 (20)*[a]	327 (24)*[b]	431 (27)*[a]
	T	13228 (866)[c]	391 (29)	450 (23)*[a]	723 (37)	598 (27)	677.3 (57)	670 (48)
HVA (ng ml^{-1})	P	139 (12)	4.72 (0.33)	4.49 (0.44)	8.67 (0.77)	5.02 (0.34)	5.57 (0.72)	6.22 (0.56)
	M	86 (9)**[a]	2.84 (0.53)	2.56 (0.37)[b]	6.06 (0.7)[d]	3.38 (0.63)[c]	3.14 (0.34)[a]	3.93 (0.7)#
	T	111 (9)[c]	3.67 (0.35)	3.37 (0.29)	7.04 (0.77)	4.18 (0.46)	4.31 (0.29)[d]	5.67 (0.4)
5-HIAA (ng ml^{-1})	P	125 (5)	4.49 (0.17)	4.64 (0.2)	5.96 (0.25)	5.71 (0.74)	5.02 (0.13)	5.26 (0.2)
	M	108 (5)**	3.53 (0.15)	3.37 (0.15)**[c]	5.45 (0.2)	4.74 (0.34)	4.08 (0.21)***[c]	4.98 (0.23)
	T	123 (6)	4.08 (0.18)	4.6 (0.24)	6.14 (0.2)	6.29 (0.87)	5.15 (0.3)	5.4 (0.27)

Plasma 5-HIAA concentrations varied much throughout the day. The rises in 5-HIAA concentration appeared mostly after meals. Toloxatone did not affect at all plasma 5-HIAA levels. AUC and nadir plasma 5-HIAA concentrations on moclobemide were significantly lower than those observed on toloxatone.

The two MAOIs did not affect the plasma concentration of noradrenaline.

Intratreatment comparisons were done to find out whether plasma concentrations at $t0$ and $t24$ h were identical or not. This analysis showed that on placebo plasma DHPG, HVA and 5-HIAA concentrations were significantly higher at $t0$ than at $t24$ h ($P = 0.04$, 0.0023 and 0.0024, respectively). Moclobemide completely blocked this increase at $t0$ of plasma DHPG, HVA and 5-HIAA since on moclobemide there was no difference between concentrations of $t0$ and $t24$ h. Toloxatone inhibited the increase at $t0$ of plasma DHPG and HVA but did not affect that of 5-HIAA.

Urinary excretion of normetanephrine and 3-methoxytyramine Moclobemide significantly increased urinary excretion of normetanephrine compared both to placebo and toloxatone (Figure 3). During the day (from 0 to 12 h) toloxatone did not rise urinary excretion of normetanephrine but it increased excretion of this metabolite of noradrenaline during the night (fraction from 12 to 24 h).

Urinary excretion of 3-methoxytyramine was increased only by moclobemide and then only during the night.

There was a great inter- and intraindividual variability on placebo for urinary excretion of both monoamine metabolites and there was no significant difference between the fractions of 0 to 12 h and of 12 to 24 h.

Psychometric evaluations (Table 3)

Vigilance No treatment effect was observed on critical flicker fusion frequency or digit substitution symbol test. Total reaction time and motor reaction time were not modified by the treatments. A treatment effect was observed for recognition time ($P = 0.0079$) at $t0$. Further analysis showed that recognition time was shorter on moclobemide than on placebo or toloxatone. This difference although statistically significant may be clinically irrelevant.

Memory tasks Neither moclobemide nor toloxatone affected short-term or long-term memory in these healthy subjects.

VAS were not modified by the two MAOIs nor was the quality of sleep changed. The MAOIs did not shorten or prolong sleeping time

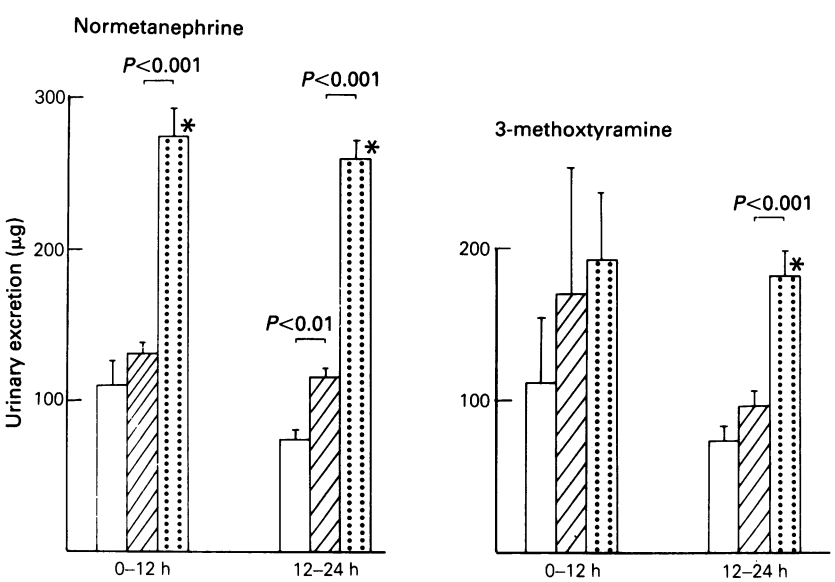

Figure 3 Urinary excretion (mean ± s.e. mean) of normetanephrine and 3-methoxytyramine on day 8 after administration of moclobemide (▦), toloxatone (▨) and placebo (□) for 1 week. *moclobemide *vs* placebo $P < 0.001$.

Table 3 Critical flicker fusion frequency (c.f.f.), choice reaction time (total: CRT-T, recognition time: CRT-R, motor reaction time: CRT-M), digit substitution symbol test (DSST) and memory tasks (mean (± s.e. mean)) before the morning dose (T0) and just before the second (T1) and third drug intake (T2) on day 8 after 1 week of drug administration (* $P < 0.01$ moclobemide *vs* toloxatone and placebo).

	Moclobemide			*Toloxatone*			*Placebo*		
C.f.f.	30.59	29.90	29.69	30.55	30.15	29.94	30.47	29.94	29.93
(Hz)	(0.58)	(0.53)	(0.58)	(0.57)	(0.57)	(0.73)	(0.59)	(0.59)	(0.73)
CRT-T	359.1	363.1	363.9	361.3	359.9	350.7	362.9	363.9	357.5
(ms)	(9.9)	(9.4)	(12.2)	(9.2)	(10.4)	(9.8)	(7.7)	(8.1)	(8.6)
CRT-R	267.9*	273.2	275.1	277.2	275.6	269.4	273.9	277	273.2
(ms)	(6.9)	(5.7)	(8.2)	(6.1)	(7.1)	(7.7)	(6.6)	(6.6)	(7.6)
CRT-M	91.2	89.9	88.8	84.2	84.3	81.3	89	86.1	84.3
(ms)	(5.8)	(6.2)	(7.3)	(6)	(7)	(5.6)	(5.7)	(4.9)	(4.9)
DSST	119.9	120.3	120.9	122.3	120.8	122.7	120.5	120.2	120.7
(total score)	(3.5)	(3.5)	(3.6)	(3.3)	(3.2)	(3.3)	(3.4)	(3.6)	(3.4)
DSST (Number of symbols	119.5	120.2	120.7	121.4	120.2	122.3	119.7	119.2	120.1
correctly copied)	(3.5)	(3.5)	(3.6)	(3.8)	(3.3)	(3.4)	(3.5)	(3.9)	(3.5)
Coupled words	6.5	6.1	5.6	6.6	6.4	5.9	6.2	6	5.9
(correct responses)	(0.2)	(0.3)	(0.3)	(0.2)	(0.3)	(0.2)	(0.3)	(0.3)	(0.2)
Free recall of pictures	15.4	11.7	13.3	15.2	12	11.9	14.2	11.7	12
(correct responses)	(0.8)	(1.4)	(1.3)	(0.6)	(1.2)	(1.5)	(0.9)	(1.2)	(1.3)
Recognition of pictures	19.5	19.7	19.7	19.7	19.6	19.7	19.8	19	19.2
(correct responses)	(0.2)	(0.1)	(0.2)	(0.2)	(0.2)	(0.1)	(0.1)	(0.3)	(0.3)

or the time necessary to fall asleep. There was no more awakening during the night on active treatments than on placebo.

Discussion

The main objective of this study was the comparison of the MAO-A inhibiting properties of moclobemide, toloxatone and placebo after long-term administration in healthy subjects. Secondary objectives included evaluation of tolerance and assessment of psychometric performance, subjective feelings and sleep characteristics in order to find out whether these reversible MAOIs impair different psychological functions.

Both reversible MAOIs significantly inhibited the deamination of noradrenaline and dopamine. Both drugs decreased plasma DHPG and HVA concentrations, increased urinary excretion of normetanephrine. The effect of moclobemide on these parameters, which reflect MAO inhibition (Waldmeier, 1985; Wiesel *et al.*, 1985) was more pronounced than that of toloxatone. The overall fall in plasma DHPG was 3.5 fold greater and the overall diminution in plasma HVA 1.3 fold greater on moclobemide than on toloxatone. Urinary excretion of normetanephrine and 3-methoxytyramine was more than twofold greater on moclobemide than on toloxatone. The maximal MAO inhibiting effect (nadir plasma concentrations of monoamine metabolites) of moclobemide was more important than that of toloxatone. Furthermore, while the MAO inhibiting effect of toloxatone did not last until the next drug intake (6 h later), moclobemide lead to a MAO inhibition throughout the day.

There were some differences in the plasma DHPG and HVA responses to these reversible MAOIs. MAO inhibition as reflected by plasma HVA levels showed a prolonged, constant blockage put plasma DHPG mirrored more rapid changes especially evident with toloxatone. Plasma turnover of DHPG is faster than that of HVA (Eisenhofer *et al.*, 1989; Elchisak *et al.*, 1982) and therefore decrease in MAO inhibition (increase in DHPG) is reflected more rapidly in plasma DHPG than in plasma HVA concentration. Therefore plasma DHPG may be a more rapid and perhaps more sensitive indicator of MAO-A inhibition than plasma HVA concentration.

Plasma 5-HIAA concentrations varied considerably. This may be the result of dietary non-compliance. However, the effect of moclobemide on the deamination of 5-HT is known to be moderate (Koulu *et al.*, 1989) probably because the affinity of the drug to the binding sites of the MAO is less than that of serotonin.

Intratreatment comparisons of plasma monoamine metabolites showed that on placebo $t0$ values were significantly higher than $t24$ h values. $t0$ blood samples were drawn 30 min after subjects arrived at the department while the $t24$ h samples were drawn next morning when subjects were still in the resting position. Thus, $t0$ values represent usual, every day life conditions and $t24$ h values experimental conditions. These different experimental conditions might result in the morning elevation of plasma monoamine metabolites when subjects were arriving and were taking placebo. This morning elevation observed only at $t0$ was completely abolished by moclobemide and to a lesser extent by toloxatone.

The subjects tolerated well both MAOIs, however, more subjects reported adverse effects on active treatment than on placebo. No adverse effect was reported by more than two subjects and neither drug caused orthostatic hypotension. The presence of epigastric pain in two subjects on moclobemide was somewhat surprising. Stomach pain or epigastric discomfort have been reported with a frequency of 2.3% in double blind (n = 1262) and 1.9% in open trials (n = 941) with moclobemide. This drug induced incidence was never significantly different from that observed with placebo (Roche, unpublished data). It seems, however, that reversible MAOIs are well tolerated by healthy subjects (Berlin *et al.*, 1989; Grind *et al.*, 1986; Wijnands *et al.*, 1989).

An overall drug effect occurred for both supine and standing heart rate but no difference could be demonstrated between the active treatments. In clinical studies moclobemide has been found not to modify heart rate and it seems to be devoid of anticholinergic properties (Berlin *et al.*, 1990).

The reversible MAOIs used in this study, did not impair either vigilance or memory tasks. Impairment of psychomotor function on antidepressant drugs other than MAOIs is very well documented (Hindmarch, 1980, 1988; Hindmarch & Subhan, 1986). Doses given in therapeutics and administered to healthy subjects lead to substantial deterioration of vigilance and/or memory functions (Bye *et al.*, 1978; Curran *et al.*, 1986; Seppälä & Linnoila, 1983). In the present study, both MAOIs were given in therapeutic doses and no impairment in psychometric performance and memory occurred. MAO inhibition *per se* seems not to affect

psychometric tests since during the psychometric assessments MAO inhibition was the lowest on toloxatone and not different from other time points on moclobemide.

This new class of MAOIs which inhibit the enzyme in a reversible and selective manner, show negligible interaction with tyramine (Berlin *et al.*, 1989; Bieck *et al.*, 1989) and the risk of hypertensive reaction therefore may be less. Drugs in this class (brofaromine, moclobemide, amiflamine, toloxatone) have a short duration of action (Mann *et al.*, 1984). However, the question may arise whether these reversible, short-acting MAOIs possess clinical efficacy. It has been postulated that greater than 80% MAO inhibition is necessary to rise intracellular concentration of neurotransmitter amines and to lead to clinical efficacy (McDaniel, 1986). Clinical studies have shown that moclobemide is superior to placebo (Casacchia *et al.*, 1984) and is as effective as clomipramine (Larsen *et al.*, 1984) and amitriptyline (Norman *et al.*, 1985) in treating depressive patients. This indicates that the MAO inhibiting effect of moclobemide is sufficient to elicit clinical improvement at a dose used in the present study. However, this mean therapeutic dose exerted a 44% inhibition of the deamination of noradrenaline. Therefore it seems that a 40–50% MAO inhibition is sufficient to lead to improvement of clinical symptoms. Further clinical studies are needed to establish a correlation between the evolution of clinical symptoms and biochemical parameters reflecting MAO inhibition and find an eventual MAO inhibition threshold which would be necessary to obtain to have a clinical efficacy.

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