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Inhibition of monoamine oxidase by moclobemide: effects on monoamine metabolism and secretion of anterior pituitary hormones and cortisol in healthy volunteers

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1 Single oral doses (100, 200 and 300 mg) of moclobemide, a reversible inhibitor of monoamine oxidase (MAO) with predominant effects on the A-type of the enzyme, were administered to eight young, healthy male volunteers in a double-blind, random-order, placebo-controlled study. The investigation was thereafter continued in an open fashion by administering a single 10 mg dose of the MAO-B inhibitor deprenyl to the same subjects.

2 Deamination of catecholamines was powerfully and dose-dependently inhibited by moclobemide, as evidenced by up to 40% decreases in the urinary excretion of deaminated catecholamine metabolites, corresponding increases in the excretion of non-deaminated, methylated metabolites, and up to 79% average decreases in the plasma concentration of 3,4-dihydroxyphenylglycol (DHPG), a deaminated metabolite of noradrenaline (NA), and up to 75% average decreases in the plasma concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC), a deaminated metabolite of dopamine. The urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA) was only slightly reduced. In contrast, deprenyl, in a dose which almost totally inhibited MAO-B activity in blood platelets, did not appreciably affect the plasma concentrations of DHPG or DOPAC.

3 Due to the rapid, reversible, dose-dependent and MAO-A specific effect of moclobemide on plasma concentrations of DHPG, it is suggested that DHPG in plasma may be a useful indicator of the magnitude and duration of MAO-A inhibition in man.

4 Sympatho-adrenal function at rest was not significantly altered by moclobemide, as judged by unchanged plasma catecholamine concentrations and stable blood pressure and heart rate recordings.

5 Monoamine oxidase type B activity in blood platelets was slightly (less than 30%) and transiently inhibited after moclobemide.

6 The secretion of prolactin was dose-dependently stimulated by moclobemide, whereas the plasma concentrations of growth hormone (hGH) and cortisol remained unchanged.

Keywords MAO inhibitors moclobemide monoamine metabolites prolactin somatotropin cortisol

Introduction

Monoamine oxidase (MAO) occurs in two forms, MAO-A and MAO-B, which differ in substrate specificity and in sensitivity to some inhibitors (Johnston, 1968). MAO-A preferentially deaminates noradrenaline (NA) and serotonin (5-HT), and is irreversibly inhibited

by clorgyline (Houslay *et al.*, 1976; Murphy *et al.*, 1984, 1987; Mann *et al.*, 1984). MAO-B preferentially deaminates phenylethylamine, benzylamine and, in man, dopamine (DA), and is selectively inhibited by deprenyl (Glover *et al.*, 1977; Riederer & Youdim, 1986; Murphy *et al.*, 1987). The tissue localization of the two forms of the enzyme may also significantly affect the inhibitory selectivity of drugs *in vivo*. The enzyme responsible for the oxidative deamination of catecholamines in sympathetic nerve endings appears to be of the MAO-A subtype (Murphy *et al.*, 1987).

Inhibitors of MAO have long been used for the treatment of depression, but side-effects, particularly hypertension associated with concomitant ingestion of foods containing tyramine (cheese effect), have limited their use. Classical MAO inhibitors, like tranylcypromine and phenelzine, do not exhibit substrate specificity, i.e. they inhibit both forms of MAO. Inactivation of the enzyme by these drugs is also irreversible, leading to a long duration of action. Recently, a number of reversible and MAO-A-selective inhibitors have been developed, including moclobemide, amiflamine and cimoxatone (Mann *et al.*, 1984; Murphy *et al.*, 1984; Jarrott & Vajda, 1987).

Moclobemide (Ro 11-1163) is a novel inhibitor of MAO, distinguished by the reversibility of its action and its predominant effect on the A-type of the enzyme (MAO-A) (Da Prada *et al.*, 1981; Keller *et al.*, 1987). Clinical phase III trials have shown that the drug possesses clear antidepressant therapeutic efficacy comparable with that of some classical tricyclic drugs (Larsen *et al.*, 1984; Casacchia *et al.*, 1984; Norman *et al.*, 1985; Lensch *et al.*, 1987). Compared with older irreversible and non-selective MAO inhibitors, the tyramine-potentiating effect of moclobemide is relatively slight (Korn *et al.*, 1988).

The present study was carried out in healthy male volunteers and was designed to assess the effects of acute administration of moclobemide on several biochemical parameters, which have been considered to reflect monoamine metabolism and/or activity of monoaminergic neurons. These included plasma concentrations of the catecholamines noradrenaline (NA) and adrenaline, and their deaminated metabolite, 3,4-dihydroxyphenylethylglycol (DHPG), and 3,4-dihydroxyphenylacetic acid (DOPAC), a deaminated metabolite of dopamine (DA), urinary excretion of deaminated and methylated, non-deaminated metabolites of the monoamines, and MAO-B activity in blood platelets. In addition, we investigated changes in the release of prolactin, human growth hormone (hGH) and cortisol after acute moclobemide administration, since these hormones may be useful indicators of the effects of drugs on monoaminergic neurotransmission (Checkley, 1980; Koulu, 1986).

Methods

Subjects

Eight healthy male volunteers participated after written informed consent. They were 23-27 years old and within 15% of their ideal weight (mean 76 kg, range 63–92 kg). Two were smokers. The health of the subjects was ascertained by detailed medical history, physical examination, clinical chemistry tests and ECG. They had taken no medications in the 2 weeks preceding this study. Alcoholic beverages were prohibited for 36 h prior to each session, and food, smoking, caffeinated beverages and chocolate were not allowed after 22.00 h on the preceding night. The study was conducted in accordance with the guidelines of the declaration of the World Medical Assembly of Helsinki and Tokyo, concerning ethics in experimentation in humans, and was approved by the Ethics Committee of Turku University Hospital and the Finnish National Board of Health.

Design of the study

The experiment was carried out as a double-blind, randomized, placebo-controlled study with a Latin square design. Each subject received, as a single dose, 100 mg, 200 mg or 300 mg moclobemide or matching placebo tablets (supplied by Roche, Basle, Switzerland) at intervals of 4 to 10 days.

Study outline

Each session started at 08.00 h, when the subjects arrived at the department. They were first asked to void their bladders. A polyethylene cannula was inserted into a vein in the cubital fossa and maintained patent with a dilute solution of heparin. The contralateral arm was used for indirect measurements of blood pressure and heart rate with an automated oscillometric device (Nippon Colin 203Y). The first blood samples and recordings were taken after a minimum of 15 min of supine rest had elapsed since the completion of these preparations. At time zero (0 h), the subjects took three similar tablets (containing either placebo, 100 mg, 200 mg or 300 mg moclobemide) with 150 ml of water. The

subjects remained supine for the first 3 h of each session, after which a standardized lunch (low in tyramine content) was served. Recordings were carried out and blood samples collected until 8 h after the beginning of each session, whereafter the subjects were allowed to leave the department. The subjects returned next morning to the department, again after an overnight fast and a 24 h blood sample was taken through an indwelling venous catheter, placed in an antecubital vein at least 30 min earlier.

Blood samples were collected at the times indicated in Figures 1–7 for the determination of the concentrations of the catecholamines NA and adrenaline, the deaminated catecholamine metabolites DHPG and DOPAC, cortisol and the anterior pituitary hormones prolactin and hGH in plasma, and for the assessment of MAO-B activity in blood platelets. Urine was collected in fractions of 0–3, 3–8, and 8–24 h into plastic containers with 15 ml 3 N HCl, and immediately chilled to 4° C. The fractions were mixed and their volume and pH recorded, and three 50 ml aliquots were stored at −20° C until subsequent analysis.

Chemical determinations

Blood for the chemical determinations was collected into chilled polycarbonate tubes containing Na_2EDTA, and was promptly chilled and centrifuged at 0–4° C. The plasma samples were stored at −70° C. Endogenous catecholamines in plasma were determined using high performance liquid chromatography with coulometric electrochemical detection (h.p.l.c.-EC), as reported previously (Scheinin *et al.*, 1987). The selective two-phase electrochemical detection provided by the present instrument (Coulochem 5100A, ESA Inc., USA, operated at +0.30 V oxidation and −0.27 V reduction) made it possible to separate and quantitate DHPG and DOPAC simultaneously with NA and adrenaline. Intra-assay coefficients of variation were 2% for NA, 4% for DHPG, 10% for adrenaline and 15% for DOPAC in the relevant concentration ranges. All samples from one experimental session were analyzed in one assay.

H.p.l.c.-EC was also used to quantitate monoamine metabolite levels in urine. The deaminated catecholamine metabolites 4-hydroxy-3-methoxymandelic acid (HMMA) and homovanillic acid (HVA) were analyzed after solid-phase extraction on Sephadex G-10 according to the procedure described by Bremmelgaard (1985). The deaminated metabolite of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), was quantitated as described by Westerink *et al.*, (1982). The non-deaminated, methylated catecholamine metabolites metanephrine, normetanephrine, and 3-methoxytyramine were determined after acid hydrolysis according to Jouve *et al.* (1983). All urine samples from one experimental subject (12 samples) were analyzed within 1 day. The urinary excretion of these monoamine metabolites was normalized for time and urine production by expressing the results in relation to the excretion of creatinine in the same sample.

Concentrations of prolactin, hGH and cortisol in plasma were determined using commercially available solid-phase radioimmunoassays (Spectria®, Farmos-Group Ltd, Turku, Finland), with intra-assay coefficients of variation of 5% at 4 ng ml^{-1} and 4% at 30 ng ml^{-1} for prolactin, 8% at 3 ng ml^{-1} and 3% at 17 ng ml^{-1} for hGH, and 4.5% at 164 nmol l^{-1} and 3% at 314 nmol l^{-1} for cortisol.

Monoamine oxidase in platelets

Blood platelets were separated from 5 ml venous samples collected at the times indicated in Figure 4. The blood was prevented from clotting with 4 mM Na_2EDTA and chilled immediately in ice. All subsequent steps were carried out at 0–4° C in order to prevent the dissociation of moclobemide and its active metabolites from the enzyme (Keller *et al.*, 1987). The blood samples were centrifuged at 160 *g* for 30 min, and the platelet-rich supernatant was removed. An aliquot was reserved for automated cell counting. The average platelet yield was 282 ± 103 (s.d.) × 10^9 l^{-1} and the purity 97.2%. The platelets were precipitated by centrifugation at 2000 *g* for 15 min. The resulting pellet was washed once with 0.1 M potassium phosphate buffer (pH 7.4) containing 25 units ml^{-1} heparin. The final pellet was covered with 0.5 ml of the same buffer (without heparin) and stored at −70° C until assayed. Just before use, the platelets were sonicated in an MSE Ultrasonic Disintegrator (100 W model, 5 μm amplitude, 60 sc).

Monoamine oxidase type B (MAO-B) activity in the platelets was assayed radiochemically as described by Keller *et al.* (1987), with slight modifications. The reaction was carried out in 0.1 M potassium phosphate buffer (pH 7.4, total volume 0.5 ml). The mixture contained 100 μl of the platelet homogenate (about 0.2 mg protein) and 50 μl of the substrate [^{14}C]-phenylethylamine (^{14}C-PEA, Amersham CFA.627, specific activity 2.22 GBq/mmol) supplemented with unlabelled PEA to yield a final concentration of 10

Table 1 Number of subjects reporting subjective drug effects after placebo (=C), 100 mg (=M100), 200 mg (=M200) and 300 mg (=M300) moclobemide

	Treatments							
	0–3 h				*4–8 h*			
Effects	*C*	*M100*	*M200*	*M300*	*C*	*M100*	*M200*	*M300*
Restlessness	1	–	–	2	–	–	–	–
Dizziness	–	1	1	–	–	–	–	–
Lightheadedness	–	1	1	–	–	–	–	–
Flush	–	–	1	–	–	–	–	–
Tiredness	–	2	1	1	1	2	1	1
Increased vigilance	–	–	–	1	–	–	–	–
Headache	–	–	–	1	–	2	1	–
Perspiration	–	–	–	1	–	–	–	–

μM PEA in the reaction mixture. Enzyme activity was expressed as nmol of deaminated metabolites formed h^{-1} mg^{-1} protein. Protein content was measured according to Peterson (1977) with bovine serum albumin as standard. An aliquot of pooled platelet homogenate was included in all sets of samples and used as a control sample. The inter-assay coefficient of variation for the MAO-B determination was 2.6%.

Deprenyl experiment

After completing the double-blind moclobemide study, the investigation was continued by administering a single 10 mg dose of deprenyl (Eldepryl, Farmos Group Ltd, Turku, Finland) orally to the same subjects in order to assess the possible contribution of MAO-B inhibition to the observed effects of moclobemide on the plasma levels of DHPG, DOPAC and prolactin. This experiment was carried out identically to the moclobemide sessions.

Statistical analysis

The statistical analysis was performed using either analysis of covariance (ANCOVA) for repeated measurements, with two within-factors (dose and time) and the individual 0 h value as covariate, or analysis of variance (ANOVA), computed with BMDP2V programs (BMDP Statistical Software Inc, USA). Separate analyses were performed for the first three hour period after drug administration (ANCOVA) and for the later time points (ANOVA) in order to eliminate the confounding effects of the lunch break on the results. ANOVA was used for all urine data. When pooled orthogonal components showed nonsphericity, Greenhouse-Geisser probability values were used (Keselman & Keselman, 1984). When variances were unequal, log-transformed data were used for DOPAC, hGH, NM, M and 3-MT, and square-root transformed data for DHPG, prolactin and MAO-B activity in platelets. When a statistically significant drug effect or dose × time interaction was present, the analysis was continued by performing separate ANCOVAs or ANOVAs for each pair of different dose levels, or time points, in order to characterize the dose- or time-dependency of the observed effects in more detail. The deprenyl results were compared with the placebo session in an analogous manner.

Results

Subjective drug effects

In general, all treatments were well tolerated (see Table 1). Moclobemide appeared to cause slight sympathomimetic-like subjective effects in three of the eight subjects. Falling asleep was disturbed in two subjects, but this effect was not clearly dose-related.

Blood pressure and heart rate

Systolic and diastolic blood pressure, and heart rate remained stable during the sessions (data not presented). There were no differences between moclobemide and placebo treatments (Tables 2 and 3).

DHPG, DOPAC and catecholamines in plasma

Moclobemide powerfully decreased the concentration of DHPG in plasma (Figure 1 and Tables 3 and 4). The decrease was dose-dependent, being about 55% after 100 mg (from a mean ± s.d. basal level of 5.49 ± 0.99 nmol l^{-1} to 2.52 ± 1.49 nmol l^{-1} at 120 min; $P < 0.001$;

Table 2 Analysis of covariance (ANCOVA) (for the 0–3 h interval, 0 h value as covariate) with two within-factors: effects of dose and time. Square-root transformed data was used for DHPG and prolactin, and log-transformed data for hGH and DOPAC, BP = blood pressure

Variable		*ANCOVA (0–3 h)*		
		Factor 1 = dose	*Factor 2 = time*	*Interaction*
DHPG	*F*	80.64	89.96	12.25
	P	<0.0001	<0.0001	0.0003
DOPAC	*F*	11.17	35.33	4.17
	P	0.0002	<0.0001	0.009
NA	*F*	0.43	1.16	1.06
	P	0.73	0.35	0.40
Adrenaline	*F*	0.44	1.11	0.74
	P	0.73	0.37	0.57
Prolactin	*F*	39.67	51.03	8.00
	P	<0.0001	<0.0001	0.0006
hGH	*F*	2.68	2.54	2.29
	P	0.13	0.046	0.11
Cortisol	*F*	0.81	10.58	1.02
	P	0.51	<0.0001	0.42
Systolic	*F*	0.67	1.99	0.90
BP	*P*	0.58	0.10	0.48
Diastolic	*F*	1.02	3.27	0.55
BP	*P*	0.40	0.060	0.72
Heart rate	*F*	1.38	1.35	1.35
	P	0.28	0.27	0.27

Table 3 Analysis of variance (ANOVA) (for the comparison of the later time points (6 h, 8 h and 24 h) with two within-factors: effect of dose and time. Square-root transformed data was used for DHPG and prolactin, and log-transformed data for hGH and DOPAC. BP = blood pressure

Variable		*ANOVA*		
		Factor 1 = dose	*Factor 2 = time*	*Interaction*
DHPG	*F*	22.55	28.75	10.35
	P	<0.0001	<0.0001	0.0033
DOPAC	*F*	18.12	10.45	1.02
	P	<0.0001	0.0017	0.42
NA	*F*	0.72	3.74	1.16
	P	0.50	0.050	0.35
Adrenaline	*F*	0.61	1.49	0.63
	P	0.61	0.26	0.54
Prolactin	*F*	1.77	23.80	0.54
	P	0.18	<0.0001	0.77
hGH	*F*	0.32	1.70	1.68
	P	0.81	0.22	0.15
Cortisol	*F*	1.50	22.06	0.20
	P	0.24	<0.0001	0.97
Systolic	*F*	0.84	1.07	1.04
BP	*P*	0.48	0.38	0.40
Diastolic	*F*	0.25	3.72	1.21
BP	*P*	0.86	0.027	0.33
Heart rate	*F*	1.08	2.68	1.20
	P	0.38	0.073	0.33

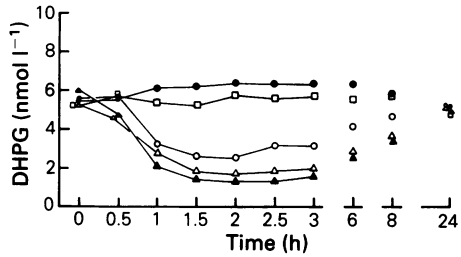

Figure 1 Mean DHPG concentrations in plasma after placebo, moclobemide and deprenyl. Symbols: ●, placebo; ○, 100 mg moclobemide; △, 200 mg moclobemide; ▲, 300 mg moclobemide; □, 10 mg deprenyl. Standard deviations have been omitted for clarity (see text for typical examples).

ANCOVA), 67% after 200 mg (from 5.21 ± 0.98 nmol l^{-1} to 1.73 ± 0.94 nmol l^{-1} at 120 min; $P < 0.001$; ANCOVA) and 79% after 300 mg moclobemide (from 5.97 ± 1.38 nmol l^{-1} to 1.27 ± 0.69 nmol l^{-1} at 120 min; $P < 0.001$; ANCOVA), respectively.

The decrease in the concentration of DHPG in plasma after moclobemide was quite rapid, being near-maximal at 1 h and maximal at 2 h. The DHPG levels remained low for several hours; 6 h after moclobemide administration, plasma DHPG levels were still reduced by 26% after 100 mg moclobemide, by 48% after 200 mg moclobemide and by 58% after 300 mg moclobemide, compared with a 15% increase in the placebo session. Eight hours after moclobemide, plasma DHPG levels were reduced by 17% after 100 mg ($F = 16.73$, $P = 0.0046$; ANOVA), by 34% after 200 mg ($F = 105.3$, $P < 0.0001$; ANOVA) and by 40% after 300 mg ($F = 46.30$, $P = 0.0003$; ANOVA), compared with a 7% increase after placebo. At 24 h, plasma DHPG levels had returned to baseline after all treatments.

DOPAC in plasma was dose-dependently reduced after moclobemide (see Tables 3 and 4 for statistical assessments and Figure 2). A maximal average reduction of 75% from baseline was seen 6 h after the 300 mg dose (from a mean basal level of 20.2 ± 6.0 nmol l^{-1} to 4.8 ± 2.3 nmol l^{-1}), whereas plasma DOPAC levels remained relatively stable after placebo administration (see Figure 2). Plasma DOPAC was still significantly depressed 24 h after dosing ($P = 0.003$, ANOVA; 42% average reduction from baseline after 300 mg).

Moclobemide did not affect the concentrations of NA or adrenaline in plasma (Figure 3 and Tables 3 and 4).

Table 4 Urinary excretion of the deaminated monoamine metabolites 4-hydroxy-3-methoxymandelic acid (HMMA), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) after placebo, 100 mg, 200 mg and 300 mg of moclobemide, and one-way ANOVA. Means ± s.d.

4-hydroxy-3-methoxymandelic acid (HMMA) (mmol mol^{-1} creatinine)

Urine fraction	*Control*	*Moclobemide 100 mg*	*200 mg*	*300 mg*	*ANOVA* F	P
0–3 h	1.53 ± 0.66	1.30 ± 0.59	1.17 ± 0.37	1.18 ± 0.40	3.36	0.038
3–8 h	1.66 ± 0.53	1.05 ± 0.33	0.95 ± 0.27	0.93 ± 0.29	21.92	<0.0001
8–24 h	1.84 ± 0.84	1.81 ± 0.91	1.59 ± 0.54	1.48 ± 0.41	2.16	0.18

Homovanillic acid (HVA) (mmol mol^{-1} creatinine)

Urine fraction	*Control*	*Moclobemide 100 mg*	*200 mg*	*300 mg*	*ANOVA* F	P
0–3 h	2.17 ± 0.53	2.48 ± 1.01	2.33 ± 1.13	1.97 ± 0.60	1.02	0.40
3–8 h	1.76 ± 0.33	1.45 ± 0.45	1.44 ± 0.73	1.05 ± 0.42	5.73	0.005
8–24 h	1.90 ± 0.35	1.72 ± 0.43	1.60 ± 0.42	1.41 ± 0.45	4.98	0.009

5-hydroxyindoleacetic acid (5-HIAA) (mmol mol^{-1} creatinine)

Urine fraction	*Control*	*Moclobemide 100 mg*	*200 mg*	*300 mg*	*ANOVA* F	P
0–3 h	1.66 ± 0.36	1.47 ± 0.14	1.43 ± 0.32	1.27 ± 0.28	3.01	0.053
3–8 h	1.27 ± 0.33	1.12 ± 0.14	1.04 ± 0.36	1.04 ± 0.11	1.77	0.22
8–24 h	1.69 ± 0.60	1.62 ± 0.43	1.76 ± 0.48	1.64 ± 0.64	0.29	0.83

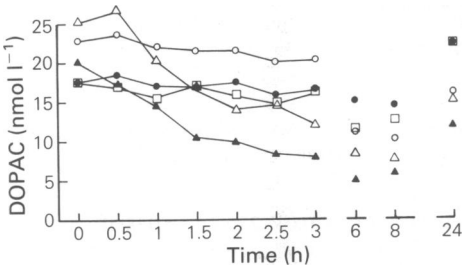

Figure 2 Mean DOPAC concentrations in plasma after placebo, moclobemide and deprenyl. Symbols as in Figure 1.

Urinary excretion of monoamine metabolites

The urinary excretion of the deaminated catecholamine metabolites, HMMA and HVA, was significantly and dose-dependently reduced by moclobemide (Table 4). The average excretion of HMMA was reduced by 23% in the 0–3 h urine fraction after 300 mg moclobemide and by 44% in the 3-8 h urine fraction. The excretion of HVA was reduced by 40% in the 3-8 h urine fraction after 300 mg moclobemide. The excretion of 5-HIAA was not statistically significantly affected by moclobemide (Table 4). Urine volumes and creatinine excretion were not affected by the drug (NS; ANOVA).

Moclobemide increased the urinary excretion of the methylated, non-deaminated catecholamine metabolites. The excretion of normetanephrine in the 3–8 h urine fraction was increased by 114%, that of metanephrine by 50% and that of 3-methoxytyramine by 180% after 300 mg moclobemide (Table 5).

MAO-B activity in blood platelets

One subject had to be excluded from the statistical analysis of platelet MAO-B activity due to low platelet recovery in several samples in all four sessions. MAO-B activity in blood platelets was inhibited maximally 27% after moclobemide administration (Figure 4 and Table 6): the inhibition was not statistically significant at 1 h, clearly significant at 2 h ($F = 4.15$, $P = 0.02$; ANOVA), and not significant thereafter. Statistically significant effects were found after 200 mg and 300 mg moclobemide, whereas 100 mg moclobemide did not differ significantly from placebo. After 100 mg, mean MAO-B activity in platelets was maximally decreased by 11% (from 22.4 ± 9.4 nmol h^{-1} mg^{-1} protein to 19.9 ± 7.7 nmol h^{-1} mg^{-1} protein; NS; ANCOVA), by 27% after 200 mg moclobemide (from 21.4 ± 9.3 nmol h^{-1} mg^{-1} protein to 15.5 ± 5.6 nmol h^{-1} mg^{-1} protein; $P < 0.05$; ANCOVA), and by 23% after 300 mg moclobemide (from 19.9 ± 7.9 nmol h^{-1} mg^{-1} protein to 15.3 nmol h^{-1} mg^{-1} protein; $P < 0.01$; ANCOVA).

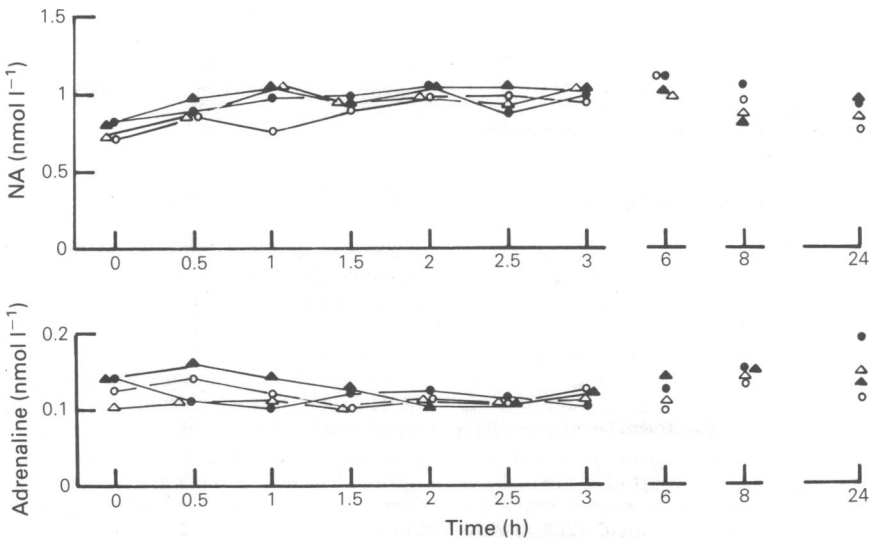

Figure 3 Mean NA and adrenaline concentrations in plasma after placebo and moclobemide. Symbols as in Figure 1.

Table 5 Urinary excretion of the methylated, non-deaminated catecholamine metabolites normetanephrine, metanephrine and 3-methoxytyramine after placebo, 100 mg, 200 mg and 300 mg of moclobemide, and one-way ANOVA. Means ± s.d.

Normetanephrine (mmol mol^{-1} creatinine)

Urine fraction	*Control*	*Moclobemide 100 mg*	*200 mg*	*300 mg*	*ANOVA F*	*P*
0–3 h	0.07 ± 0.03	0.13 ± 0.16	0.08 ± 0.02	0.09 ± 0.02	2.05	0.17
3–8 h	0.07 ± 0.01	0.10 ± 0.02	0.11 ± 0.03	0.15 ± 0.03	23.84	<0.0001
8–24 h	0.11 ± 0.11	0.15 ± 0.18	0.13 ± 0.07	0.18 ± 0.09	2.44	0.14

Metanephrine (mmol mol^{-1} creatinine)

Urine fraction	*Control*	*Moclobemide 100 mg*	*200 mg*	*300 mg*	*ANOVA F*	*P*
0–3 h	0.05 ± 0.03	0.08 ± 0.10	0.05 ± 0.02	0.05 ± 0.02	1.13	0.36
3–8 h	0.04 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.06 ± 0.03	18.26	<0.0001
8–24 h	0.03 ± 0.01	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	1.40	0.28

3-methoxytyramine (mmol mol^{-1} creatinine)

Urine fraction	*Control*	*Moclobemide 100 mg*	*200 mg*	*300 mg*	*ANOVA F*	*P*
0–3 h	0.05 ± 0.03	0.11 ± 0.14	0.08 ± 0.02	0.07 ± 0.02	2.62	0.077
3–8 h	0.05 ± 0.01	0.10 ± 0.02	0.11 ± 0.03	0.14 ± 0.03	51.22	<0.0001
8–24 h	0.07 ± 0.03	0.07 ± 0.03	0.08 ± 0.03	0.09 ± 0.01	1.27	0.31

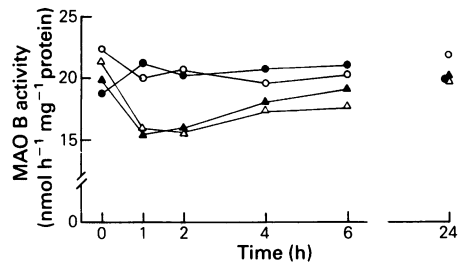

Figure 4 Mean MAO-B activity in blood platelets after placebo and moclobemide. Symbols as in Figure 1.

Table 6 Analysis of covariance (ANCOVA, all time points) of the effect of moclobemide on MAO-B activity in platelets. Square-root transformed data was used (n = 7)

		ANCOVA Factor 1 = dose	*Factor 2 = time*	*Interaction*
MAO-B activity	*F*	7.89	7.45	2.31
	P	0.020	0.0005	0.08

Anterior pituitary hormones and cortisol in plasma

Moclobemide increased prolactin levels in plasma (Figure 5 and Tables 2 and 3). The stimulatory influence on prolactin secretion was dose-dependent, and the duration of the effect was short (less than 3 h). Plasma prolactin concentrations were increased from a mean basal level of 6.3 ± 2.2 ng ml^{-1} to a maximum of 6.4 ± 3.56 ng ml^{-1} after 100 mg moclobemide (NS; ANCOVA), from 6.4 ± 2.1 ng ml^{-1} to 11.4 ± 4.6 ng ml^{-1} after 200 mg ($P < 0.001$; ANCOVA), and from 6.4 ± 2.7 ng ml^{-1} to 23.5 ± 7.1 ng ml^{-1} after 300 mg ($P < 0.001$; ANCOVA). After placebo treatment, plasma prolactin concentrations showed a decreasing tendency (from a mean basal value of 6.1 ± 2.9 ng ml^{-1} to 3.5 ± 2.1 ng ml^{-1} at 2.5 h).

Moclobemide did not significantly stimulate hGH secretion, although the average plasma hGH level showed a slight increase after the largest moclobemide dose: plasma hGH levels increased from a mean basal level of 0.8 ± 1.6 ng ml^{-1} to 4.7 ± 8.0 ng ml^{-1} at 90 min after 300 mg moclobemide, compared with an increase from 0.7 ± 1.8 ng ml^{-1} to 1.0 ± 0.8 ng ml^{-1} at 150 min after placebo (NS; ANCOVA) (Figure 6 and Tables 2 and 3).

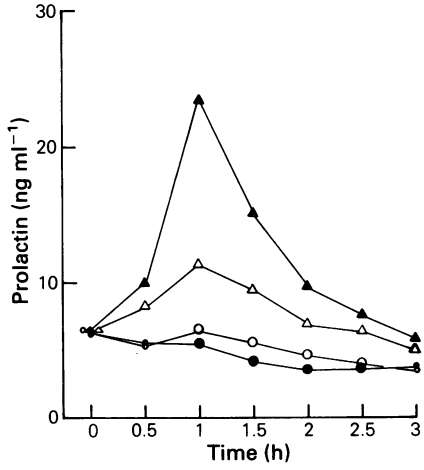

Figure 5 Mean prolactin concentrations in plasma after placebo and moclobemide. Symbols as in Figure 1.

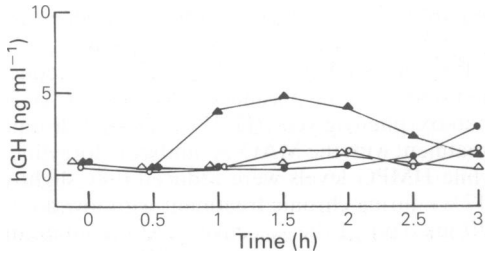

Figure 6 Mean hGH concentrations in plasma after placebo and moclobemide. Symbols as in Figure 1.

Plasma cortisol showed a diurnal variation with a nadir at 3–6 h after dosing, between 11.00 h and 14.00 h. This pattern was uninfluenced by moclobemide (see Tables 3 and 4 for statistical assessments and Figure 7).

Deprenyl experiment

Deprenyl inhibited MAO-B activity in blood platelets maximally 94%: the mean MAO-B activity decreased from 23.8 ± 6.3 to 1.5 ± 0.7 nmol h^{-1} mg^{-1} protein at 2 h after deprenyl ($P < 0.0001$, ANOVA), and remained inhibited by 90% ($P < 0.0001$; ANOVA) 24 h after deprenyl.

Plasma DHPG levels remained stable after a single oral dose of deprenyl (see Figure 1), whereas they tended to increase slightly after placebo (from 5.44 ± 1.04 nmol l^{-1} to 6.31 ± 1.67 nmol l^{-1} at 120 min). When deprenyl was compared with placebo, there was no drug effect ($F = 1.13$, $P = 0.33$; ANCOVA), but the drug × time interaction was statistically significant ($F = 2.73$, $P = 0.035$; ANCOVA).

There were only time-related changes in plasma DOPAC levels after deprenyl and placebo. Plasma DOPAC levels decreased from a mean basal level of 17.5 ± 5.4 nmol l^{-1} to 11.5 ± 1.8 nmol l^{-1} at 6 h after deprenyl, and from 17.5 ± 7.5 nmol l^{-1} to 14.4 ± 4.8 nmol l^{-1} at 8 h after placebo ($F = 0.86$, $P = 0.39$ for drug effect; $F = 25.42$, $P < 0.0001$ for time effect; $F =$

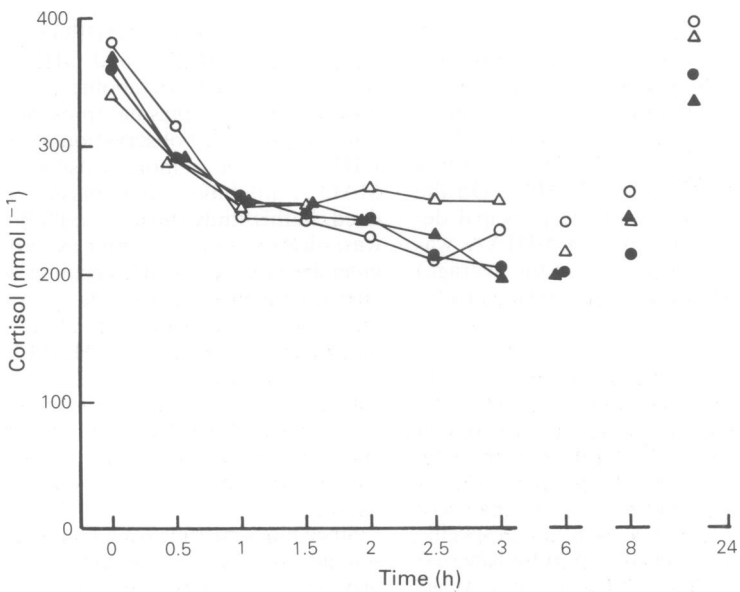

Figure 7 Mean cortisol concentrations in plasma after placebo and moclobemide. Symbols as in Figure 1.

0.91, $P = 0.42$ for drug × time interaction; ANOVA).

Plasma prolactin concentrations showed similar time-related changes after deprenyl and placebo: the mean plasma prolactin level decreased from 5.5 ± 3.2 to 3.0 ± 0.8 ng ml^{-1} at 180 min after deprenyl, and from 6.1 ± 2.8 to 3.5 ± 2.1 ng ml^{-1} at 150 min after placebo ($F = 0.70$, $P = 0.43$ for drug effect; $F = 12.96$, $P < 0.0001$ for time effect; $F = 0.85$, $P = 0.53$ for drug × time interaction; ANCOVA).

Discussion

Single oral doses of up to 300 mg moclobemide were well tolerated by the eight healthy male subjects participating in this study. Three of the volunteers reported slight and transient sympathomimetic-like subjective drug-related symptoms. Blood pressure and heart rate were unaffected by the drug in our supine subjects; this is in line with previous reports with 150 mg single oral doses (Gasic *et al.*, 1983), and 2 weeks' treatment of healthy subjects with 100 mg three times daily (Korn *et al.*, 1988). The plasma concentrations of NA and adrenaline were also unaffected by the drug. It is thus concluded that moclobemide, in the dose range used in this study, was devoid of significant effects on sympatho-adrenal function in resting volunteers.

The metabolic profile of the catecholamines was markedly altered by moclobemide. While the urinary excretion of HMMA (deaminated metabolite of NA and adrenaline) and HVA (deaminated metabolite of dopamine) was reduced by approximately 40% in the 3–8 h urine fraction, the excretion of the non-deaminated catecholamine metabolites normetanephrine (from NA), metanephrine (from adrenaline) and 3-methoxytyramine (from dopamine) was simultaneously increased by 50–180%. On the other hand, the excretion of the principal deaminated metabolite of serotonin, 5-HIAA, was reduced only slightly (by 23%, on the average) in the first 3 h urine fraction after 300 mg moclobemide, only approaching statistical significance ($P = 0.053$, ANOVA). These results provide evidence for substantial inhibition of MAO activity *in vivo* in our subjects, but do not permit any firm conclusions regarding the subtype of MAO inhibited, nor of the organs in the body where MAO activity was inhibited. Previous clinical studies have demonstrated this change in the metabolic profile of NA after type-A specific (clorgyline) and non-specific MAO-inhibitors, but not after selective inhibition of MAO-B (Robinson & Kurtz, 1987). The smaller proportional effect on the deamination of 5-HT compared with NA has also been reported previously after the selective type-A inhibitor, clorgyline (Robinson & Kurtz, 1987).

Although moclobemide has been considered a selective and reversible inhibitor of MAO-A, there is evidence that active metabolites with inhibitory activity against MAO-B are formed from moclobemide in man (Da Prada *et al.*, 1986; Fuchs *et al.*, 1986). The present study is in accordance with the results of Da Prada *et al.* (1986) confirming the inhibitory effect of moclobemide on MAO-B activity in platelets. The average inhibition of MAO-B activity in platelets was 20–30% in our subjects, lasting only a few hours after 200 mg and 300 mg moclobemide. Due to the reversible nature of MAO-B inhibition by the metabolites of moclobemide, the present assay method of MAO-B activity may actually underestimate the degree of enzyme inhibition *in vivo*. Further studies are required to clarify the role of MAO-B inhibition in the biochemical effects of moclobemide.

Previous studies have shown marked reductions (86%) in the plasma levels of 4-hydroxy-3-methoxyphenylglycol (HMPG) during chronic treatment with the MAO-A inhibitor clorgyline, while HMPG levels were reduced only slightly (17%) during chronic treatment with low doses (10 mg/day) of the selective MAO-B inhibitor deprenyl, suggesting plasma HMPG as a valid indicator of MAO-A inhibition in man (Pickar *et al.*, 1981). DHPG is an immediate precursor of HMPG, requiring only methylation by catechol-*O*-methyltransferase (COMT), which is abundant in all tissues. Plasma DHPG levels are lower than those of HMPG, and DHPG appears to constitute a small metabolite pool with a fast turnover rate in plasma (present results and other unpublished observations). Thus, plasma DHPG may be a more sensitive indicator of MAO-A inhibition than plasma HMPG. Indeed, in this study the plasma DHPG reduction was already highly significant after 100 mg moclobemide, was nearly maximal already one h after drug administration, and was quantitatively much more impressing (79% after 300 mg) than the reduction in urinary HMMA excretion. Plasma DHPG levels were still significantly depressed 8 h after the 300 mg dose, but had returned to baseline at 24 h. The DHPG change may be useful also in the assessment of the duration of MAO-A inhibition in human subjects.

Since moclobemide (or a metabolite of it) had a significant inhibitory effect on the activity of MAO-B in blood platelets, it was important to clarify a possible contribution of MAO-B in-

hibition in the action of moclobemide on plasma DHPG levels. Therefore, we administered deprenyl, a selective and irreversible inhibitor of MAO-B, to our volunteers. The acute administration of 10 mg of deprenyl did not change plasma levels of DHPG, which suggests that plasma DHPG may be a specific indicator of MAO-A inhibition in man at least in acute studies. This is in line with previous studies in rats, using clorgyline, harmaline, a reversible MAO-A inhibitor, and deprenyl; DHPG in rat plasma was decreased by more than 90% after clorgyline and harmaline and unaffected by the relatively large acute dose (3 mg kg^{-1}) of deprenyl (Brown & Monks, 1983, 1984). The same authors also reported on a preliminary human experiment, where 40 mg of cimoxatone, a reversible MAO-A inhibitor, reduced plasma DHPG levels by 75%, whereas plasma DHPG was unaltered after 5 days' treatment with deprenyl 10 mg daily (Brown & Monks, 1984). On the other hand, chronic treatment of elderly humans with larger doses of deprenyl (30–60 mg/day) has been reported to reduce plasma DHPG levels by 70%, a decrement comparable with that seen after treatment with tranylcypromine (75%), a non-selective MAO inhibitor (Eisenhofer *et al.*, 1986). This may reflect loss of MAO-B selectivity for higher doses of deprenyl upon repeated administration (Murphy *et al.*, 1987; Sunderland *et al.*, 1987), or alternatively, a more complex relationship between the pharmacological effects of deprenyl and plasma DHPG levels (Liebowitz *et al.*, 1985). Intravenous NA infusions, blockade of neuronal NA reuptake, and neuronal NA depletion with guanethidine have previously been used to demonstrate that DHPG in human plasma is closely related to intraneuronal NA stores and metabolism (Izzo *et al.*, 1985).

The concentration of DOPAC in plasma was also significantly reduced by moclobemide, but this change appeared more slowly and variably than the DHPG decrease. On the other hand, deprenyl did not change DOPAC levels in plasma, which suggests that also plasma DOPAC levels may reflect MAO-A inhibition and intraneuronal catecholamine metabolism. Plasma DOPAC levels were still reduced 24 h after the administration of moclobemide, which contrasts with the DHPG results.

Moclobemide stimulated prolactin secretion in a dose-dependent fashion. In contrast, the secretion of hGH and cortisol was not significantly affected. Since hGH and cortisol secretion, as well as plasma concentrations of catecholamines remained unaltered, it is unlikely that the effect of moclobemide on prolactin secretion would be a nonspecific stress effect. The present study does not provide direct pharmacological evidence of the specific mechanisms mediating the prolactin-releasing action of moclobemide. The secretion of prolactin from the anterior pituitary is under tonic inhibitory control exerted by hypothalamic dopaminergic neurons (Tuomisto & Männistö, 1985). Although moclobemide is a benzamide derivative, it has negligible affinity to DA D_1- and D_2-receptors (own unpublished results). Acute inhibition of MAO-A by moclobemide could be expected to intensify noradrenergic and serotonergic neurotransmission at the synaptic level (Murphy *et al.*, 1984). Noradrenergic mechanisms may facilitate prolactin release, but the evidence in man is only fragmentary and partially contradictory (Tuomisto & Männistö, 1985). Instead, there is ample evidence that serotonergic neurons play an important stimulatory role in the regulation of prolactin release (Tuomisto & Männistö, 1985). Thus, 5-HT precursors, L-tryptophan and 5-hydroxytryptophan have stimulatory effects on prolactin secretion (Charney *et al.*, 1982; Winokur *et al.*, 1986; Lancranjan *et al.*, 1977). Tricyclic antidepressants, e.g. clomipramine, amitriptyline, imipramine and desipramine, potentiate L-tryptophan-stimulated prolactin release (Charney *et al.*, 1984; Anderson & Cowen, 1986) and, given alone in sufficiently large acute doses, also induce secretion of prolactin (Calil *et al.*, 1984; Laakmann *et al.*, 1985, 1986; Nutt *et al.*, 1987). Furthermore, fenfluramine, which releases 5-HT and inhibits its neuronal reuptake, stimulates prolactin secretion in man (Quattrone *et al.*, 1983; Lewis & Sherman, 1985), as also does the putative 5-HT receptor agonist *m*-chlorophenylpiperazine (Mueller *et al.*, 1986). It would be tempting to speculate that the stimulatory effect of moclobemide on prolactin secretion is mediated via serotonergic neurons, but further studies are needed to test this hypothesis. Other MAO inhibitors (clorgyline and pargyline) have also been shown to increase plasma prolactin levels during chronic treatment of depressed patients (Slater *et al.*, 1977). It is concluded that moclobemide has a short-acting, stimulatory effect on prolactin secretion in man. Since moclobemide could be used as a challenge test in neuroendocrine studies, it is important to clarify which neurotransmitter mechanisms are involved in its action.

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