

Effects of reboxetine and desipramine on the kinetics of the pupillary light reflex

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- 1 The aim of the study was to examine the effects of single doses of two antidepressants (desipramine and reboxetine) on three kinetic parameters (latency, amplitude, 75% recovery time) of the pupillary light reflex response.
- 2 Six healthy male volunteers participated in three experimental sessions at bi-weekly intervals. Each session was associated with one of three treatment conditions (placebo, desipramine 100 mg, reboxetine 4 mg). Subjects were allocated to sessions and treatments double-blind according to a Latin Square design.
- 3 Pupil diameter was measured in the dark with binocular television pupillometry. Reflex responses were evoked by 12 light stimuli (5.3×10^{-5} – 3.5 mW cm^{-2} ; 500 ms), and the kinetic parameters of each response were recorded.
- 4 The amplitude and 75% recovery time were positively, and latency was negatively correlated with the logarithm of light stimulus intensity. In the presence of the antidepressants the latency was prolonged, the amplitude was reduced and the 75% recovery time was shortened. There was a positive linear relationship between reflex amplitude and recovery time under all three treatment conditions; this relationship was not significantly affected by the antidepressants.
- 5 The effects of the antidepressants on latency and amplitude are consistent with the blockade of muscarinic cholinergic receptors in the iris, whereas the shortening of the recovery time appears to be secondary to the reduction in amplitude.

Keywords reboxetine desipramine pupil light reflex

Introduction

The pupillary light reflex is the constriction of the pupil in response to a light stimulus. The effector organ is a smooth muscle diaphragm, the iris, which receives a dual sympathetic/parasympathetic innervation. Both innervations contribute to the light reflex: while the latency and amplitude of the reflex response are almost exclusively determined by activity in the parasympathetic [1–3], the time required for the pupil to redilate to its original size after the cessation of the light stimulus ('recovery time') is significantly influenced by activity in the sympathetic nerve supply to the iris [2, 4–6]. Thus it has been shown that drugs that block muscarinic cholinergic receptors in the iris (e.g. tropicamide) prolong the latency and reduce the amplitude of the pupillary light reflex [3], whereas drugs that reduce sympathetic outflow (e.g. clonidine) prolong the recovery time [5], and variables that enhance sympathetic activity (e.g. high ambient tem-

perature) shorten the recovery time of the reflex response [6].

Many antidepressant drugs are known to influence both sympathetically and parasympathetically mediated tissue responses in the periphery [7]. The most common effects of tricyclic antidepressants are sympathetic potentiation (due to the blockade of noradrenaline uptake and prejunctional release-modulating α_2 -adrenoceptors), sympathetic inhibition (due to the blockade of postjunctional α_1 -adrenoceptors), and parasympathetic inhibition (due to the blockade of postjunctional muscarinic cholinergic receptors).

In the present study we examined the effects of single doses of reboxetine and desipramine on the kinetic parameters (latency, amplitude, recovery time) of the pupillary light reflex. Reboxetine is a novel antidepressant with potent noradrenaline uptake

blocking and relatively weak muscarinic cholinceptor blocking properties [8], whereas desipramine is an established tricyclic antidepressant with a similar profile of action on the noradrenaline uptake mechanism and on muscarinic cholinceptors [9]. In addition, by exploring the sensitivity of the kinetic parameters of the pupillary light reflex to drugs with predictable actions, we aimed at validating the use of these parameters in detecting the effects of drugs on peripheral autonomic mechanisms. A preliminary report of our findings has been presented to the British Pharmacological Society [10].

Methods

Subjects

Six healthy drug-free male volunteers aged 19–21 years (mean 20.5 years) and weighing 55–75 kg (mean 67.1 kg) participated in the study. The study was approved by the Ethics Committee of South Manchester Health Authority. All subjects were informed of the nature of the study and gave their written consent. The subjects underwent a medical examination before inclusion in the study and their general practitioners were contacted and asked to report any medical condition which might exclude the subject from the study.

Drugs

One dose of reboxetine (4 mg), one dose of desipramine (100 mg) and one dose of lactose placebo were ingested by each subject. The drugs were prepared in identical capsules for double-blind administration: each session involved ingestion of one capsule. The drugs were allocated to subjects and sessions according to a Latin Square design.

Apparatus

Pupil diameter was measured in darkness using a binocular infrared television pupillometer (Applied Science Laboratories, Waltham, MA, USA). Prior to assessment of pupil diameter the subjects wore red goggles for 15 min (dark adaptation). The pupillary light reflex was evoked by a light source constructed in our laboratory. This consisted of three green light emitting diodes (peak wavelength 565 nm) glued together to form a common compact light source. The light source was mounted on a headband and positioned 1 cm in front of the cornea of the right eye. The diodes were driven by a current source controlled by computer. The computer could be programmed to pass current pulses of predetermined intensity and duration through the diodes and also to determine the time intervals between the applications of successive stimuli. The following twelve light intensities were used: 5.3×10^{-5} , 1.5×10^{-4} , 5.0×10^{-4} , 1.7×10^{-3} , 5.3×10^{-3} , 1.5×10^{-2} , 4.6×10^{-2} , 0.13, 0.36, 0.86, 1.86 and 3.5 mW cm^{-2} (measured 1

cm from the light source). The stimulus duration was 500 ms, and the interval between successive stimuli was 20 s.

Procedure

Each subject participated in three experimental sessions, at bi-weekly intervals. Sessions always took place in the morning. On an experimental day the subject was allowed a light breakfast (including decaffeinated coffee) at 08.00 h. The subject was asked to report to the laboratory at 09.00 h and after 0.5 h of acclimatizing to laboratory conditions (temperature, dark adaptation for 15 min) the pre-drug pupillometric test was carried out. The test took 10 min to complete. The subjects then ingested the drug capsule. The post-drug test was carried out 3 h after drug taking. The time point of this test was chosen to coincide with the time when the plasma levels of the antidepressants were at their maxima. It has been reported that the peak plasma level of desipramine is attained 2–4 h after drug-taking [11] and the peak plasma level of reboxetine is obtained 3–6 h after drug-taking [8].

After dark adaptation the resting pupil size was measured and recorded on computer disk. This was followed by recording changes in pupil diameter in response to light stimulation. Each response was displayed on the computer screen and a cursor was used to determine the position of relevant points on the response curve; the distance between the relevant points was measured by the computer. The following points were used: onset of light stimulus, onset of response, (negative) peak of the response, and point at which the size of the pupil had recovered to 75% of the full response amplitude. The following kinetic measures were obtained from the relevant points of the response curve: latency (time elapsed between the onset of stimulus and onset of response), amplitude (distance between resting pupil size and the deepest trough of the response), 75% recovery time (time taken from the peak of the response to obtain 75% recovery).

Analysis of data

Each kinetic parameter was plotted against the logarithm of the light stimulus intensity to obtain the light intensity/latency, light intensity/amplitude and light intensity/recovery time curves. The effects of the antidepressants were obtained by comparing pupillary measures taken after the ingestion of the antidepressants with those taken after the ingestion of placebo, in the right eye.

Analysis of variance, with individual comparisons, was used for the statistical analysis of the data. In each one-factor analysis of variance, *F*-ratios were calculated for the overall main effect; in each two-factor analysis of variance *F*-ratios were calculated for each overall main effect and the interaction effect; probability values were adjusted using the Huynh-Feldt correction. When a significant overall main effect of drug treatment was identified,

individual comparisons with a single control were made between the two active drug treatments (i.e. reboxetine and desipramine) and placebo using Student's *t*-test with Dunnett's correction for multiple comparisons with a single control (d.f. = 10, k = 3). An *a priori* criterion of $P < 0.05$ was used to identify significant effects. The relationship between log light intensity and each parameter of the light reflex response, and the relationship between response amplitude and recovery time were analyzed using the product moment correlation coefficient (d.f. = 10), and best fit linear functions were derived with the method of least squares. Unpaired *t*-test was used to compare the slope and intercept values for each active drug and placebo.

Results

Resting pupil diameter

The dark-adapted resting pupil sizes (mm; mean \pm s.e. mean), 3 h after drug taking, were as follows: placebo 7.67 (\pm 0.22), desipramine 7.83 (\pm 0.35), reboxetine 8.32 (\pm 0.25). There was a significant main effect of drug treatment ($F = 10.93$, d.f. = 2,10); individual comparisons revealed that pupil diameter was significantly greater after reboxetine than after placebo ($t = 4.50$).

Kinetic parameters of light reflex

Figure 1 shows the light intensity/latency, light intensity/amplitude and light intensity/75% recovery time curves under the three treatment conditions. Each kinetic parameter was linearly related to the logarithm of light intensity: there were statistically

significant positive correlations between light intensity and response amplitude, between light intensity and recovery time, and negative correlations between light intensity and latency (Table 1). It is apparent from Figure 1 that the antidepressants prolonged the latency, reduced the amplitude and shortened the recovery time of the pupillary light reflex response. Two-way analysis of variance revealed that there was a significant main effect of both factors (light intensity, drug treatment) in the case of each kinetic parameter (Table 2). In the case of response amplitude there was also a significant interaction effect, reflecting the convergence of the functions obtained under the three treatment conditions at lower light intensities. A similar trend is apparent in the case of 75% recovery time, although in this case the adjusted probability value fell just short of statistical significance (Huyhn-Feldt $P = 0.067$). These trends are also revealed by the regression analyses which show that the slopes were reduced in the presence of the two active treatments in the case of amplitude and 75% recovery time (Table 1). Individual comparisons of the two acute treatments with placebo indicated that the effects of both antidepressants were statistically significant in the case of all three parameters: latency (desipramine: $t = 3.62$, reboxetine: $t = 4.98$); amplitude (desipramine: $t = 8.23$, reboxetine: $t = 8.99$); 75% recovery time (desipramine: $t = 5.57$, reboxetine: $t = 4.82$).

Relationship between response amplitude and recovery time

The relationship between reflex response amplitude and 75% recovery time, at each light intensity value studied, is shown in Figure 2; the results of the linear regression analysis are displayed in Table 3. It is apparent that there was a statistically significant posi-

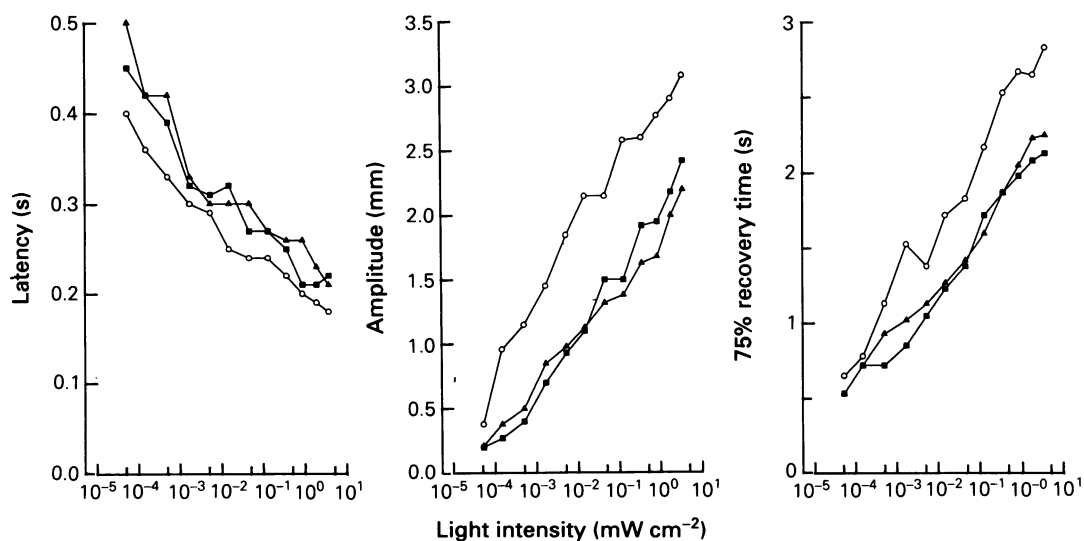


Figure 1 Relationship between light stimulus intensity and the latency, amplitude and recovery time of the pupillary light reflex response. Each point corresponds to the mean obtained in the group of six subjects. The three lines correspond to the three treatment conditions (open circles: placebo; closed squares: desipramine 100 mg; closed triangles: reboxetine 4 mg). For statistical analysis see Tables 1 and 2.

Table 1 Slopes (95% confidence interval, CI) of best-fit linear functions and product-moment correlation coefficients (r) derived for the log light intensity/kinetic parameter relationships under each treatment condition (see Figure 1)

Parameter	Slope (95% CI)	r^*
Latency		
Placebo	-0.0185 (-0.0176, -0.0195)	-0.987
Desipramine	-0.0212 (-0.0198, -0.0226)	-0.978
Reboxetine	-0.0223 (-0.0199, -0.0246)	-0.949
Amplitude		
Placebo	0.2265 (0.2136, 0.2394)	0.984
Desipramine	0.2052 (0.1985, 0.2145)	0.990
Reboxetine	0.1667 (0.1601, 0.1731)	0.993
75% recovery time		
Placebo	0.1990 (0.1896, 0.2084)	0.989
Desipramine	0.1546 (0.1474, 0.1619)	0.989
Reboxetine	0.1545 (0.1476, 0.1614)	0.990

* $P < 0.001$ (in each case).

Table 2 F -ratios obtained from two-way analysis of variance of data included in the light intensity/kinetic parameter curves (see Figure 1)

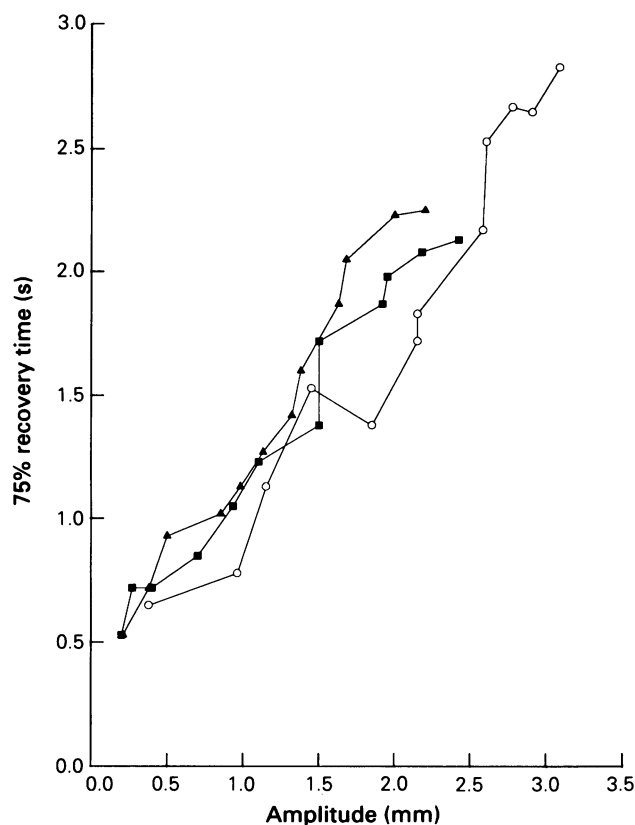
	Latency	Amplitude	75% recovery time
Light intensity (d.f. = 11,55)	129.83*	48.71*	47.53*
Drug treatment (d.f. = 2,10)	13.25*	34.18*	18.27*
Interaction (d.f. = 22,110)	1.31	3.01*	1.71

* $P < 0.05$.

tive correlation between response amplitude and 75% recovery time in the case of each treatment condition. There were no statistically significant differences between the slopes and intercepts of the regression lines obtained in the presence of either of the antidepressants or placebo (unpaired t -test: desipramine vs placebo (slope: $t = 1.24$, intercept $t = 1.86$); reboxetine vs placebo (slope: $t = 0.58$, intercept: $t = 1.39$)).

Discussion

Both antidepressants caused a small increase in dark-adapted resting pupil size; this increase, however, reached statistical significance only in the case of reboxetine. The mydriatic effect of the antidepressants could reflect the potentiation of the effect of endogenously released noradrenaline resulting from uptake blockade [7], since both antidepressants are known to be potent inhibitors of noradrenaline uptake [8, 9]. Furthermore, the mydriatic effect could have been enhanced by the blockade of muscarinic cholinergic receptors since both desipramine [9] and reboxetine (see below) have some anticholinergic properties.

**Figure 2** Relationship between the amplitude and 75% recovery time of the light reflex responses evoked by the range of light stimulus intensities shown in Figure 1. Conventions are the same as in Figure 1. For statistical analysis see Table 3.**Table 3** Results of linear regression analysis (least squares, product moment correlation) of data displayed in Figure 2

	Slope \pm s.e. estimate	Intercept \pm s.e. estimate	Correlation coefficient
Placebo	0.86 \pm 0.07	0.11 \pm 0.15	0.97*
Desipramine	0.76 \pm 0.04	0.41 \pm 0.06	0.99*
Reboxetine	0.91 \pm 0.05	0.34 \pm 0.07	0.99*

* $P < 0.001$.

The present results confirm our previous finding that the kinetic parameters of the light reflex are linearly related to the logarithm of light stimulus intensity [4, 5]. The two antidepressants studied affected all three kinetic parameters of the light reflex: the latency was prolonged, the amplitude was reduced and the 75% recovery time was shortened. The effects on the latency and the amplitude are the same as observed following local instillation of anticholinergic drugs [3], and suggest the blockade of muscarinic cholinergic receptors by the antidepressants in the eye. Although there is evidence that desipramine can interact with muscarinic cholinergic receptors [9], reboxetine has been reported to have only very low affinity for this receptor [8]. However, in a previous study we have found that single doses of both

desipramine and reboxetine can reduce salivation and antagonize carbachol-evoked sweating in human volunteers (Szabadi, *et al.*, unpublished observations), indicating that both antidepressants can block muscarinic cholinergic receptors in humans *in vivo*.

The reduction in recovery time of the light reflex in the presence of the antidepressants could be consistent with sympathetic potentiation [6] resulting from noradrenaline uptake blockade. Although this interpretation is consistent with the known pharmacological profile of both antidepressants [8, 9], it was necessary to exclude the possibility that the reduction in recovery time was not merely a reflection of the reduction in the amplitude of the light reflex response ('smaller responses recover faster'). Therefore, we carried out the analysis shown in Figure 2 and Table 3. This analysis revealed a close positive linear relationship between response amplitude and recovery time under all three treatment conditions. Moreover, the slopes and intercepts of the fitted regression lines did not differ significantly in the presence of the antidepressants from those observed in the presence of placebo, indicating that the observed reduction in recovery time (Figure 1, Table 2) is likely to be the reflection of the reduction in reflex response amplitude. It is possible that the reduction in amplitude in the presence of the antidepressants militated against the detection of any effect on the sympathetic input, since previous studies have suggested that a sympathetic contribution to the recovery phase of the light reflex response becomes apparent only at larger response sizes [5, 6]. In theory, the range of response amplitudes in the presence of the antidepressants could have been

extended to the same level as observed in the presence of placebo by the application of more intense light stimuli. However, in practice this approach could not be adopted because of the subjective discomfort caused by the brighter stimuli.

In conclusion, the present experiment demonstrates that the kinetic parameters of the pupillary light reflex provide a sensitive pharmacological test system for the detection of the effects of single doses of systemically taken drugs on cholinergic (and potentially also on noradrenergic) neurotransmission. In the case of drugs, such as the antidepressants, that affect both cholinergic and noradrenergic activity, it may be difficult to detect an effect on the noradrenergic system, since an alteration of the recovery time may be related to a change in amplitude. However, if the predicted changes in amplitude and recovery time are in a different direction to that seen in the present study, it may be possible to draw more definite conclusions about the change in noradrenergic activity. For example, if a reduction in amplitude is accompanied by an elongation of the recovery time, one may conclude that the drug has shown both anti-cholinergic and anti-noradrenergic activity, or if the drug increases the amplitude and shortens the recovery time, this may be interpreted as evidence for the cholinomimetic and noradrenaline potentiating effects of the drug. Further experiments are needed to explore these possibilities.

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