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ORIGINAL INVESTIGATION

Comparison of ketanserin, buspirone and propranolol on arousal, pupil size and autonomic function in healthy volunteers

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Abstract
Rationale The human pupil may be a suitable physiological test system for the assessment of excessive daytime sleepiness (EDS), but pupillometric assessment could be confounded by medication for comorbid hypertension and mood disorders.
Objectives We examined the profile of the 5HT-2/α1/H1 antagonist ketanserin, the 5HT1a agonist buspirone and the beta adrenoceptor antagonist propranolol on pupillary and other measures of arousal.
Materials and methods Ketanserin (20 mg), buspirone (10 mg) and propranolol (40 mg) were administered in three independent experiments according to a crossover, placebo-controlled, double-blind design. Resting pupil diameter (RPD) was sampled over 5-min in darkness with infrared pupillometry. Tests also included critical flicker fusion frequency (CFFF), visual analogue scales (VAS), the pupillary light reflex and heart rate/blood pressure.
Results Ketanserin reduced RPD, CFFF, VAS-rated arousal and blood pressure and increased the light reflex amplitude. Buspirone reduced RPD and blood pressure. Propranolol reduced heart rate but had no effects on pupillary functions or any arousal measure.
Conclusions Ketanserin but not propranolol had a fully sedative profile and may confound pupillometric assessment of EDS. Beta adrenergic receptors do not appear to participate in arousal and pupillary functions, while 5HT1a receptors reduce pupil size without affecting arousal. Pupil size may not be used unequivocally as an index of the level of alertness in the case of drug-induced changes, when drugs interfere with the central pupil control mechanism in ways that are unrelated to their effects on arousal.

Keywords Pupil size · Light reflex · Arousal · Pupillary alertness test · Critical flicker fusion frequency · Drug-induced sedation

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Introduction

It has long been known that any decrease in arousal is accompanied by a decrease in pupil diameter (Loewenfeld 1993), and assessment of pupil diameter is routinely used by anaesthetists when gauging the depth of anaesthesia (Aitkenhead et al. 2001). Pupil size in darkness has been successfully used as a single physiological measure of arousal in patients suffering from excessive daytime sleepiness (EDS) due to obstructive sleep apnea (Bitsios et al. 2006); compared to age- and sex-matched controls, the sleepy patients showed smaller pupil size, which correlated with objective indexes of apnea severity and subjective measures of sleepiness, the differences becoming more apparent during the afternoon circadian nadir (Bitsios et al. 2006). Moreover, pupil size was sensitive to the alerting effects of modafinil in patients with EDS as a result of obstructive sleep apnea (Nikolaou et al. 2008).

Recent evidence points to the importance of metabolic factors, hypertension and depression in the aetiology of EDS (Bixler et al. 2005), and treatment for these conditions is not uncommon among these patients. If monitoring of resting pupil size is to be more regularly incorporated in future studies as a clinical tool for the objective assessment

of EDS, it would be important to understand the pupillary effects of the various drugs prescribed for these patients. In this study, we sought to determine the effects of single doses of ketanserin, buspirone and propranolol on pupillary behaviour of healthy subjects in three separate experiments. If these drugs alter pupil size, they might interfere with the pupillometric determination of alertness in patients suffering from EDS (Bitsios et al. 2006; Nikolaou et al. 2008) if the above drugs are prescribed for treatment of comorbid hypertension or mood disorders.

Ketanserin is an anti-hypertensive agent with sympatholytic effects, via central 5HT2-mediated modulation of the sympathetic system (Cameron et al. 1987). It is also an α_1 adrenergic and histamine receptor antagonist (Dollery 1999). Ketanserin 20 mg has been reported to reduce critical flicker fusion frequency (CFFF; Graham et al. 2002) and sustained attention (Wingen et al. 2007) and it is considered a sedative drug (Dollery 1999) although clinically, its effects on arousal may not be as profound (Herrmann and Baumgartner 1986). Propranolol is another widely used antihypertensive agent with sympatholytic properties via peripheral beta adrenoceptor blockade on the vascular bed. It can also behave as a 5HT1a antagonist and a 5HT1b agonist in the rat cortex (Pierson et al. 1989). Propranolol is not considered a sedative drug (Currie et al. 1988), and there are mixed reports regarding its ability to reduce arousal; impaired psychomotor performance has been reported after single doses (Landauer et al. 1979; Salem and McDevitt 1984), but other studies have failed to show such effects (Currie et al. 1988; Harmer et al. 2001; Ogle et al. 1976; Tyrer and Lader 1974). Buspirone is a non-sedative anxiolytic and a partial agonist at the 5-HT1A receptor (Andrade and Nicoll 1987), with some affinity for the dopamine D2 receptor (Jann 1988; Peroutka 1985; Riblet et al. 1982). Buspirone has a dose-dependent miotic effect in healthy human subjects (Fanciullacci et al. 1995; Phillips et al. 1999), but the mechanism remains unclear (Phillips et al. 1999).

A reduction in pupil size by a drug may be due to the reduction of the sympathetic input to the iris, increase of the parasympathetic input to the iris or both. In order to examine the relative contributions of the sympathetic and the parasympathetic systems in a putative effect of these drugs on pupil size, we examined their effect on the pupillary light reflex. The pupillary light reflex may help to elucidate the effects of a drug on the sympathetic and parasympathetic inputs to the iris, since the time course of the light reflex response is determined by the successive activation of the parasympathetic and sympathetic inputs; the amplitude reflects activation of the midbrain parasympathetic Edinger–Westphal nucleus (Barbur 2004; Gamlin et al. 1997), while the recovery time reflects mainly sympathetic activation, which resumes at the end of the light stimulus and recovers the pupil to its original levels (Bitsios et al. 1998a; Loewenfeld 1999; Smith and Smith 1999). However, because recovery time also depends, by definition, on initial (baseline) pupil diameter (Loewenfeld 1993) and is, in the experience of our lab, susceptible to blinks, we measured only latency and amplitude of the light reflex. Finally, we also examined the effects of these drugs on other measures of arousal such as CFFF (Smith and Misiak 1976) and visual analogue scales (VAS) (Bond and Lader 1974) and cardiovascular indices of autonomic function such as heart rate and blood pressure.

Materials and methods

Subjects

In all three experiments, subjects were between 18 and 30 years old, with a body mass index (BMI) in the normal range. Inclusion criteria included written informed consent, absence of personal history of head trauma, medical and neurological conditions or use of prescribed and recreational drugs and absence of personal or family (up to second-degree relatives) history of Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition Axis I disorders. All participants underwent physical and psychiatric assessment using the Mini-International Neuropsychiatric Interview (Sheehan et al. 1998), an ophthalmological examination and a urine drug screen test. All subjects were regular caffeine (two to three cups of coffee per day on average) and occasional alcohol consumers. Subjects' demographic characteristics in experiments I (ketanserin), II (buspirone) and III (propranolol) are shown in Table 1. The study was approved by the University of Crete Ethics Committee.

Design and drugs

Ketanserin, buspirone and propranolol were administered in a placebo-controlled, within-subject design in three separate experiments, using separate groups of subjects but identical experimental procedures. In each experiment, subjects participated in two weekly sessions (returning to the laboratory at the same time each week for each session). Subjects were allocated to treatments and sessions according to a double-blind, balanced, crossover design. Ketanserin 20 mg, buspirone 10 mg, propranolol 40 mg and placebo were prepared in identical capsules and administered orally. The choice of dose of each drug was based on centrally bioactive doses reported in published studies [ketanserin (Graham et al. 2002), buspirone (Phillips et al. 1999) and propranolol (Grillon et al. 2004)].

Table 1 Subjects' demographic characteristics in each experiment

	Experiment I, Ketanserin	Experiment II, Buspirone	Experiment III, Propranolol	*p* value
Sample size	12	12	12	NA
Male/female[a]	6:6	6:6	6:6	1
Age (years)	24.8(4.0)	25.3(3.0)	24.8(3.6)	>0.9
BMI	24.3(3.9)	23.5(3.1)	22.2(2.9)	>0.3
Education (years)	16.3(2.4)	17.3(1.6)	17.3(1.8)	>0.4
Smokers/non-smokers[a]	4:8	4:8	8:4	>0.1

Figures in brackets are SD

[a] For these measures, chi-square comparisons were applied. All other variables were analysed with one-way ANOVAs

Tests and apparatus

Resting pupil diameter

A binocular infrared video pupillometer (Procyon P2000D, Procyon, London, UK; sampling rate, 25 Hz; spatial resolution, >0.05 mm; accuracy, >±3%) was used to monitor RPD in darkness. Our methodology of recording pupil diameter [5-min Pupillary Alertness Test (5-min PAT)] has been described in detail previously (Bitsios et al. 2006, Nikolaou et al. 2008). Pupil diameter was sampled for 15 consecutive 20-s periods, and thus, the total monitoring time was 300 s. The outcome measures were the average RPDs for each one of the 15 20-s periods and the collapsed RPD for the entire 300-s recording. Data were stored for off-line cleaning from spontaneous blinks, scoring and statistical analysis.

The light reflex

The light reflex was elicited and recorded in darkness, following testing with the 5-min PAT. The stimuli were light flashes of 200-ms duration delivered via a light-emitting diode, presented to the subject's right and left eye in an alternating fashion, as a white disc of 8° diameter, providing 'full retinal field' light stimulation (at four levels of stimulus luminance, 0.35, 5, 50 and 140 cd m^{-2}), while the non-stimulated eye was fixating a target dot projected at a distance of approximately 10 m. Each one of the 4 levels of stimulus luminance was presented in a block of three stimuli, the average of which was the response for that luminance level. The inter-stimulus interval within blocks was fixed at 7 s. Therefore, the total time for the elicitation and recording of the light reflex was 80 s. Stimulus presentation was computer controlled, and pupillary measures were digitised and stored for off-line analysis. Using the automated manufacturers' software, the parameters studied were light reflex latency (i.e. the time elapsed from onset of the light flash until the onset of a pupillary response) and light reflex response amplitude [i.e. the difference between the baseline (defined as the mean pupil diameter recorded over 500 ms before the onset of the light stimulus) and the diameter reached at the trough of the pupillary response to the light stimulus].

Critical flicker fusion frequency The Leeds Psychomotor Tester (Psychopharma, Surrey, UK) was used to collect CFFF measurements, defined as the frequency at which a flickering light appears to be continuous (Smith and Misiak 1976). The CFFF is sensitive to sedative drugs. Subjects viewed the stimulus through a 2-mm 'artificial pupil'. The CFFF test was conducted conventionally, with eight threshold measurements collected per session: four with increasing frequencies and four with decreasing frequencies. The mean of the eight measurements was taken as the value of the CFFF (see Samuels et al. 2006).

Visual analogue scales A computerised version of the VAS was used to collect self-ratings of alertness, contentedness and anxiety. Nine contrasting statements were rated along a continuous 10-cm line to represent the participant's subjective alertness (Norris 1971). The ratings on the nine items were multiplied by their respective factor loadings based on a factor analysis carried out by Bond and Lader (1974) and the mean of the weighted values entered the analysis.

Cardiovascular measures

Blood pressure and heart rate recordings were taken in the sitting position using an electroaneroid sphygmomanometer.

Procedure

After arrival in the laboratory, each subject had a 15-min acclimatisation period, after which the pretreatment tests (recordings of heart rate, blood pressure and VAS) were

carried out. The testing was completed in 5 min. On completion of pretreatment tests, the subjects ingested the capsule containing either the active drug or placebo. The pretreatment tests were repeated post-ingestion, together with recordings of the 5-min PAT and the pupillary light reflex (post-treatment tests). The time course of the sessions was based on the pharmacokinetics of the active drugs: t_{max} is 1 h following oral administration of single doses of propranolol (Hardman et al. 2001), ketanserin (Brogden and Sorkin 1990) and buspirone (Sakr and Andheria 2001).

Data analysis

In each experiment, the average RPDs for each one of the 15 20-s periods were analysed with a mixed model analysis of variance (ANOVA) with period (15 levels) as the within- and order (placebo then drug and drug then placebo), treatment (placebo and active drug), gender (male and female) and smoking status (smokers and non-smokers) as the between-subject factors. Light reflex variables (latency and amplitude) were analysed with separate 2×4 (treatment×luminance level) repeated measures ANOVAs. The pre-post treatment differences in CFFF, VAS and cardiovascular measures were subjected to paired samples *t* tests.

Results

Resting pupil diameter (5-min PAT) Figure 1 shows the RPD values for each one of the 15 20-s periods for the drug treatment conditions in experiments I, II and III. In all three experiments, RPDs were becoming progressively smaller from the first to the 15th period in all treatment conditions. In experiment I (ketanserin), a 2×2×2×2×15 (order × treatment × gender × smoking status × period) ANOVA showed significant main effects of treatment [$F(1,5)=13.3$, $p<0.015$] and period [$F(14,70)=3.02$, $p<0.001$]. All other main effects and interactions were non-significant (all p values >0.09). Identical analysis in experiment II (buspirone) showed significant treatment [$F(1,5)=7.0$, $p<0.05$] and period [$F(14,70)=2.9$, $p<0.002$] main effects. All other main effects and interactions were not significant (all p values >0.2). Finally, identical analysis in experiment III (propranolol) did not reveal any significant main effects or interactions (all p values >0.3). A 3×2×2×2×15 (treatment group × order × gender × smoking status × period). ANOVA of the placebo data only showed that RPDs in the three treatment groups did not differ in the placebo condition [group main effect and all interactions involving group ($F<1$)].

Light reflex In all three experiments, latency was reduced, and amplitude was increased with increasing light intensity, as evidenced by expected significant main effects of light intensity for these measures [latency; $F_{propranolol}(3,33)=26.7$, $p<0.001$; $F_{ketanserin}(3,33)=19.8$, $p<0.001$; $F_{buspirone}(3,33)=18.1$, $p<0.001$; amplitude: $F_{propranolol}(3,33)=287.0$, $p<0.001$; $F_{ketanserin}(3,33)=102.1$, $p<0.001$; $F_{buspirone}(3,33)=71.3$, $p<0.001$] (Fig. 2).

There was no significant treatment main effect for latency in any experiment (all $p>0.1$). Ketanserin increased light reflex amplitude as evidenced by a significant treatment main effect [$F(1,11)=8.1$, $p=0.016$] in experiment I, while propranolol and buspirone had no effect on this measure [$F(1,11)=1.2$, $p>0.3$, and $F<1$, respectively]. There was no significant treatment by light intensity interaction for any measure in any experiment.

Critical flicker fusion frequency The post-pre treatment differences are shown in Table 2. There were significant treatment effects in experiment I (ketanserin) ($t=3.48$, *df* 11, $p<0.005$) but not in experiments II (buspirone) and III (propranolol; $t=0.15$, *df* 11, $p>0.88$ and $t=-1.27$, *df* 11, $p>0.23$, respectively).

VAS alertness The post-pre treatment differences are shown in Table 2. There were significant treatment effects in experiment I (ketanserin; $t=3.1$, *df* 11, $p<0.01$) but not in experiments II (buspirone) and III (propranolol; $t=-0.34$, *df* 11, $p>0.7$ and $t=-0.04$, *df* 11, $p>0.9$, respectively).

Cardiovascular measures The post–pretreatment differences in heart rate, systolic and diastolic blood pressure are shown in Table 2. Heart rate was significantly reduced in experiment III (propranolol; $t=2.6$, *df* 11, $p<0.025$), while diastolic but not systolic blood pressure was significantly reduced in experiments I (ketanserin) and II (buspirone; $t=3.55$, *df* 11, $p<0.005$; and $t=2.28$ *df*:11, $p<0.05$ respectively).

Discussion

Table 2 shows that in the doses used, all treatments showed some evidence of bioactivity. Ketanserin reduced diastolic blood pressure, consistent with its antihypertensive properties, which are thought to be centrally mediated via the suppression of sympathetic nerve activity (McCall and Schuette 1984; Ramage 1985). Ketanserin reduced RPD and increased the light reflex amplitude, suggesting a role of 5HT2 receptors in mediating pupil size and the light reflex. Two previous reports failed to show an effect of ketanserin on the pupil (Costagliola et al. 1991; Tekat et al. 2001), but the observed ketanserin-induced miosis in our study is in agreement with previously reported miotic effects of the selective 5HT2 antagonists ICI 169,369 and ICI 170,809 (Millson et al. 1991; Millson et al. 1992).

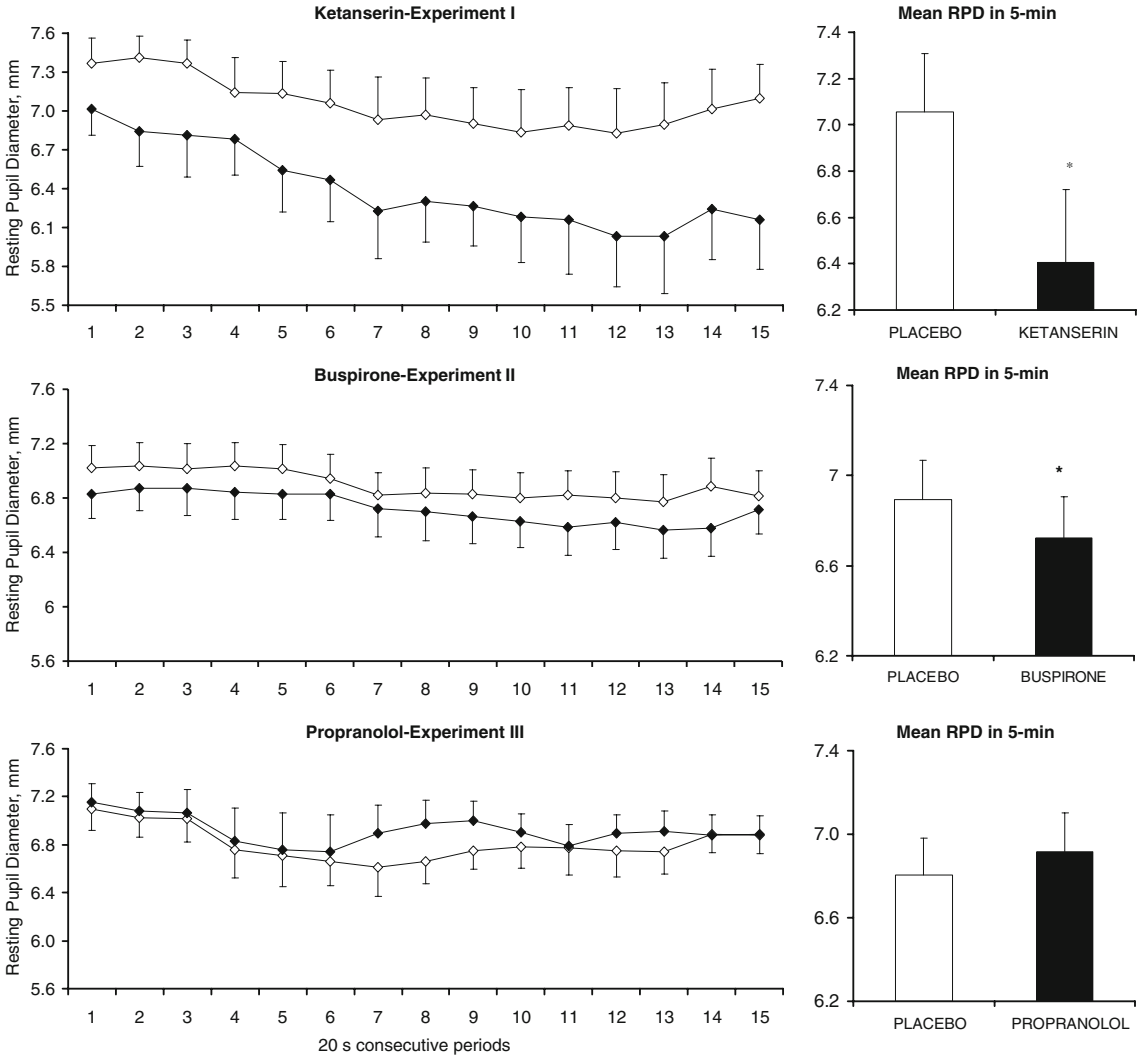

Fig. 1 Resting Pupil Diameter (RPD) values for the 15 20-s periods (*left*) and mean RPD in 5 min (*right*) for the placebo (*white diamonds*) and the drug treatment conditions (*black diamonds*) in the three experiments

Although preclinical studies in rabbits would support a direct involvement of 5-HT2 receptors in controlling pupillary responses (Tobin et al. 1988), local application of ketanserin has been reported to have no effect in human subjects (Costagliola et al. 1993). Therefore, the observed pupillary effects of ketanserin cannot be easily attributed to peripheral effects of the drug on the iris. The reduction in pupil size by ketanserin is consistent with a postulated sympatholytic effect of this drug, but this cannot account for the increase in light reflex amplitude, since the latter is a parasympathetically mediated response (Loewenfeld 1993). This pattern of effects on pupillary size and kinetics is similar to that observed by clonidine (Bitsios et al. 1998a). Clonidine is an a2-adrenoceptor agonist whose effects in

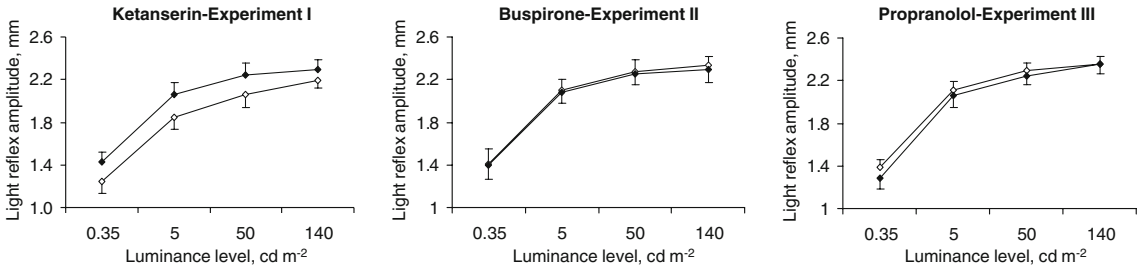

Fig. 2 Light reflex amplitude for the placebo (*white diamonds*) and the drug treatment conditions (*black diamonds*) in the three experiments

Table 2 Critical flicker fusion frequency (CFFF), visual analogue scales (VAS), heart rate and blood pressure post–pretreatment differences [group means(SD)] in each experiment

Test	Experiment I		Experiment II		Experiment III	
	Placebo	Ketanserin	Placebo	Buspirone	Placebo	Propranolol
Δ CFFF	0.13 (0.4)	−1.4 (1.7)*	−0.09 (0.8)	−0.16 (1.7)	−0.2 (0.7)	0.1 (1.5)
Δ VAS alertness	−0.0 (0.06)	−0.13 (0.1)*	−0.0 (0.04)	0.0 (0.06)	−0.0 (0.04)	−0.0 (0.08)
Δ Heart rate	−3.3 (6.6)	−4.7 (5.6)	−7.7 (4.7)	−3.7 (6.5)	−5.3 (6.6)	−12.7 (9.0)*
Δ Systolic BP	−6.3 (7.1)	−10.0 (6.7)	−2.1 (5.0)	−4.6 (5.8)	−7.9 (8.4)	−7.5 (6.9)
Δ Diastolic BP	5.0 (7.4)	−5.0 (8.3)*	1.3 (5.7)	−2.9 (3.3)*	−2.1 (5.4)	0.0 (8.5)

*$p<0.05$

man are generally attributed to a sympatholytic action resulting from stimulation of pre-synaptic inhibitory a2-adrenoceptors located on the cell bodies and dendrites of central noradrenergic neurones; these neurones have an excitatory effect on sympathetic function (Szabadi and Bradshaw 1996). Central noradrenergic neurones located in the locus coeruleus send an inhibitory projection to the Edinger–Westphal nucleus (Szabadi and Bradshaw 1996). Thus, the 'switching off' of the central noradrenergic neurones due to the activation of inhibitory a2-adrenoceptors by clonidine results not only in a decrease in sympathetic outflow but also in the removal of the noradrenergic inhibition of the pupillary light reflex, leading to an increased miotic response of the pupil after a light stimulus. The observed clonidine-like effects of ketanserin on pupil size and kinetics raise the possibility that this drug may attenuate central inhibition of Edinger–Westphal neurones, leading to an enhancement of the effect of light on the pupil. Ketanserin showed a sedative profile in the CFFF and VAS-rated alertness, and it is interesting in this respect that this drug, similarly to clonidine, reduced salivation and alertness and had a clonidine-like profile in the waking electroencephalogram (Reimann et al. 1986). All the above, taken together, strengthen the notion that pupil size is a physiological correlate of central arousal levels and suggest that ketanserin reduces alertness and pupil size, via an action on the 'arousal/pupil control interface', which is likely to be the locus coeruleus and its connections (Szabadi and Bradshaw 1996; Hou et al. 2005, Hou et al. 2007b).

The precise mechanism and neuronal circuitry involved remain to be elucidated, but it is likely that these ketanserin effects were mediated through antagonism at the 5-HT2 receptor for which the drug shows high affinity (p*K*i of 9.5; Branchek et al. 1990). However, a contribution from antagonistic activity at the α_1-adrenoceptor cannot be excluded, since ketanserin has appreciable affinity (p*K*i, 8.0; Israilova et al. 2002) for these receptors as well and such antagonism could have caused miosis by an action on the α_1-rich iris dilator muscle or in the pre-ganglionic sympathetic neurons (Szabadi and Bradshaw 1996). In this context, it is important that local application of ketanserin in the iris did not cause miosis (Costagliola et al. 1993) because it speaks against participation of the α_1-adrenoceptor in the ketanserin-induced miosis observed in the present study. Finally, ketanserin also possesses some anti-histaminergic activity (Dollery 1999), which could have also contributed to its sedative and miotic effects. Indeed, antagonism of H1 histamine receptors by diphenhydramine resulted in sedation and miosis (Hou et al. 2006, 2007a).

Buspirone reduced RPD consistent with previous observations (Fanciullacci et al. 1995; Phillips et al. 1999). In contrast to ketanserin, the buspirone-induced miosis is unlikely to be related to any sedative properties of this drug, since buspirone is not a sedative agent (Newton et al. 1982), and it did not affect the VAS and CFFF measures of alertness used in this study. Buspirone also reduced diastolic blood pressure consistent with previous results (Fanciullacci et al. 1995) and in agreement with the effect of flenoxisan, another 5HT1a agonist. This drug decreased blood pressure in hypertensive patients and normotensive subjects without reflex tachycardia (De Voogd and Prager 1990).

Buspirone exhibits affinity for dopamine D2 receptors; however, this is 16-fold lower than that for 5HT1a receptors, and therefore, while a contribution from the D2 receptors cannot be entirely excluded, it is likely that the observed effects of buspirone were primarily the result of activity at the 5HT1a receptor. 5-HT1a receptor agonists reduce sympathetic outflow (Connor et al. 1991; Ramage and Fozard 1987), an effect that is reversed by 5-HT antagonists (McCall et al. 1987). Therefore, the observed buspirone-induced miosis and hypotension are fully consistent with a central sympatholytic effect of the drug. It is surprising that we could not find any effect of buspirone on the amplitude of the pupillary light reflex, since previous work (Phillips et al. 1999) has shown that the buspirone-induced miosis was light dependent consistent with the involvement of the light reflex mechanism. It is possible that this was a result of the low buspirone dose (10 mg) used in the present study, since the light dependent miosis

in the study of Phillips et al. (1999) was observed with 20 mg but not 5 or 10 mg of buspirone. Buspirone effects on the light reflex are informative in this respect, as buspirone did not affect light reflex amplitude, a parasympathetically mediated response. Thus, the miosis caused by buspirone, at least for the dose used in the present study, is likely to have been mediated by a reduction in sympathetic activity rather than an increase in parasympathetic activity.

The way in which the activation of central 5-HT1a receptors may lead to a sympatholytic effect is not clear, but it could be mediated via central noradrenergic neurons, since the latter receive modulatory input from the serotonergic system (Maeda et al. 1991; Vertes and Kocsis 1994). On the other hand, there is evidence that the periaqueductal grey (PAG) may have an integrative function in the sympathetic and parasympathetic control of the pupil (Klooster and Vrensen 1998) and may also participate in the modulation of the cardiac sympathetic function (Farkas et al. 1998); indeed, stimulation of the 5-HT1a-rich (Brandao et al. 1991; Pazos and Palacios 1985; Pompeiano et al. 1992) and highly responsive to 5HT1a agonists (Behbehani et al. 1993) dorsomedial PAG produces hypotension without tachycardia (Pajolla and de Aguiar Correa 2004; Pajolla et al. 2005) in line with our observations. Therefore, it is also possible that the pupillary and cardiovascular effects of buspirone are mediated via a direct drug effect in the PAG, but in any case, the neuronal circuitry involved and the location of the 5-HT1a receptors within this circuitry remain to be elucidated.

Consistent with its beta-blocking properties, propranolol reduced heart rate, suggesting bioactivity at 40 mg, but it did not affect pupil size and kinetics or any other measure of alertness, suggesting that beta adrenergic receptors are not involved in central regulation of arousal and pupillary functions. These data favour the view that propranolol is not a sedative drug (Currie et al. 1988; Harmer et al. 2001; Ogle et al. 1976; Tyrer and Lader 1974). It is worth noting that no active treatment affected the light reflex latency, which is an index of speed of signal processing by the retina and the integrity of the afferent branch of the reflex (Loewenfeld 1993). This suggests that treatments did not interfere with afferent light signal processes.

These results contribute to our understanding of the central pupillary control, but they also show that pupil size may not be used unequivocally as an index of the level of alertness in the case of drug-induced changes, when the drugs directly influence, by a separate pharmacological action, the pupil control mechanism, e.g. buspirone (this study), pramipexole (Samuels et al. 2006) and diazepam (Bitsios et al. 1998b; Hou et al. 2006; Hou et al. 2007b for a discussion on the relationship between drug-induced sedation and miosis). These considerations aside and given that pharmacological sedation in normal individuals may not be assumed to equate to the EDS seen in patient populations, elucidation of the central pupillary control via drug-induced sedation may be of relevance to sleep disorders characterised by reduced alertness and small pupils (Bitsios et al. 2006; Nikolaou et al. 2008). The practical implication of our study is that the pupillometric assessment of alertness in patients with EDS may be confounded by buspirone treatment for a comorbid anxiety/mood disorder or by ketanserin but not propranolol treatment for comorbid hypertension. Our study also suggests that due to its sedative properties, ketanserin may not be the drug of choice for the treatment of comorbid hypertension in patients suffering from EDS.

In conclusion, 5HT2 but not 5HT1a receptors or beta adrenoceptors appear to be involved in central regulation of arousal together with pupillary functions, although 5HT1a receptors may participate in central pupillary control alone. More detailed dose–response studies are required, but these results help to elucidate the central regulation of pupil size and alertness and they are informative to (a) drug studies using the pupil as a test system of alertness and (b) clinical studies using the pupil as a test system of alertness in patients with sleep disorders, who are concurrently medicated with ketanserin, propranolol or buspirone.

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