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ORIGINAL INVESTIGATION

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Comparison of the antidepressants reboxetine, fluvoxamine and amitriptyline upon spontaneous pupillary fluctuations in healthy human volunteers

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Abstract *Rationale:* Spontaneous fluctuations in the size of the pupil in darkness are a recognised index of "sleepiness". *Objective:* To evaluate the effects of single oral doses of three antidepressants: reboxetine (4 mg), a selective noradrenaline reuptake inhibitor, fluvoxamine (100 mg), a selective serotonin reuptake inhibitor, and amitriptyline (100 mg), a tricyclic antidepressant of known sedative property, upon spontaneous pupillary fluctuations in healthy male volunteers (*n*=16). *Methods*: Using the recently developed pupillographic sleepiness test (PST), resting pupil diameter was recorded and two measures of pupillary fluctuations were obtained: total power obtained from a fast Fourier transform and spectral analysis, and the pupillary unrest index (PUI), a cumulative measure of changes in pupil size. Subjects also rated themselves on a battery of visual analogue scales for "alertness", "anxiety" and "contentedness". *Results*: Resting pupil diameter was enhanced by reboxetine, but remained unaffected by the other two antidepressants. Amitriptyline, consistent with its sedative property, increased the total power of pupillary fluctuations and showed a tendency to increase PUI. These pupillary effects of amitriptyline were paralleled by reduced scores on the "alertness", "contentedness" and "anxiety" self ratings. Neither fluvoxamine nor reboxetine affected pupillary fatigue waves or subjective ratings of "alertness". Reboxetine caused a small reduction in subjectively rated "anxiety". *Conclusions:* The mydriatic effect of reboxetine may be due to noradrenaline reuptake blockade in the iris and/or in the central nervous system. The enhancement of pupillary fatigue waves by the sedative antidepressant amitriptyline, but not by the non-sedative antidepressants fluvoxamine and reboxetine, indicates that the PST is a suitable quantitative objective test for the detection of drug-induced changes in the level of arousal.

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Introduction

Spontaneous changes in pupil size in the dark are characteristic of a person's level of vigilance through the spectrum from alertness (large stable pupils) to sleep (small mobile pupils). Lowenstein et al. (1963) first characterized these pupillary oscillations and found that they were accentuated in drowsy subjects. These "fatigue waves" are characterized as being of low frequency and high amplitude. Although it was recognised early that this phenomenon of "pupillary unrest", as recorded by pupillography, could be useful in the clinical situation, e.g. for the assessment of narcoleptic patients (Yoss et al. 1970), progress in this area was hampered by the lack of availability of a quantitative method for measuring pupillary fatigue waves. Recently, the "pupillographic sleepiness test" (PST) has been developed for the quantitative assessment of pupillary fatigue waves (Lüdtke et al. 1998). The PST involves obtaining a power spectrum of the pupillary fluctuations from an 11-min record, following the fast Fourier transform of the data by computer. The PST has been used to quantify daytime sleepiness both in healthy sleep-deprived subjects (Wilhelm et al. 1998a) and patients suffering from sleep disorders (Wilhelm et al. 1998b). Apart from one early qualitative observation showing that the alerting drug benzedrine abolished pupillary fatigue waves in one sleepy subject (Lowenstein et al. 1963), there are no reports as to the effects of centrally acting drugs upon pupillary fatigue waves. In this study, we used the PST to compare the effects of single doses of three antidepressants, with differential propensity to cause sedation, on pupillary fatigue waves in healthy human volunteers. The three antidepressants were amitriptyline, a tricyclic antidepressant of known sedative property (Szabadi and Bradshaw 1995), the selective serotonin reuptake inhibitor (SSRI) fluvox-

amine and the noradrenaline reuptake inhibitor (NARI) reboxetine, two antidepressants with little clinical sedative potential (Szabadi and Bradshaw 1995). Furthermore, there are some indications in the literature that reboxetine may have "alerting" properties: it has been reported to activate the electroencephalogram in healthy volunteers (Herrmann and Fuder 1998) and display an "anti-fatigue" effect in depressed patients (Dubini et al. 1997). Therefore our prediction was that amitriptyline would increase and reboxetine may decrease pupillary fatigue waves while fluvoxamine would have no effect on pupillary fluctuations.

Materials and methods

Subjects

Sixteen healthy drug-free male volunteers aged 21.8±0.8 years and weighing 77.1±2.7 kg were recruited for this experiment. All volunteers gave their written informed consent following a verbal explanation of the study and after reading a detailed information sheet. Each subject completed a brief medical history and underwent a complete physical examination before inclusion in the study. All subjects reported compliance with the request to abstain from caffeine-containing beverages on each test day and to be "well rested". The study protocol was approved by the University of Nottingham Medical School Ethics Committee.

Drugs

Reboxetine mesylate (4 mg), fluvoxamine maleate (100 mg), amitriptyline hydrochloride (100 mg) and placebo were prepared in identical capsules and administered orally.

Design

Each subject participated in four experimental sessions at weekly intervals (returning to the laboratory at the same time each week). Subjects were allocated to treatment sessions according to a double-blind, balanced cross-over design.

Apparatus and tests

A monocular infrared video pupillometer (AMTech, Weinheim Germany) was used to record pupil diameter in the dark. During each recording session subjects wore goggles with infrared filters, in order to make sure that no light entered the eye, except that of the pupillographic light source. White noise [60 dB(A)] was presented binaurally via headphones in order to mask extraneous sounds. Subjects were asked to fixate on an infrared light-emitting diode positioned 80 cm in front of them, which was used as the pupillographic light source. Data were captured by dedicated software (AMTech, Weinheim, Germany) and stored for off-line analysis. A battery of 16 visual analogue self-rating scales (VAS) (Bond and Lader 1974) was also administered. These scales consist of 10-cm lines with the poles of the lines allocated to opposite properties (e.g. "alert" and "drowsy"). Both the order of presentation and the positions of the poles (left versus right) of the scales were randomized between subjects and sessions. The ratings on the 16 scales were grouped under the headings of "alertness", "anxiety" and "contentedness" based on the factor analysis of Bond and Lader (1974).

Procedure

After a 30-min acclimatization period, subjects completed the VAS. Subjects were then placed in a darkened room where they put on headphones and infrared goggles and underwent the PST for a period of 11 min. Treatments were administered immediately following the PST. Post-drug measurements (VAS and PST) were made 3 h after drug ingestion. All post-treatment measurements were carried out in the afternoon (between 1200 and 1700 hours), and only the results of these measurements were included in the data analysis.

Pupillography

Pupil diameter was measured continuously during the 11-min recording period at a frequency of 25 Hz. Software driven artifact-rejection was applied to the raw data in order to remove blinks and high-frequency noise. Oscillations of pupil diameter at frequencies of ≤0.8 Hz were captured and subjected to fast Fourier transform. Absolute changes in pupil diameter were also recorded and subjected to low pass filtering. This was achieved by sampling for 0.64-s periods (16 data points at 25 Hz); the cumulative difference between successive 0.64-s samples over 1 min yields the pupillary unrest index PUI (mm min^{-1}). For further details, see Lüdtke et al. (1998).

Data analysis

The following measures were obtained from each subject: resting pupil diameter, power of pupillary oscillations, PUI, and self-rated "alertness", "anxiety" and "contentedness". The pupillary measures were average values obtained during the 11-min recording period. The self-rated values of "alertness", "anxiety" and "contentedness" were derived from the VAS scores after weighting with their loadings on these factors: see Bond and Lader (1974). Post-treatment scores on each variable were subjected to one-way analysis of variance with repeated measures, with treatment condition as a within-subject factor. When a significant effect of treatment was found, individual comparisons were made between each active treatment and placebo using Dunnett's test with an a priori criterion of $P<0.05$ (df=36; k=4; criterion t=2.44).

Results

The data from three subjects were excluded from statistical analysis. One subject's pupil diameter was in excess of 10 mm after reboxetine, 10 mm being the maximum for the recording equipment. Two other subjects failed to remain awake during the amitriptyline condition, and thus the recording could not be completed.

Pupil diameter

Figure 1 shows post-treatment pupil diameter after each drug treatment condition. One-way ANOVA revealed a significant treatment effect [$F(3,36)=8.39$, $P<0.0001$]. Reboxetine significantly increased pupil diameter, compared to placebo (t=3.15, $P<0.01$).

Total power of pupil fluctuations

Figure 2 shows the power of pupil fluctuations obtained after the four treatments. One-way ANOVA revealed a significant treatment effect [$F(3,36)=8.41$, $P<0.0001$]. Amitriptyline significantly increased the power of pupillary oscillations, compared to placebo (t=2.57, $P<0.05$).

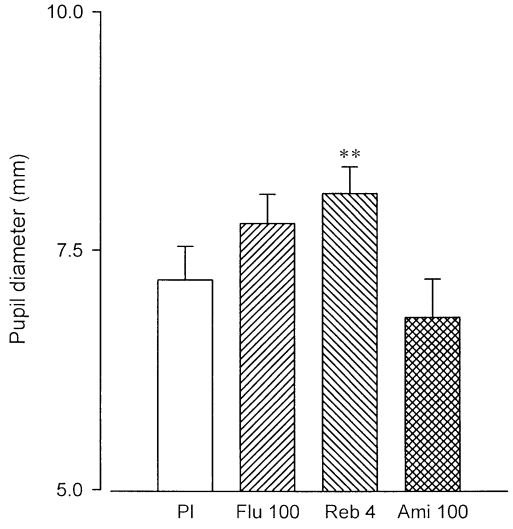

Fig. 1 Absolute pupil diameter (mm) in darkness following the administration of placebo, fluvoxamine (100 mg), reboxetine (4 mg) and amitriptyline (100 mg). Each *column* refers to the mean (n=13), *vertical bars* are SEM. Significance of difference between active treatment and placebo: $^{**}P<0.01$ (see text for details of statistical analysis)

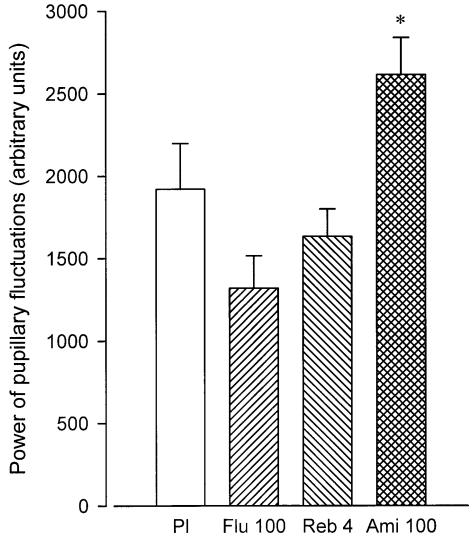

Fig. 2 Total power of pupillary fluctuations at frequencies <0.8 Hz (arbitrary units) following drug administration (see Fig. 1 for convention). Significance of difference between active treatment and placebo: $^{*}P<0.05$ (see text for details of statistical analysis)

Pupillary unrest index

Post-treatment PUI data are shown in Figure 3. One-way ANOVA revealed a significant treatment effect [$F(3,36)$= 6.26, P<0.01]. The effects of fluvoxamine and reboxetine did not differ significantly from that of placebo. Amitriptyline showed a tendency to increase PUI; however, the difference between the effects of amitriptyline and placebo on

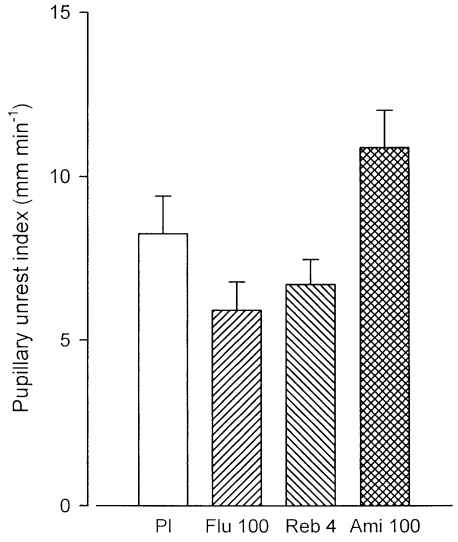

Fig. 3 Pupillary unrest index (mm min^{-1}) following drug administration (see Fig. 1 for convention)

PUI did not reach statistical significance (t=2.13, 0.05<P<0.1).

Visual analogue scales

Table 1 shows the post-treatment "alertness", "contentedness" and "anxiety" scores. One way-ANOVA showed a significant treatment effect upon subjectively rated "alertness" [$F(3,36)$=12.26, P<0.0001], "anxiety" [$F(3,36)$= 3.79, P<0.05] and "contentedness" [$F(3,36)$=6.63, P<0.01]. Comparison of each active treatment with placebo revealed a reduction in "alertness" (t=5.57, P<0.01), "anxiety" (t=2.89, P<0.05) and "contentedness" (t=4.24, P<0.01) ratings following the administration of amitriptyline. Reboxetine reduced subjectively rated "anxiety" (t=2.69, P<0.05). Fluvoxamine had no significant effect on any of the subjective ratings.

Discussion

Amitriptyline did not have any significant effect on resting pupil diameter. This is in agreement with previous findings that amitriptyline can cause either a small increase or a small decrease or no change in pupil diameter, probably reflecting the balance between mydriasis resulting from muscarinic cholinoceptor blockade and miosis resulting from α_1-adrenoceptor blockade (Szabadi and Bradshaw 1986). Amitriptyline enhanced pupillary fatigue waves as evidenced by an increase in the power of the oscillations recorded in the low (<0.8 Hz) frequency band and a tendency to increase PUI. These changes in pupillary behaviour were paralleled by the subjects' self-rated scores: there was a decrease in "alertness", "contentedness" and "anxiety" following the administration of amitriptyline. The decrease in "contentedness" is

Table 1 Effects of treatments (mean±SEM) upon subjectively rated "alertness", "anxiety" and "contentedness"

Factor rating (mm)	Placebo	Fluvoxamine	Reboxetine	Amitriptyline
Alertness rating (mm)	41.99±2.35	39.39±3.01	33.53±3.48	22.32±2.40**
Anxiety rating (mm)	28.17±3.11	24.51±3.09	18.88±1.99*	18.18±2.20*
Contentedness rating (mm)	50.84±1.68	48.44±2.75	46.97±2.50	39.94±2.19**

Significance of difference between active treatment and placebo [ANOVA (repeated measures) followed by Dunnett's corrected *t*-test]: *P<0.05, **P<0.01

likely to reflect the dysphoria experienced by the subjects, which in turn may be related both to sedation and dryness of the mouth. Amitriptyline also reduced "anxiety" scores. These observations are consistent with numerous reports including both healthy volunteers (Longmore et al. 1988; Theofilopoulos et al. 1989; Hindmarch et al. 1992; Kerr et al. 1996) and depressed patients (Reimherr et al. 1990; Bakish et al. 1992) showing that amitriptyline has sedative properties. This property of the drug is reflected in impaired psychomotor functions and can seriously interfere with the subjects' daytime functioning. However, this property is taken advantage of when the drug is administered at bedtime in an attempt to promote night-time sleep (Szabadi and Bradshaw 1995; British Medical Association/Royal Pharmaceutical Society of Great Britain 1999).

Fluvoxamine, like amitriptyline, failed to affect resting pupil diameter, in agreement with previous observations (Wilson et al. 1983; Flett et al. 1992). In contrast to amitriptyline, fluvoxamine did not increase pupillary fatigue waves, suggesting the absence of sedation. This finding is consistent with reports in healthy volunteers showing the lack of effect of fluvoxamine on psychomotor functions (Saletu et al. 1983; Hindmarch et al. 1992). The lack of effect of fluvoxamine on pupillary fatigue waves was paralleled by the failure of this drug to affect subjectively rated alertness.

Reboxetine increased resting pupil diameter, in agreement with previous observations (Szabadi et al. 1997). The mydriatic effect of reboxetine is likely to reflect noradrenaline re-uptake blockade at noradrenergic nerve endings, possibly both in the periphery and in the central nervous system. In the iris, noradrenaline uptake blockade would potentiate the effect of noradrenaline at α_1-adrenoceptors on the dilator pupillae muscle, thus leading to enhancement of pupil diameter. In the central nervous system, the blockade of noradrenaline reuptake at noradrenergic terminals on the Edinger-Westphal nucleus would enhance the effect of noradrenaline on inhibitory α_2-adrenoceptors, leading to an increase in the tonic noradrenergic inhibition of the parasympathetic innervation of the constrictor pupillae muscle (Szabadi and Bradshaw 1996).

Reboxetine had no effect on pupillary fatigue waves, arguing against a sedative effect of this drug. In agreement with this observation, it has been shown in healthy volunteers that reboxetine fails to affect psychomotor functions (Hindmarch 1997; Herrmann and Fuder 1998). The lack of effect of reboxetine on pupillary fatigue waves was paralleled by the failure of this drug to affect subjectively rated alertness. In fact, the only effect of this drug on the visual analogue scales was a small reduction in "anxiety".

As reboxetine is a selective and potent noradrenaline reuptake inhibitor (Brunello and Racagni 1998), it might have been expected that it would display an "alerting" effect manifesting as a reduction in pupillary fatigue waves. Such an alerting effect would be due to the activation of the central noradrenergic system, which has been implicated in the regulation of arousal mechanisms (Robbins and Everitt 1995). Indeed, an alerting ("vigilance-enhancing") effect of reboxetine was detected in another study using pharmaco-encephalography (Herrmann and Fuder 1998). Furthermore, an "anti-fatigue" effect of reboxetine was described in depressed patients manifesting as an increase in the levels of social motivation and activity (Dubini et al. 1997), which again may reflect the activation of the central noradrenergic system by the drug. However, no alerting effect of a single dose of reboxetine on pupillary fluctuations was detected in the present experiment. A possible explanation for this may be a "baseline effect": our well-rested healthy volunteer subjects had low levels of pupillary fluctuations in the absence of any medication that could not be further decreased by reboxetine. In order to detect the "anti-fatigue" effect of reboxetine on pupillary fluctuations, it may be necessary to test the drug in "sleepy" subjects after such manipulations as sleep-deprivation or the administration of a sedative drug.

The PST has been developed for the monitoring of the level of arousal in patient groups suffering from sleep disorders (e.g. narcolepsy, sleep apnoea). The present results show that the PST is also a suitable quantitative test for the detection of drug-induced changes in the level of arousal.

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