

Relationship between sedation and pupillary function: comparison of diazepam and diphenhydramine

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Aims

To examine the relationship between sedation and pupillary function by comparing the effects of diazepam and diphenhydramine on arousal and pupillary activity.

Methods

Fifteen male volunteers participated in three weekly sessions in which they received (i) diazepam 10 mg, (ii) diphenhydramine 75 mg and (iii) placebo, according to a balanced, double-blind protocol. Pupil diameter was measured with infrared pupillometry under four luminance levels. Alertness was assessed by visual analogue scales (VAS) and by critical flicker fusion frequency (CFFF). Blood pressure, heart rate and skin conductance were recorded by conventional methods. Data were analysed with analysis of variance (ANOVA) with multiple comparisons.

Results

There were significant effects of ambient luminance ($F_{3,42} = 305.7$, $P < 0.001$) and treatment condition ($F_{2,28} = 9.0$, $P < 0.01$) on pupil diameter; diphenhydramine caused miosis at all luminance levels ($P < 0.05$). The light reflex response was not affected. Both active drugs reduced the pre-post treatment changes compared with placebo [mean difference from placebo (95% confidence interval)]: in CFFF (Hz), diazepam -0.73 (-1.63 , 0.17), diphenhydramine -1.46 (-2.40 , -0.52); and VAS alertness (mm), diazepam -11.49 (-19.19 , -3.79), diphenhydramine -19.83 (-27.46 , -12.20). There were significant effects of both session ($F_{2,26} = 145.1$, $P < 0.001$) and treatment ($F_{2,26} = 5.5$, $P < 0.01$) on skin conductance; skin conductance was reduced by both drugs ($P < 0.05$).

Conclusions

The miosis by diphenhydramine and the reduction in skin conductance by both drugs may indicate central sympatholytic effects. A lack of a sympatholytic effect of diazepam on the pupil may be due to the masking of the miosis by mydriasis resulting from the inhibition of the parasympathetic output to the iris.

Introduction

It is generally accepted that there is a close relationship between the level of arousal of the central nervous system and pupil diameter: any decrease in arousal is accompanied by a decrease in pupil diameter. In fact, it has been stated that sedative drugs 'all decrease pupillary diameter in proportion to their sedative-hypnotic

effects: the closer the recipient drifts toward somnolence, the smaller the pupils' [1]. This relationship may not hold in the case of drugs which, apart from causing sedation, may directly influence, by a separate pharmacological action, the pupil control mechanism. An example of such a class of drug may be the first-generation antihistamines. Many of these drugs, apart

from causing sedation, presumably due to the blockade of central H1 histamine receptors, also have anticholinergic effects, due to their affinity for muscarinic cholinergic receptors. Thus the sedation caused by these drugs is expected to induce miosis, whereas their atropine-like effect on the iris is predicted to lead to mydriasis. Another class of drug not conforming to the general rule of an association between sedation and miosis are the benzodiazepines. These drugs, although highly sedative, have been reported not to influence pupillary diameter [2–6].

Diphenhydramine is a first-generation antihistamine [7], which has high affinity for H1 histamine receptors [8, 9], without any affinity for H2 histamine receptors [10]. It has been shown that diphenhydramine also possesses anticholinergic effects [7] due to its affinity for muscarinic cholinergic receptors [11, 12]. The drug has marked sedative effects, probably reflecting the blockade of central H1 histamine receptors and, to some extent, central muscarinic cholinergic receptors [13]. Indeed, the drug is available in the UK as an over-the-counter hypnotic [14].

Diazepam is one of the prototypical benzodiazepines with potent anxiolytic and sedative properties [7]. These effects of diazepam are generally attributed to the potentiation of central GABA-mediated inhibition [15]. In the present study, we compared the effects of single doses of diphenhydramine and diazepam on level of arousal and some indices of pupillary function (pupil diameter, pupillary light reflex response) in a group of healthy male volunteers. Nonpupillary autonomic functions (cardiovascular functions, sweat gland activity assessed by measuring skin conductance) were also monitored. Some of these results have been communicated to the British Association for Psychopharmacology [16] and published in a preliminary form [17].

Materials and methods

Subjects

Fifteen healthy drug-free male volunteers, between 18 and 30 years old, with a body mass index (BMI) within the normal range, were recruited for this experiment. All subjects 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 and hearing threshold measurement before inclusion in the study. All subjects denied any history of illicit drug use or excessive consumption of alcohol in general and agreed to remain free of prescribed drugs during the period of the study. Furthermore, they agreed

not to drink any alcohol during the 48 h before each visit. Their caffeine intake was less than 6 cups of coffee or tea per day in general and they were required not to consume any caffeine-containing drinks during the 24 h before each visit and during the experimental sessions. All subjects were nonsmokers or light smokers (i.e. less than 5 cigarettes per day). All subjects reported compliance with these requirements on each test day and stated that they were well rested at the start of each experimental session. The study protocol was approved by the University of Nottingham Medical School Ethics Committee.

Drugs

Diazepam 10 mg, diphenhydramine 75 mg and placebo were prepared in identical capsules and administered orally. The doses of the drugs were selected on the basis of published information and also experience in our laboratory. Diazepam 10 mg consistently evokes a robust sedative effect in laboratory conditions [6, 7, 18]. Although most studies have used smaller single doses (25 mg or 50 mg) of diphenhydramine [19], it has been reported that a higher dose of 75 mg is required to evoke reproducible robust sedative effects [20]. Furthermore, it was found in a previous study in our laboratory that single doses of diazepam 10 mg and diphenhydramine 75 mg were approximately equi-sedative [21].

Tests and apparatus

Measures of alertness The level of arousal was assessed using critical flicker fusion frequency (CFFF), which is defined as the frequency at which a flickering light gives rise to the subjective sensation of a steady light [22, 23]. A Flicker Fusion Monitor, model 1199 (System 696 Ltd, London, UK) was used (for details see [23]). Subjects viewed the stimulus through a 2-mm 'artificial pupil'. Four measurements of the threshold were made, two with increasing frequencies and two with decreasing frequencies, and the mean of the four measurements was taken.

Subjects rated their subjective state of mood by using a computerized version of the visual analogue scales (VAS) developed by Norris [24]. The ratings on the 16 scales were grouped under the headings of 'alertness', 'anxiety' and 'contentedness', based on a factor analysis carried out by Bond and Lader [25].

Pupillary functions Static pupil diameter was measured by a binocular infrared video pupillometer (Procyon Ltd, London, UK), which was used to obtain static pupil diameter readings under four luminance levels (dark-

ness, 6, 91 and 360 cd m⁻²), using a calibrated internal light source within the pupillometer. Measurements were carried out in a darkened room. Pupil diameter was first recorded in darkness and then under each of the increasing luminance levels for 2 s at 4 Hz and stored to disk for off-line analysis [26].

Light reflex response was measured by an infrared binocular television pupillometer (TVC 1015B; Applied Science Laboratories, Waltham, MA, USA). The method used here was similar to that used by Bitsios *et al.* [6, 18, 27]. The sampling rate of the pupillometer was 50 Hz and the detection accuracy was better than 0.05 mm. The light reflex response was evoked in the dark with a light flash (green, 565 nm peak wavelength) of 200 ms duration, delivered via a light emitting diode positioned 1 cm from the cornea of the subject's right eye, providing 'full face' light stimulation [28]; the incident light intensity measured 1 cm from the source was 2050 cd m⁻². Delivery of the stimuli was controlled using Spike 2 software from CED (Cambridge Electronic Design Ltd, Cambridge, UK) via a μ 1401 interface and in-house current controller. The same μ 1401 interface was used to capture the output signal from the pupillometer. The light reflex response was evoked in three blocks of three stimuli separated by 25-s intervals; the blocks were separated by 175-s intervals. For each light reflex response, initial pupil diameter (i.e. diameter of the pupil before the application of the light stimulus) and amplitude (i.e. the difference between the initial and the minimal diameters of a pupillary response to a light flash, mm) were measured [18, 27, 28].

Nonpupillary autonomic functions Blood pressure and heart rate recordings were taken in the sitting position using an electroneroid sphygmomanometer.

Skin conductance recordings were made via two 8-mm diameter silver–silver chloride surface electrodes taped to the terminal phalanges of the first two fingers of the left hand. Skin conductance was monitored via a CED 2502-SA interface connected to the same CED 1401+ computer that was used to control the light reflex stimuli. Skin conductance was measured in three blocks of four 5-s recordings at 25-s intervals in each block; the blocks were separated by 150-s intervals.

Design

Each subject participated in three experimental sessions at weekly intervals (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, cross-over design.

Procedure

After arrival in the laboratory, each subject had a 15-min acclimatization period after which the pretreatment tests (recordings of heart rate, blood pressure, CFFF, VAS and static pupil diameter) were carried out. The testing was completed in 15 min. On completion of pretreatment tests, the subjects ingested two capsules: capsule 1 (diphenhydramine or placebo) immediately, and capsule 2 (diazepam or placebo) 1 h later. One hour after the ingestion of capsule 2, the pretreatment tests were repeated, together with recordings of skin conductance and pupillary light reflex ('post-treatment test'). The time course of the session was based on the pharmacokinetic profile of the drugs used: diphenhydramine has a t_{\max} approximately 2 h following oral administration [29] and diazepam approximately 1 h following oral administration [30].

In the same sessions, eyeblink responses and auditory evoked potentials in response to acoustic stimuli delivered by a headphone were also recorded (to be reported separately).

Data analysis

In the case of static pupil diameter, the pre- and post-treatment data were subjected to separate two-way analysis of variance, the factors being luminance level and treatment condition. In the case of the light reflex response (initial pupil diameter and amplitude) and skin conductance, the post-treatment data and, in the case of all other measures, the pre-post treatment differences were subjected to one-way analysis of variance, with treatment condition as the within-subject factor. When a significant effect of treatment was found, multiple comparisons were made between each active treatment and placebo using Dunnett's test, with an *a priori* criterion of $P < 0.05$ (d.f. = 28; $k = 3$; $t \geq 1.99$).

Results

Measures of alertness

The upper graph in Figure 1 shows the effects of the treatments on CFFF. There was a significant effect of treatment condition on CFFF ($F_{2,28} = 6.0$, $P < 0.01$). Both diazepam and diphenhydramine reduced CFFF: multiple comparisons between active treatments and placebo showed that the effect of diphenhydramine was significant ($P < 0.05$); however, the effect of diazepam fell short of statistical significance. The mean [95% confidence interval (CI)] differences from the placebo condition (Hz) were: diazepam -0.73 (-1.63 , 0.17); diphenhydramine -1.46 (-2.40 , -0.52).

The lower graphs in Figure 1 show the post-treatment changes in the three VAS factors. There was a significant

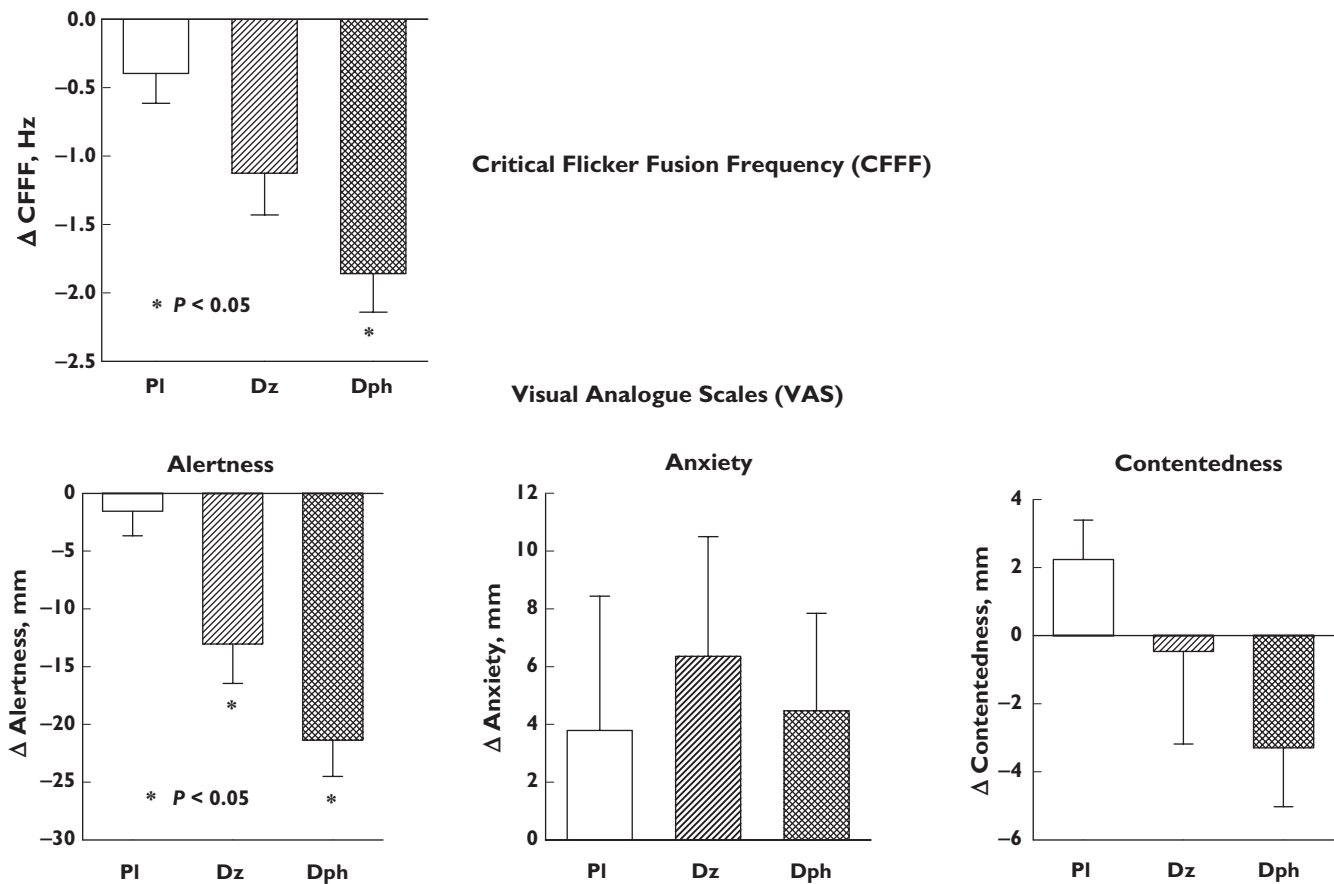


Figure 1

Critical flicker fusion frequency (CFFF) and visual analogue scales (VAS): post-treatment changes in CFFF (top) and VAS (bottom). Columns correspond to mean changes in the parameters in response to treatments, obtained in the group of 15 subjects. Treatments are indicated at the bottom of the graph (PI, placebo; Dz, diazepam; Dph, diphenhydramine). Vertical bars are standard error of the mean (SEM). CFFF, Ordinate, change from pretreatment (Hz). Diphenhydramine reduced CFFF. * $P < 0.05$ (Dunnett's test: comparison with placebo condition). VAS, Ordinate, change from pretreatment (mm). Both diazepam and diphenhydramine reduced alertness. Anxiety and contentedness were not affected. * $P < 0.05$ (Dunnett's test: comparison with placebo condition)

effect of treatment condition on alertness ($F_{2,28} = 10.9$, $P < 0.001$): both diazepam ($P < 0.05$) and diphenhydramine ($P < 0.05$) reduced alertness compared with placebo. The mean (95% CI) differences from the placebo condition (mm) were: diazepam -11.49 (-19.19 , -3.79); diphenhydramine -19.83 (-27.46 , -12.20). There was no significant effect of treatment condition on either anxiety or contentedness.

Pupillary functions

Static pupil diameter in darkness and at three luminance levels, under the three treatment conditions, both before and after treatment, are shown in Figure 2. Before treatment there was a significant effect of luminance ($F_{3,42} = 374.1$, $P < 0.001$), but not of treatment condition. After treatment, there was a significant effect of

both luminance ($F_{3,42} = 305.7$, $P < 0.001$) and treatment condition ($F_{2,28} = 9.0$, $P < 0.01$). However there was no significant treatment condition \times luminance interaction. It is apparent from Figure 3 that diphenhydramine evoked miosis at all luminance levels. Multiple comparisons between the active treatments and placebo showed that this effect was statistically significant ($P < 0.05$). Diazepam had no significant effect on pupil diameter at any luminance level.

Figure 3 shows the initial diameter and amplitude of the light reflex response, evoked by a light stimulus of 2050 cd m^{-2} , under the three post-treatment conditions. There was a significant effect of treatment condition on the initial diameter ($F_{2,28} = 6.2$, $P < 0.01$): multiple comparisons between active treatments and placebo showed that this effect was due to a reduction in pupil diameter

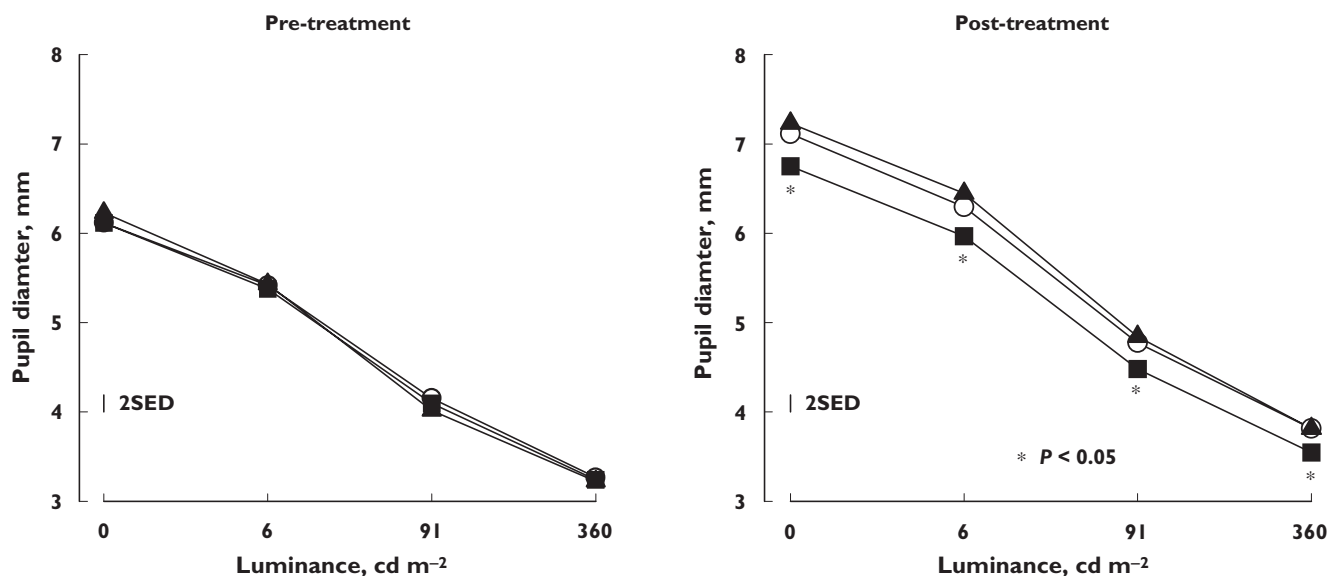


Figure 2

Static pupil diameter: relationship between level of luminance and pupil diameter, pre- and post-treatment. The three treatment conditions are indicated by different symbols (see inset). Ordinate: absolute pupil diameter (mm); abscissa: level of luminance (cd m^{-2}). Each point corresponds to the mean obtained in the group of 15 subjects; the vertical bars represent two standard errors of the difference (2 SED) obtained from the interaction term of the analysis of variance. Diphenhydramine reduced static pupil diameter at all luminance levels, whereas diazepam had no significant effect. $*P < 0.05$ (Dunnett's test: comparison with placebo condition). Placebo (○), diazepam 10 mg (▲), diphenhydramine 75 mg (■)

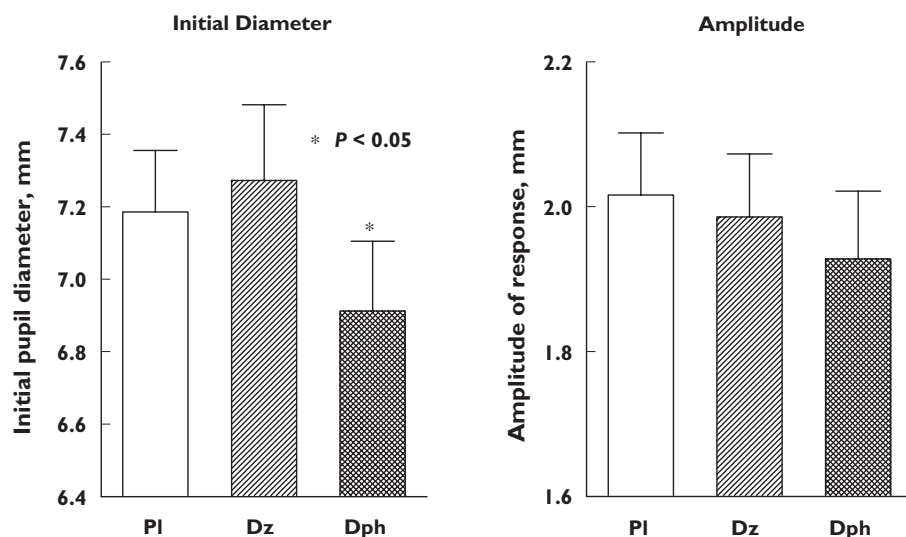


Figure 3

Light reflex response: initial diameter (left) and amplitude (right) evoked by a light stimulus of 2050 cd m^{-2} , under the three post-treatment conditions. Columns correspond to mean changes in the parameters in response to treatments, obtained in the group of 15 subjects. Treatments are indicated at the bottom of the graphs (as above). Vertical bars are SEM. Diphenhydramine reduced initial pupil diameter, whereas there was no treatment effect on amplitude after treatment. $*P < 0.05$ (Dunnett's test: comparison with placebo condition)

evoked by diphenhydramine ($P < 0.05$). There was no significant effect of treatment condition on the amplitude of the light reflex response after treatment.

As the sedation caused by the two drugs was not equivalent in the present experiment, an additional analysis was carried out using the post-treatment change in alertness as covariate. Inclusion of the covariate did not alter the significant effects of treatment ($F_{2,142} = 6.0$, $P < 0.01$) or luminance ($F_{3,142} = 206.7$, $P < 0.01$). There

was no significant treatment \times luminance interaction ($F_{6,142} = 0.5$, $P > 0.2$) and the effect of the covariate was not significant ($F_{1,142} = 1.2$, $P > 0.2$).

Nonpupillary autonomic functions

Table 1 shows pre-post-treatment changes in heart rate, systolic blood pressure, diastolic blood pressure and the post-treatment skin conductance level. There were no significant treatment effects on the cardiovascular mea-

Table 1

Pre-post treatment changes in cardiovascular measures and post-treatment skin conductance values under the three treatment conditions; mean (95% CI)

Measure	Placebo	Diazepam	Diphenhydramine
Systolic BP (mmHg)	0.20 (-5.54, 5.94)	-6.47 (-10.43, 2.51)	-0.27 (-6.86, 7.39)
Diastolic BP(mmHg)	-2.33 (-5.83, 1.16)	-2.47 (-8.45, 3.52)	0.67 (-3.91, 5.24)
Heart rate (bpm)	-3.57 (-7.64, 0.51)	-4.33 (-8.68, 0.02)	-2.77 (-8.03, 2.50)
Skin conductance (μ S)	7.71 (5.60, 9.80)	7.60 (5.03, 10.17)	5.86 (3.39, 8.32)

tures. Visual inspection of the skin conductance data indicated that there was a marked reduction of skin conductance level across sessions, irrespective of the treatments administered. Therefore the data were subjected to a repeated-measures analysis of variance appropriate for a Latin square design, with session as a within-subject factor [31]. This analysis revealed significant effects of session ($F_{2,26} = 145.1$, $P < 0.001$) and treatment ($F_{2,26} = 5.5$, $P < 0.01$). Individual comparisons of the active treatments with placebo indicated that both diazepam and diphenhydramine significantly reduced skin conductance level.

Discussion

Single doses of both diphenhydramine and diazepam showed robust sedative effects as evidenced by a reduction in CFFF following the administration of diphenhydramine, and subjectively rated alertness after treatment with either drug. This observation is in agreement with numerous previous reports demonstrating the sedative effects of diphenhydramine (for example [32–35]) and diazepam (for example [6, 36, 37]). Recent evidence suggests that the sedative effects of both drugs may be mediated by the central histaminergic system originating from the tuberomammillary nucleus of hypothalamus. The tuberomammillary nucleus is one of the most important wakefulness-promoting nuclei of the brain which increases the level of arousal via diffuse ascending fibre system activating excitatory H1 receptors of cortical neurones [38–40]. On the other hand, the tuberomammillary nucleus is under inhibitory control from the GABAergic ventrolateral preoptic nucleus of the hypothalamus, the major sleep-promoting centre [40, 41]. Thus, the sedative effect of diphenhydramine can be attributed to the blockade of excitatory H1 histamine receptors on cortical neurones, whereas the sedative effect of diazepam is likely to reflect the potentiation of the GABAergic inhibition of histaminergic neurones in the tuberomammillary nucleus.

The effects of both drugs were studied on static pupil diameter and the pupillary light reflex response. The diameter of the pupil at any time reflects the balance between the physiologically antagonistic sympathetic (pupil dilatation) and parasympathetic (pupil constriction) influence on the iris. On the other hand, the amplitude of the light reflex response is almost exclusively determined by parasympathetic activity, parasympathomimetic drugs increasing [42, 43] and parasympatholytic drugs decreasing light reflex response amplitude [44].

In the present experiment diphenhydramine caused a reduction in pupil diameter in darkness and at all three luminance levels studied, whereas diazepam had no effect on pupil diameter. The miotic effect of diphenhydramine was also apparent as a reduction in initial pupil diameter recorded prior to the onset of the light reflex response. It is unlikely that the difference in the pupillary effects of the two drugs was due to the difference in the degree of sedation caused by them since the change in pupil diameter in response to treatment remained significant after the influence of sedation had been partialled out. Neither of the drugs had any effect on the amplitude of the light reflex response. This negative observation, however, is informative since it indicates that neither drug affected the parasympathetic innervation of the iris. Thus the miosis caused by diphenhydramine is likely to have been mediated by a reduction in sympathetic activity rather than an increase in parasympathetic activity. Furthermore, there is no evidence of the blockade of muscarinic cholinergic receptors in the iris by diphenhydramine, as was predicted on the basis of the reported anticholinergic effect of the drug.

The effects of the drugs were also studied on skin conductance, an index of sweat gland activity [45, 46]. Eccrine sweat glands receive a sympathetic cholinergic innervation [47]. In the present experiment, both diazepam and diphenhydramine reduced sweat gland activity, as indicated by reductions in skin conductance evoked by both drugs. The reduction in sweat gland

activity may reflect either reduced sympathetic outflow and/or the blockade of muscarinic cholinergic receptors. It is likely that the effect of the drugs is due to a reduction in central sympathetic activity rather than to a peripheral anticholinergic effect, since diazepam has no affinity for muscarinic cholinergic receptors and diphenhydramine failed to affect pupillary light reflex amplitude, an index of parasympathetic activity. There is evidence that the central histaminergic system contributes to sympathetic activity [39, 48, 49]. The sympathetic activating effect of the central histaminergic system is largely mediated by a dense histaminergic projection to the paraventricular nucleus of the hypothalamus [50], a major source of sympathetic outflow [51]. Furthermore, the histaminergic system may also activate the noradrenergic locus coeruleus, another source of sympathetic outflow [52, 53], either directly [54] or indirectly via the paraventricular nucleus [53]. Thus, the central sympatholytic effect of diphenhydramine may reflect the blockade of excitatory H₁ histamine receptors on paraventricular nucleus and locus coeruleus neurones.

A sympatholytic effect of diazepam has been described before, but mainly as antagonism of the effects of experimentally induced increases in sympathetic activity. It has been reported that diazepam prevents sympathetic activation by hypothalamic stimulation in experimental animals [55] and the development of hyper-responsiveness of sweat glands to carbachol at high ambient temperature in human subjects [56]. The central sympatholytic effect of diazepam may reflect the potentiation of GABA on presympathetic neurones in the brain: it has been reported that the paraventricular nucleus receives a GABAergic input from the suprachiasmatic nucleus of the hypothalamus [57] and locus coeruleus [58].

In agreement with previous reports, the sympatholytic effect of diazepam was not apparent on the pupil (see above). It is noteworthy according to an early report that diazepam was effective in reducing sympathetic cardiovascular responses, such as increase in blood pressure and reflex bradycardia in experimental cats while the sympathetic pupillary response, i.e. pupil dilatation, remained unaffected [59]. The reason for the relative resistance to diazepam of the sympathetic outflow of the iris is not clear. A possible explanation may be that diazepam potentiates the GABAergic inhibition not only of the locus coeruleus but also of the preganglionic parasympathetic neurones in the Edinger–Westphal nucleus, which mediate the pupillary light reflex (pupil constriction). Indeed, inhibitory GABA_A receptors have been identified on the Edinger–Westphal nucleus [60]. Thus a reduction in sympathetic pupil dilatation may

be masked by a reduction in parasympathetic pupil constriction.

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