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A COMPARISON OF THE PHARMACODYNAMIC PROFILES OF NOMIFENSINE AND AMITRIPTYLINE IN NORMAL SUBJECTS

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1 Six healthy male volunteers participated in a double-blind placebo crossover comparison of the pharmacodynamic profiles of single oral doses of 75 mg nomifensine and 50 mg amitriptyline.

2 Nomifensine treatment did not influence salivary flow and did not significantly affect psychomotor performance (critical flicker fusion, pursuit rotor and reaction time): in addition nomifensine had no significant effect on subjective measurements of sedation and concentration.

3 By contrast, amitriptyline treatment significantly reduced salivary flow and was associated with significant sedation and reduced concentration: significant changes in psychomotor performance were also noted.

4 Plasma concentrations of amitriptyline and nomifensine were measured at 2 h. The respective median concentration values were 55.0 ng/ml and 52.0 ng/ml.

5 *Ex vivo* platelet amine uptake of dopamine (DA) and 5-hydroxytryptamine (5HT) was measured 2 h after each treatment. Both nomifensine and amitriptyline treatment significantly inhibited DA uptake to a similar extent. Amitriptyline treatment additionally inhibited 5-HT uptake.

Introduction

Nomifensine (8-amino-1,2,3,4-tetrahydro-2-methyl-phenyl-isoquinoline) is a tetraisoquinoline with psychopharmacological activity. Clinical trials have shown antidepressant efficacy comparable to amitriptyline (Grof, Saxena, Daigle & Mahutte, 1977). Pharmacologically, it shares in many respects a profile similar to tricyclic antidepressants: however, nomifensine also influences dopaminergic mechanisms, acting as a dopamine (DA) agonist (Costall, Kelly & Naylor, 1975, Hoffman, 1977). It inhibits rat synaptosomal uptake of noradrenaline, DA and 5-hydroxytryptamine (5-HT) *in vitro* (Schacht, Leven & Bäcker, 1977), and has also been shown to inhibit the *in vitro* uptake of DA and 5-HT into human platelets (Ehsanullah & Turner, 1977). The incidence of anticholinergic side-effects was reportedly less in some studies in depressed patients (McClelland, Kerr & Littler, 1977) while in other studies such a difference was not significant (Forrest, Hewett & Nicholson, 1977). Since anticholinergic effects such as dry mouth, palpitation, dizziness and drowsiness are also symptoms of depression, assessment of anticholinergic drug effects in patients can be difficult. It may therefore be of value to establish whether nomifensine in standard therapeutic doses produces anticholinergic side-effects in normal subjects. This present study was designed to examine the above question and to compare the general pharmacodynamic profile of nomifensine and amitriptyline in healthy volunteers. An opportunity was also taken to investigate platelet amine uptake following drug treatment.

Methods

Six healthy male volunteers gave their informed consent. They were instructed to refrain from taking other drug treatment throughout the study and to abstain from alcohol, tea, coffee, chocolate and nicotine up to 6 h on each treatment day. Each subject received the three treatments below in matching capsules according to a double-blind study of Latin square design with an interval of at least 1

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week between doses: (1) 75 mg nomifensine hydrogen maleate (3 × 25 mg capsules), (2) 50 mg amitriptyline hydrogen chloride (2 × 25 mg capsules + 1 placebo), (3) 3 placebo capsules. Treatment was given with 100 ml water following an overnight fast. A series of tests as described below were performed before treatment (0 h) and at 2, 4 and 6 h after treatment.

Somatic effects

Salivary flow, stimulated by sucking an acid drop, was measured according to the procedure described by Kingsley & Turner (1974).

Pupil diameter was measured directly and also by the photographic method of Sneddon & Turner (1967) under constant lighting conditions.

Supine resting pulse rate and blood pressure were measured following a 5 min rest. Radial pulse was recorded over a 30 s interval. Blood pressure was measured with the London School of Hygiene sphygmomanometer.

Subjective tests

On a series of 100 mm linear analogue rating scales, subjects were asked to mark the point on the line between the two extremes which would give an indication of sedation, concentration, mood, appetite and nausea. The extremes of the scales for sedation were 'I cannot keep awake' and 'As alert as I have ever been'; for concentration, the extremes were 'I cannot concentrate at all' and 'My mind has never been clearer'; the extremes for appetite were 'I couldn't eat a thing' and 'I could eat a horse'; the extremes for nausea were 'I feel very sick (nauseated)' and 'I don't feel sick at all'. At 0 and 24 h the subjects also indicated the quality of the previous night's sleep; the extremes of the scale were 'Last night was my worst night's sleep ever' and 'Last night was my best night's sleep ever'.

Objective tests

Critical flicker fusion (CFF) threshold was measured using the method of Turner (1968) as modified by Ogle & Turner (1974). Before the start of the study, subjects were acquainted with the methodology and the normal CFF for each subject was determined. A mean of four readings was taken on each occasion; (a) ascending threshold from 20 Hz after conditioning to 20 Hz; (b) descending threshold from 50 Hz after conditioning to 20 Hz; (c) ascending threshold from 20 Hz after conditioning to 50 Hz and (d) descending threshold from 50 Hz after conditioning to 50 Hz.

Multiple (complex) reaction time was measured by a standard procedure (Kulshrestha, Gupta, Turner & Wadsworth, 1978). A total of thirteen stimuli were given at randomized intervals. The first three readings were discarded and the subsequent ten consecutive readings were meaned.

The performance of subjects on a standard pursuit rotor (Forth Instruments) was measured as described by Ogle, Turner & Markomihelakis (1976). Before the start of the study, the performance of each subject was standardized. On each occasion each subject was tested with a speed setting which normally resulted in 50–70% of the time 'on target'. Twelve consecutive 10 s runs were made during each test period. The first six readings were discarded and the final six readings meaned.

Measurement of platelet amine uptake

Blood (30 ml) was taken by venepuncture from each subject at 0 h and 2 h and mixed with 4 ml of an acid citrate anticoagulant (Ehsanullah & Turner, 1977). Plastic syringes and tubes were used throughout the experiment to minimize platelet aggregation. Platelet rich plasma (PRP) was prepared by centrifugation at 150 *g* for 10–15 min. The plasma layer was removed and mixed gently to ensure even dispersal of the platelets. A small volume was taken for platelet counting using a thrombocounter Model C. The PRP was dispensed in 1 ml aliquots and allowed to equilibrate at 37°C for 10 min before the addition of 100 µl volumes of [^{3}H]-DA (specific activity 5 Ci/mM or [^{3}H]-5-HT (specific activity 10 Ci/mM). Labelled amines were supplied by Radiochemical Laboratories, Amersham. Amine concentrations between 10^{-8} M and 10^{-6} M were used. Incubation was continued at 37°C for 5 min in the case of [^{3}H]-5-HT and 10 min for [^{3}H]-DA and was terminated by transferring the incubation tubes to ice cold water. Platelets were spun down by centrifugation at 10,000 rev/min for 3.5 min and lysed by the addition of 1 ml 0.01 M KOH. The radioactivity released from the platelets which indicated the amount of tritiated amine taken up into the platelets was determined by liquid scintillation counting using a Packard Tricarb spectrometer. The amount of radioactive amine taken up by the platelet was corrected to d/min/10^8 platelets.

Measurement of plasma drug concentration

Blood samples were collected 2 h after treatment. The amount of unchanged nomifensine in plasma was measured by gas chromatography (Chamberlain & Hill, 1977). Conjugated nomifensine was also measured by the same method following acid hydrolysis of plasma. Plasma concentration of amitriptyline and its metabolite nortriptyline was determined by RIA (Robinson, Risby & Aherne, 1978). The results were expressed in ng-equivalents of amitriptyline.

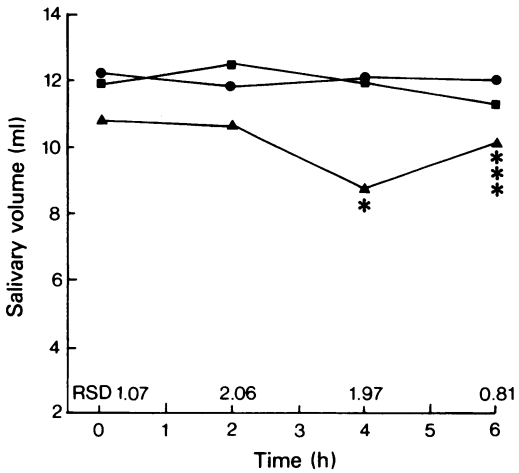

Figure 1 The mean salivary volume at 0, 2, 4 and 6 h after the administration of placebo (●), nomifensine (■) and amitriptyline (▲). *** $P < 0.01$, * $P < 0.05$. RSD residual standard deviation.

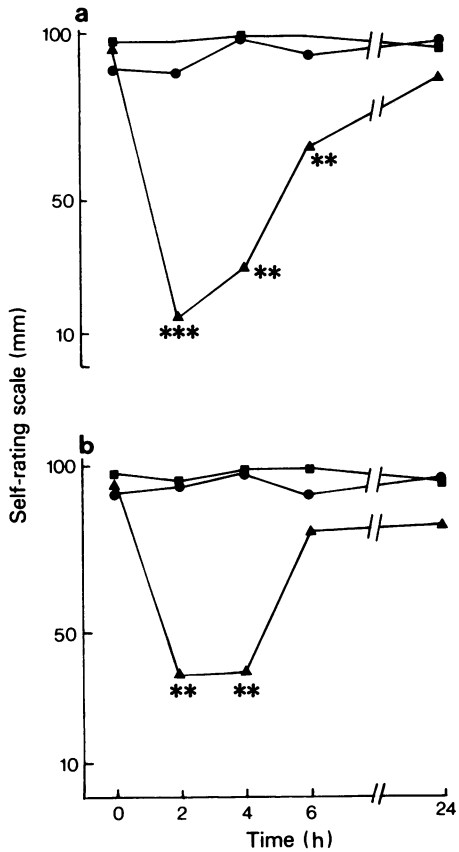

Figure 2 The median value in the self-rating scale for sedation (a) and concentration (b) at 0, 2, 4, 6 and 24 h after the administration of placebo (●), nomifensine (■) and amitriptyline (▲). *** $P < 0.01$, ** $P < 0.02$.

Statistics

Analysis of variance was used to compare the results of the somatic effects, objective psychomotor tests and platelet amine uptake. Examination of platelet amine uptake data revealed a skewed distribution, so in this instance the analysis of variance was carried out after logarithmic transformation. A wide inter-subject variation was noted in the visual analogue scores, so Friedman's two-way analysis of variance was used to detect within-subject differences between the three treatments at each time point.

Results

None of the treatments affected pupil size. However, in comparison with placebo, salivary volume was decreased following amitriptyline (Figure 1) and this difference was statistically significant at 4 h ($P < 0.05$) and 6 h ($P < 0.01$). Nomifensine and placebo did not influence salivary flow. Neither of the active treatments influenced pulse rate or blood pressure.

Among the visual analogue scales, those for sedation and concentration showed the most marked changes (Figure 2). There was a wide inter-subject variation: for clarity Figure 2 shows median values only. In comparison with placebo, nomifensine had no significant effect on subjective sedation or concentration, although two of the six subjects reported improved concentration. By contrast, amitriptyline produced a fair degree of sedation, significantly different from placebo at 2 h ($P < 0.01$), 4 h ($P < 0.02$) and 6 h ($P < 0.02$). Amitriptyline was also associated with a significant decrease in subjective concentrating ability in five out of six subjects at 2 h ($P < 0.02$) and 4 h ($P < 0.02$). Three of the six subjects also reported some degree of nausea 2 h after amitriptyline: no nausea was experienced following the other two treatments. Neither active treatment had any influence on mood or appetite, and there was no consistent effect on the quality of sleep during the night following treatment.

The results of more objective psychomotor tests were also consistent with these subjective findings. In the CFF test (Figure 3) amitriptyline treatment produced a significant decrease in CFF threshold at 2 h ($P < 0.05$), 4 h ($P < 0.02$) and 6 h ($P < 0.05$) in comparison with placebo. Nomifensine did not significantly affect CFF. Amitriptyline also significantly impaired pursuit rotor performance at

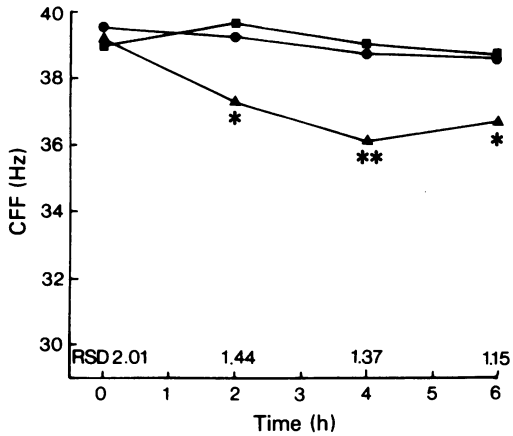

Figure 3 The mean CFF at 0, 2, 4 and 6 h after the administration of placebo (●), nomifensine (■) and amitriptyline (▲). ** $P < 0.02$, * $P < 0.05$. RSD residual standard deviation.

2 h ($P < 0.01$), 4 h ($P < 0.02$) and 6 h ($P < 0.05$), whereas following nomifensine, rotor performance improved, although not significantly (Figure 4). Reaction time data were less reproducible (Figure 4) and a steady baseline was not obtained following placebo treatment. Nevertheless, amitriptyline was found to prolong reaction time significantly at 2 h ($P < 0.05$). Nomifensine was not significantly different from placebo with respect to reaction time.

The platelet uptake of DA and 5-HT with each treatment was studied *ex vivo* at 0 h and 2 h. The results are shown in Figures 5 and 6. The asymmetric SD bars reflect the skewness of the distribution. Pretreatment (0 h) amine uptake did not change significantly over the three week study period, and the platelet count was similarly constant. Both nomifensine and amitriptyline treatment significantly ($P < 0.01$) inhibited the uptake of dopamine into platelets obtained 2 h after dosage: this effect was observed at the two larger dopamine concentrations (5×10^{-7} M and 1×10^{-6} M). Each active treatment had a similar biochemical effect on dopamine uptake. However, there appeared to be some qualitative differences between the two active treatments with respect to 5-HT platelet uptake. Amitriptyline was associated with a small but significant ($P < 0.05$) drop in 5-HT uptake between 0 and 2 h, again at the two higher 5-HT concentrations (2.5×10^{-7} M and 5×10^{-7} M), whereas placebo and nomifensine treatment gave no significant change.

Table 1 shows the plasma concentrations of amitriptyline and nomifensine, and provides evidence of drug absorption: at 2 h, the median unchanged nomifensine concentration was 55.0 ng/ml. The

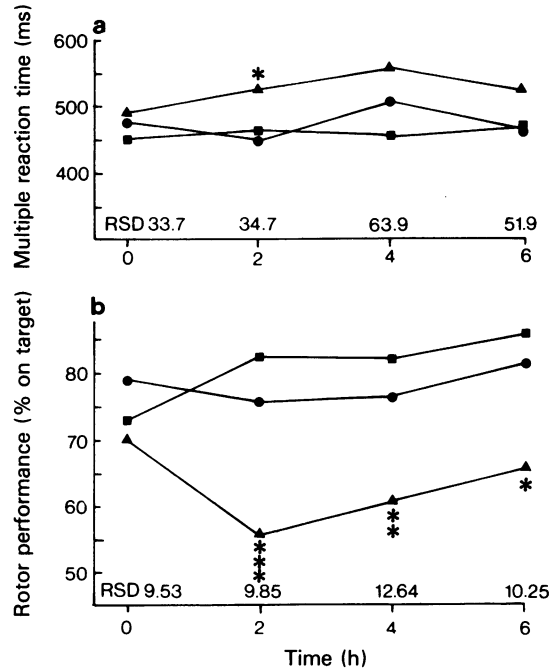

Figure 4 The mean multiple reaction time (a) and mean pursuit rotor performance (b) at 0, 2, 4 and 6 h after administration of placebo (●), nomifensine (■) and amitriptyline (▲). *** $P < 0.01$, ** $P < 0.02$, * $P < 0.05$. RSD residual standard deviation.

corresponding median amitriptyline concentration was 52.0 ng/ml. These 'amitriptyline' concentrations included measurement of nortryptyline converted to 'amitriptyline equivalents'. One subject (No. 6) had undetectable levels of unchanged nomifensine at 2 h, but the increased nomifensine levels following hydrolysis of this subject's plasma indicated the presence of circulating nomifensine conjugate.

Table 1 Plasma concentrations of nomifensine and amitriptyline at 2 h.

	Subject					
	1	2	3	4	5	6
Unconjugated nomifensine (ng/ml)	80	29	75	35	95	ND
Nomifensine following hydrolysis (μg/ml)	1.73	1.13	1.00	1.53	1.80	0.73
Amitriptyline (ng/ml)	29	81	53	46	64	51

ND = Not detected.

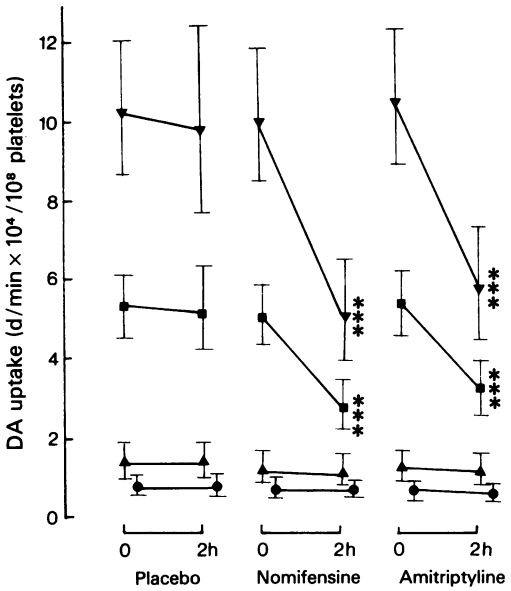

Figure 5 Platelet uptake of DA (mean ±s.d.: logarithmic transformation) following incubation with a range of DA concentrations before and 2 h after each treatment. ●, 5×10^{-8} M, ▲, 1×10^{-7} M, ■, 5×10^{-7} M, ▼, 1×10^{-6} M. *** $P < 0.01$.

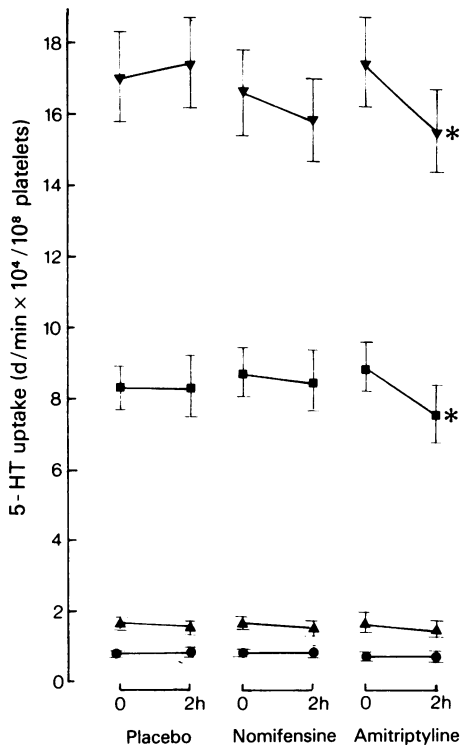

Figure 6 Platelet uptake of 5-HT (mean ± s.d.: logarithmic transformation) following incubation with a range of 5-HT concentrations before and 2 h after each treatment. ●, 2.5×10^{-8} M, ▲, 5×10^{-8} M, ■, 2.5×10^{-7} M, ▼, 5×10^{-7} M, * $P < 0.05$.

Discussion

The tricyclic group of drugs remain the most commonly used antidepressants. Treatment is often associated with varying degrees of anticholinergic side-effects and sedation (Spencer, 1977). This present study employed amitriptyline as a positive control in this respect. Lauber, Hartmann & Herman (1968) have previously commented that tricyclic antidepressants do not invariably alter pupil size: in keeping with this, of the two measures of anticholinergic effect, salivary flow appeared here to be the more sensitive: pupil size showed no drug-induced changes, but salivary flow was significantly inhibited by amitriptyline. Nomifensine treatment did not influence salivary flow: this suggests that the latter has less peripheral anticholinergic activity than amitriptyline following single doses in normal subjects.

The sedative effect of amitriptyline was confirmed in this study, using both subjective visual analogue scales and objective measurements such as CFF, pursuit rotor and reaction time. Nomifensine had no sedative effect, did not alter CFF, and did not impair performance: in fact, there was a trend for performance and concentration to be improved by nomifensine, but the differences were not statistically significant. Hindmarch & Parrott (1977) have reported a significant increase in CFF following long-term nomifensine treatment, but this stimulant effect was not noted in the present study. In view of the criticisms levelled at the accuracy of visual analogue scales (Maxwell, 1978; Nicholson, 1978) the statistical tests applied to these present data were more influenced by the direction of any change than by its magnitude. In addition, it was useful to have more objective psychomotor confirmation of positive analogue scale effects. Of the psychomotor tests employed, reaction time was only significantly altered at 2 h, whereas pursuit rotor performance and CFF were affected up to 6 h. CFF has been shown previously to be particularly sensitive to central depressant activity (Turner, 1968). Blackwell, Lipkin, Meyer, Kuzma & Boulter (1972) have suggested that the sedative effect of tricyclic antidepressants may be mediated by a central anticholinergic action. This would support the hypothesis that nomifensine possesses little central anticholinergic activity.

The human platelet has been proposed as a model of amine uptake which reflects the central biochemical effect of antidepressant drugs (Sneddon,

1973). Tricyclic antidepressants such as clomipramine and imipramine have been shown to inhibit the *in vitro* uptake of 5-HT and DA into human platelets (Turner & Ehsanullah, 1977), and nomifensine has also been shown to inhibit platelet amine uptake *in vitro* (Ehsanullah & Turner, 1977): however, there appears to be little information on amitriptyline in this respect. Ehsanullah & Turner (1977) showed significant inhibition of dopamine uptake at 10^{-6} M nomifensine, and significant inhibition of 5-HT uptake at 10^{-4} M nomifensine whereas in this present study, significant inhibition of dopamine uptake was found at plasma nomifensine concentrations between 0.3×10^{-7} M and 2.7×10^{-7} M (mean 1.4×10^{-7} M), indicating that *ex vivo* nomifensine in plasma had a greater potency than predicted by *in vitro* experiments. There are two possible explanations for this. The first is that circulating nomifensine conjugate may have hydrolysed to give unchanged nomifensine during the preparation and incubation of platelet-rich plasma. The second possibility is that there may be a nomifensine metabolite in plasma exerting a biochemical action on platelet dopamine uptake.

In keeping with *in vitro* nomifensine data, there was no significant *ex vivo* effect of nomifensine on platelet uptake of 5-HT. By contrast, amitriptyline did show a small but significant *ex vivo* inhibition of 5-HT uptake at plasma concentrations ranging from 0.9×10^{-7} M to 2.6×10^{-7} M, with in addition an inhibitory effect on dopamine platelet uptake comparable with nomifensine. These biochemical differences in platelet amine uptake between amitriptyline and nomifensine may have some bearing on their central biochemical action.

In conclusion, this study has shown significant pharmacological, psychomotor and biochemical differences between nomifensine and amitriptyline following moderately large single doses of each compound. It was useful to obtain additional plasma level data for each treatment, first to confirm drug absorption, and second to compare previous *in vitro* nomifensine data with the present *ex vivo* results. Nomifensine treatment was not associated with detectable anticholinergic side-effects and appeared to be the better tolerated treatment under these study conditions.

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