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The effects of clonidine on the fear-inhibited light reflex

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We have shown previously that pupil diameter increases and the amplitude of the pupillary light reflex is reduced when subjects are under threat of an aversive event (electric shock), and that light reflex amplitude correlates negatively with subjective anxiety. Furthermore, we have shown that the threat-induced reduction in light reflex amplitude is sensitive to the effect of the anxiolytic drug diazepam. We have suggested that the 'fear-inhibited light reflex' paradigm could be used as a laboratory model of human anxiety. In the present study, we examined whether a single oral dose (200 μg) of the sedative–sympatholytic drug clonidine would antagonize the effects of threat on the pupillary light reflex. Twelve healthy male volunteers participated in two sessions separated by seven days in which they ingested clonidine 200 mg or placebo in a double-blind, balanced, cross-over design. Light stimuli (0.43 mW/cm^2, 200 msec) were generated by a green (peak wavelength 565 nm) light-emitting diode, and pupil diameter was monitored by computerized binocular infrared television pupillometry in the dark. The light reflex response was recorded during either the anticipation of a shock ('threat' blocks) or periods in which no shocks were anticipated ('safe' blocks). The shock was a single square wave current pulse (1.5 mA, 50 msec) applied to the median nerve at the end of the experiment. Following each 'threat' or 'safe' block, subjects rated their anxiety using visual analogue scales. Two-factor ANOVA (treatment × condition) showed that clonidine treatment antagonized both the threat-induced increase in pupil diameter and the threat-induced reduction in light reflex amplitude. These effects, however, were not threat-specific since clonidine also reduced pupil diameter and enhanced light reflex amplitude in the 'safe' condition. Clonidine also reduced subjective alertness but not subjective anxiety in the 'threat' condition. These findings suggest that the mutual antagonism between clonidine and threat is likely to reflect the opposite effects of the two variables on the central noradrenergic control of pupillary functions, rather than a specific anxiolytic effect.

Key words: anxiety; clonidine; fear; healthy volunteers; light reflex; pupil

Introduction

We have shown previously (Bitsios *et al.*, 1996b) that the threat of an electric shock increases the diameter of the pupil and decreases the amplitude of the light reflex response, compared to periods when the subjects are resting. The reduction in light reflex response amplitude is accompanied by increases in subjectively rated alertness and anxiety, as measured by visual analogue scales (VASs). Furthermore, only light reflex amplitude, but not pupil diameter, is correlated with anxiety, indicating a possible dissociation between the two pupillary measures. We have termed the phenomenon of the attenuation of the light reflex response by threat the 'fear-inhibited light reflex'. We have also shown that the paradigm of the fear-inhibited light reflex is sensitive to the anxiolytic drug diazepam: single doses (5 and 10 mg) of this drug effectively and dose-dependently antagonize the threat-evoked attenuation of light reflex amplitude and increase of subjectively rated anxiety, without affecting the threat-evoked increases in resting pupil diameter and subjectively rated alertness (Bitsios *et al.*, 1998).

In the present study, we examined the effects of the sympatholytic/sedative drug clonidine on the fear-inhibited light reflex. It could be predicted on the basis of the 'noradrenergic model' of anxiety (Redmond, 1981; Libet and Gleason, 1994; Charney *et al.*, 1995) that clonidine would also have some anxiolytic effect. This model postulates that an increase in central noradrenergic activity may underlie pathological anxiety, and clonidine, by blocking inhibitory somatodendritic α_2-adrenoceptors on central noradrenergic neurones, 'switches off' the central noradrenergic system (see Szabadi and Bradshaw, 1996). In agreement with this prediction, it has been shown that clonidine has an antagonistic effect in the fear-potentiated startle reflex paradigm which has been proposed as a laboratory model of anxiety (Davis *et al.*, 1979, 1993). It should be noted, however, that in this paradigm clonidine does not only antagonize the effect of threat, but also suppresses the baseline startle reflex response (Davis *et al.*, 1993; Abduljawad *et al.*, 1997). There is also some evidence for an anxiolytic effect of clonidine in patients suffering from different forms of anxiety disorder (Redmond,

1982; MacDonald *et al.*, 1988; Coupland *et al.*, 1996; Ahmed and Takeshita, 1996).

Some of the results reported here have been communicated to the British Pharmacological Society (Bitsios *et al.*, 1996c).

Materials and methods

Subjects

Twelve healthy male volunteers aged 18–24 years (mean ± SD 19.8 ± 1.7 years) and weighing 54–92 kg (mean ± SD 71.5 ± 9.5 kg) participated in the study. Subjects were all medication-free and were requested to avoid drinking alcohol, coffee and other caffeine-containing beverages for at least 12 h before the experimental session. All of them were occasional caffeine and occasional social alcohol consumers. The instructions given to the subjects prior to the experiment are described in detail under Procedures. They were all tested in the morning (9.00–13.00 hours). The study protocol was approved by the University of Nottingham Medical School Ethics Committee. All volunteers gave their written consent following a verbal explanation of the study and after reading a detailed information sheet.

Drugs

Clonidine hydrochloride 200 mg and placebo were administered orally.

Experimental design

All subjects participated first in a training session and 1 or 2 days later in two experimental sessions separated by seven days, which were associated with clonidine hydrochloride 200 μg or with placebo (for details, see Procedures). Subjects were allocated to drug treatment conditions and experimental sessions according to a double-blind balanced design.

Tests and apparatus

Pupillometry

An infrared binocular television pupillometer (TVP 1015B Applied Science Laboratories, Waltham, MA, USA) was used for the recording of the light reflex in darkness, in previously dark-adapted eyes. The stimuli were light flashes (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; the incident light intensity measured 1 cm from the source was 0.43 mW/cm^2. The recordings took place in a dark, sound-attenuated room and the subjects fixed their gaze on a dim red spot of light positioned approximately 2.5 m in front of them. Stimulus presentation was controlled by a microcomputer, and pupillary measures were digitized and stored on a floppy disk for off-line analysis. The parameters studied were: initial pupil diameter (i.e. diameter of the pupil before the application of the light stimulus); amplitude of light reflex response (i.e. the difference between the initial and minimal pupil diameters); and recovery time of the light reflex (i.e. the time needed for the constricted pupil to recover to 75 percent of its initial diameter).

Cardiovascular measures

Systolic and diastolic blood pressure and heart rate (sitting) were measured with an electroaneroid sphygmomanometer (Bosch, Gojo-Prestige) (for details see Procedures).

Electrical stimulation

A constant current square pulse (1.5 mA, 50 msec) was delivered to the skin overlying the median nerve of the left wrist through disposable silver surface electrodes using a Grass stimulator (SD 9) (for details of shock delivery, see Procedures).

Subjective ratings

The subjects' mood and feelings were self-rated on a battery of 16 visual analogue scales (VASs) (Aitken, 1969; Norris, 1971) on several occasions throughout the session (for details, see Procedures).

Procedures

Training session

Upon their arrival in the laboratory, the subjects received a detailed description of all procedures and a demonstration of all apparatus. Then the subjects underwent a brief training session (application of a few light flashes in the dark to evoke the pupillary light reflex), in order to familiarize them with pupillometry.

Experimental sessions

Figure 1 shows the design of the experimental session. At the beginning of each experimental session, after a 10-min rest period, the subjects' blood pressure (systolic and diastolic) and heart rate were measured and then they ingested the capsule. The subjects entered the adaptation phase 100 min after ingestion of the capsule. During the adaptation phase the subjects first wore red goggles for 15 min in order to adapt to dim red illumination. Following this, the light reflex was elicited in darkness with twelve light flashes, in order to familiarize them with pupillometry (5 min). At the end of the adaptation phase (2 h after treatment), subjects' blood pressure (systolic and diastolic) and heart rate were measured again, the electrodes were applied on the subjects' left wrists, and the main phase was started.

The main phase comprised seven identical consecutive blocks of three light flashes of the same intensity and duration (21 light flashes in total, per session). In the main phase, responses in each block were recorded either during anticipation of an electric shock (THREAT condition) or without anticipation (SAFE condition) (see Fig. 1). The first block was always associated with the SAFE condition ('initial' SAFE block), responses recorded in this block were not entered in the analysis. After recording responses from the initial SAFE block, half of the subjects started with a SAFE block, and the remaining half with a THREAT block. The SAFE and THREAT conditions alternated regularly in the remaining six blocks. Subjects were asked to rate their mood and feelings during the SAFE and THREAT blocks retrospectively, at the end of each SAFE and THREAT block, using the 16 mood and feelings VASs. The interblock interval was 90–120 sec, to allow sufficient time for the completion of the VASs. The main phase lasted approximately 20 min. The experimental session

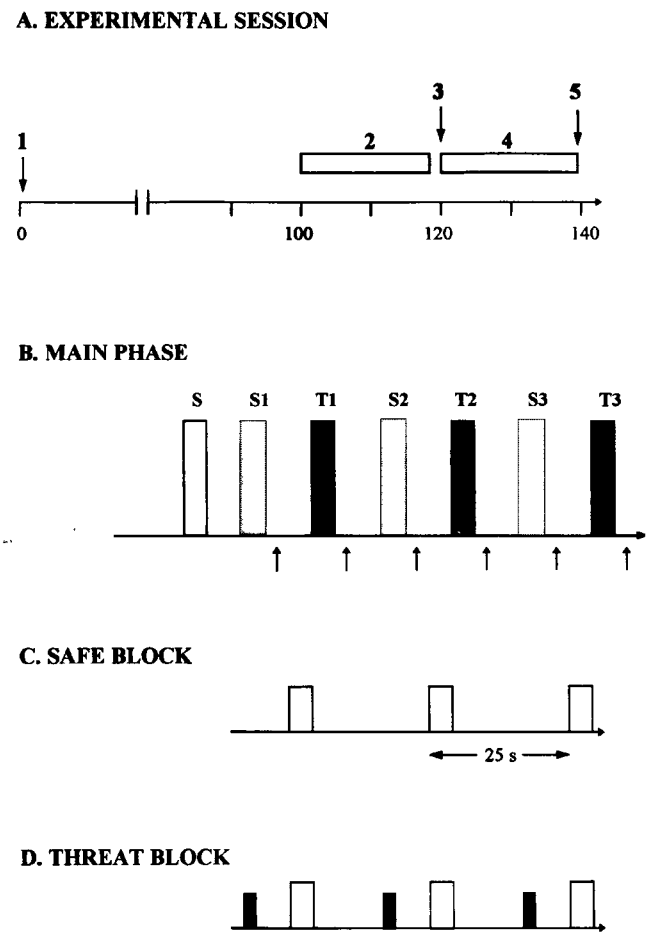

Figure 1 Design of experiment. There were two experimental sessions (A) of identical time-courses. 1: Blood pressure and heart rate measurements; ingestion of capsule; instructions. 2: Adaptation phase; at the end of the phase blood pressure and heart rate measurements. 3: Placement of electrodes; instructions repeated (emphasized in Session 2). 4: Main phase. 5: Shock delivery (Session 1: no shock; Session 2: one shock of 1.5 mA), blood pressure and heart rate measurements. The main phase (B) (see 4, in A, above) consisted of alternating SAFE (S) (light shaded columns) and THREAT (T) (closed columns) blocks. The unnumbered open column indicates the initial safe block. Arrows indicate the administration of visual analogue scales. The SAFE block (C) consisted of three light stimuli (0.43 mW/cm^2, 200 msec) at 25 sec intervals (open columns). The THREAT block (D) consisted of three light stimuli (0.43 mW/cm^2, 200 msec) at 25 sec intervals (open columns) preceded by a warning tone (closed columns) applied 3 sec prior to the light stimulus. See text for details

ended with a debriefing interview after which systolic and diastolic blood pressure and heart rate were measured.

Instructions to subjects

Thirty seconds prior to the onset of each block, the subjects were informed about the nature of the condition with which the block was associated. In the SAFE condition the subjects were told that no electric shocks would be administered. In the THREAT blocks the subjects were instructed to anticipate a total of one to three electric shocks, delivered to their left wrists during the 3 sec elapsing between a 500 msec warning tone and a light flash (see Fig. 1). The subjects did not know the exact number of shocks, or in which THREAT block(s) they would occur. The shocks were described by the experimenter as mildly painful stimuli inducing a short-lived localized unpleasant sensation on the wrist. It was shown previously (Bitsios *et al.*, 1996a), that it was the threat of the shock, rather than the delivery of the shock, which was responsible for the changes in light reflex amplitude. Therefore, no shock was delivered in the first session. To restore threat in the second session it was emphasized to the subjects that 'although shock delivery had been judged unnecessary the first time, one to three electric shocks' as previously instructed, 'would now definitely occur'. In fact only one 1.5 mA shock was delivered at the end of the second session which, as has been shown previously (Bitsios *et al.*, 1996b), is not judged as painful by the subjects.

Data reduction and data analysis

The pupillary measures (initial pupil diameter, light reflex amplitude and 75 percent recovery time) for each block were obtained by averaging the light reflex responses by computer, and taking the measures from the averaged response. The raw values of the VASs (mm) for each item and each subject were weighted by multiplication with their respective factor loading, and the weighted values for each item and subject were then allocated to 'alertness', and 'anxiety' factors, based on a principal component analysis (Bond and Lader, 1974). The mean of the weighted group values for each factor was entered in the statistical analysis.

Data for each pupillary and VAS measure were collapsed across blocks for the two conditions (SAFE, THREAT) and the two treatments. Two-way analysis of variance with treatment (placebo or clonidine) and condition (SAFE or THREAT) as the within-subject factors, with repeated measures on both factors, was used to analyse the pupillary and VAS measures. In the case of a significant interaction, placebo and clonidine treatments were compared under each condition with the least significant difference test (criterion, $p < 0.05$). The relationship between changes in light reflex amplitude and initial pupil diameter (baseline), and the relationship between 75 percent recovery time and light reflex amplitude were analysed by analysis of covariance (treatment × condition with initial diameter or amplitude, respectively, as the covariate).

For the assessment of the effects of treatment on cardiovascular measures, the post- pre-treatment differences were taken for each subject and occasion (i.e. 2 h after ingestion of capsule, completion of experimental session), in every session. Student's *t*-test for paired data was used to compare the post-pre-drug differences in the cardiovascular measures across the two occasions.

Results

Pupillary measures

Initial pupil diameter, amplitude of light reflex response and 75 percent recovery time (group means) for each of the three SAFE and three THREAT occasions, and the collapsed data

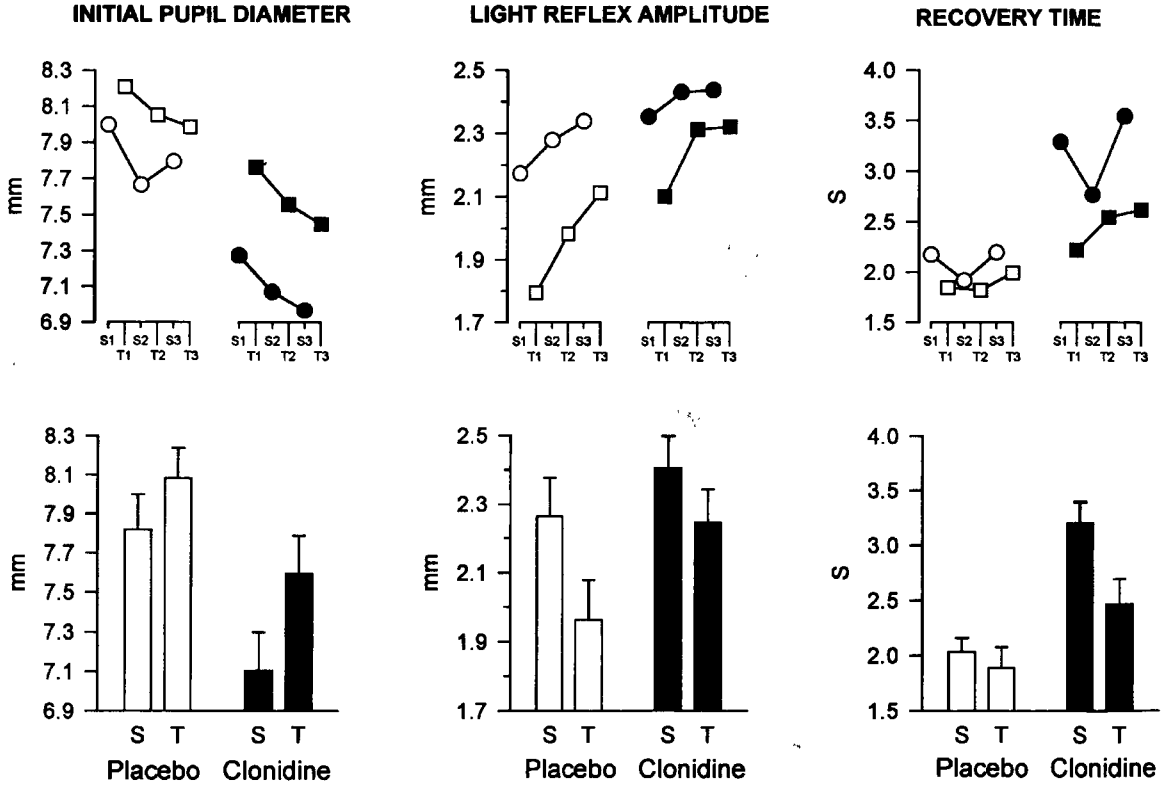

Figure 2 Initial pupil diameter (left panel), light reflex amplitude (middle panel) and 75 percent recovery time (right panel) recorded in main phase of the two experimental sessions. Top panels: ordinate: pupil diameter (mm), light reflex amplitude (mm) and recovery time (sec); abscissa: sequential blocks (S: SAFE; T: THREAT). The data points are mean values obtained in the group of 12 subjects. Open symbols: placebo; closed symbols: clonidine 200 μg; circles: SAFE condition; squares: THREAT condition. Initial pupil diameter increased in the THREAT blocks and slightly declined in the course of the session. Light reflex amplitude decreased in the THREAT blocks and slightly increased in the course of the session. Recovery time was shortened in the THREAT blocks but it did not change consistently in the course of the session. Bottom panels: ordinate: pupil diameter (mm); amplitude of light reflex response (mm); 75 percent recovery time (sec). The bars represent data averaged across the three blocks for the two conditions (S: SAFE; T: THREAT) and the two treatments (open columns: placebo; closed columns: clonidine 200 μg). The height of each bar corresponds to the mean value ($\pm$SEM) obtained in the group of 12 subjects. Treatment with clonidine reduced initial pupil diameter, increased light reflex amplitude and prolonged recovery time in both the SAFE and the THREAT conditions. Treatment with clonidine antagonized the effects of threat on all pupillary measures. See text for statistical analysis

(group means) averaged across the blocks for the two conditions and the two treatments, are displayed in Fig 2.

It can be seen that initial pupil diameter was greater under the THREAT than under the SAFE condition, under both treatments. It can also be seen that there was a general reduction of initial diameter in both conditions when the subjects received clonidine treatment. Analysis of variance of the initial pupil diameter data revealed significant main effects of treatment [$F(1,11)=21.02$; $p<0.001$] and condition [$F(1,11)=24.03$; $p<0.001$] but no significant interaction [$F(1,11)=4.74$; $p>0.05$].

There was a progressive increase in amplitudes during the experimental session with both treatments, but in each block amplitude was smaller under the THREAT than under the SAFE condition, under both treatments. However, it can also be seen that the amplitude in the clonidine/THREAT condition was shifted to placebo/SAFE levels. Analysis of variance of the amplitude data revealed significant main effects of treatment [$F(1,11)=39.7$; $p<0.001$] and condition [$F(1,11)=35.5$; $p<0.001$] as well as significant treatment $\times$ condition interaction [$F(1,11)=11.1$; $p<0.001$]. *Post-hoc* comparisons with the least significant difference test showed that clonidine treatment was associated with a significant increase in the response amplitude under both the SAFE and the THREAT conditions. In order to compare the magnitude of the effects of threat in the presence of placebo and clonidine, the differences were calculated between each subject's light reflex response amplitudes in the SAFE and THREAT conditions and recovery times following placebo and clonidine treatments. These differences were compared between the placebo and clonidine treatments with Student's *t*-test (paired comparisons). This analysis showed that the effect of threat on the response amplitude was significantly smaller in the presence of clonidine than in the presence of placebo ($t=3.33$; df$=11$; $p<0.01$).

It can be seen that the 75 percent recovery time was shorter under the THREAT than under the SAFE condition, under both treatments. It can also be seen that there was a general prolongation of recovery time in both conditions when the subjects received clonidine treatment. Analysis of variance of the recovery time data revealed significant treatment [$F(1,11)=36.7$; $p<0.001$] but not condition main effects [$F(1,11)=4.3$; $p>0.05$] and significant treatment $\times$ condition interaction [$F(1,11)=6.6$; $p<0.05$]. *Post-hoc* comparisons with the least significant

difference test showed that clonidine treatment was associated with a significant increase in the recovery time under both the SAFE and the THREAT conditions.

In order to address the possibility that the changes in light reflex amplitude were secondary to changes in initial pupil diameter and the changes in 75 percent recovery time were secondary to changes in amplitude, analyses of covariance of the amplitude data (treatment × condition with initial diameter as the covariate) and of the recovery time data (treatment × condition with amplitude as the covariate) were carried out. These analyses did not reveal any significant effect of the regression in the case of treatment [$F(1,10)=1.5$; $p>0.1$], condition ($F<1$) or the interaction ($F<1$) for the amplitude data, nor a significant effect of the regression in the case of treatment or interaction ($F<1$) for the recovery time data.

Subjective ratings

The results obtained with the mood/feelings VASs for 'anxiety' and 'alertness', are shown in Fig. 3. It is apparent that both 'anxiety' and 'alertness' were greater under the THREAT than under the SAFE condition, and that clonidine treatment caused a general decline of 'alertness' in both the SAFE and THREAT conditions, compared to that recorded after placebo. Indeed, analysis of variance of the 'alertness' data revealed significant treatment [$F(1,11)=10.8$; $p<0.01$] and condition [$F(1,11)=19.3$; $p<0.001$] main effects without significant treatment × condition interaction ($F<1$). Analysis of variance of the 'anxiety' data revealed only a significant condition main effect [$F(1,11)=29.3$; $p<0.001$]. There was no significant effect of treatment [$F(1,11)=1.5$; $p>0.1$] or treatment × condition interaction ($F<1$).

Effects of treatment on cardiovascular measures

The effects of treatment on heart rate, systolic and diastolic blood pressure, are shown in Fig. 4. It can be seen that 2 h after ingestion, clonidine significantly reduced heart rate, systolic and diastolic blood pressures, compared to placebo treatment ($p<0.001$), as revealed by Student's t-test for paired data, and that these measures were still reduced at the end of the session ($p<0.001$).

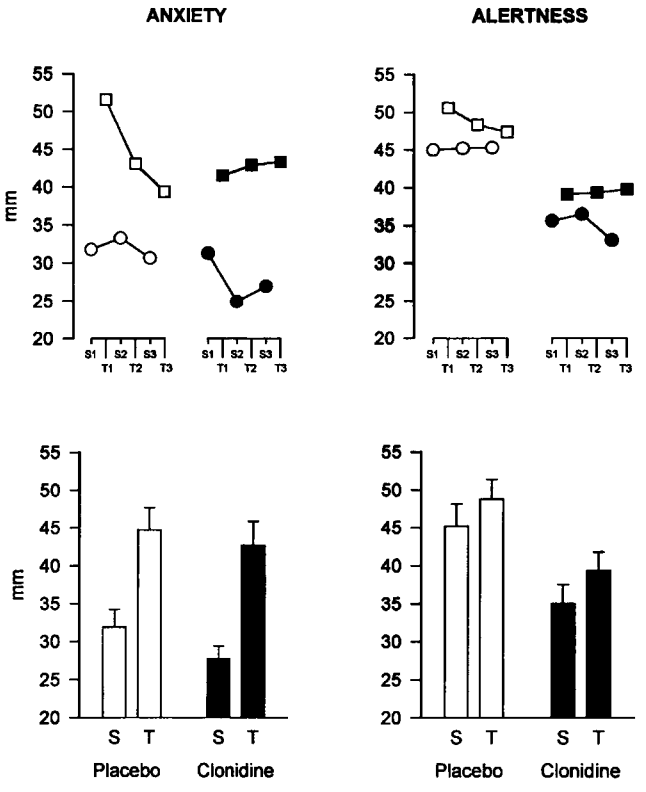

Figure 3 Subjective ratings on a battery of visual analogue scales of 'anxiety' (left panel), and 'alertness' (right panel) recorded in main phase of the two experimental sessions. Top panels: ordinate: score (mm); abscissa: sequential blocks (S: SAFE; T: THREAT). The data points are mean values obtained in the group of 12 subjects. Open symbols: placebo; closed symbols: clonidine 200 μg; circles: SAFE condition; squares: THREAT condition. Both anxiety and alertness increased in the THREAT blocks and did not change consistently in the course of the session. Bottom panels: ordinate, score (mm); the bars represent data averaged across the three blocks for the two conditions (S: SAFE; T: THREAT) and the two treatments (open columns: placebo; closed columns: clonidine 200 μg). The height of each bar corresponds to the mean score (±SEM) obtained in the group of 12 subjects. Treatment with clonidine had no effect on anxiety but reduced alertness in both the SAFE and THREAT conditions. See text for statistical analysis

Discussion

It is generally accepted that the predominant action of clonidine in man is the stimulation of inhibitory α_2-adrenoceptors ('autoreceptors') on central noradrenergic neurones, resulting in the sedative and sympatholytic effects of the drug (Szabadi and Bradshaw, 1996). Recent evidence indicates that the autoreceptors on central noradrenergic neurones belong to the α_{2A}-subtype (MacDonald *et al.*, 1997). Clonidine also interacts with α_2-adrenoceptors ('heteroreceptors') on cells innervated by noradrenergic neurones, both in the central nervous system and the periphery. Furthermore, the drug possesses some partial agonistic activity at α_1-adrenoceptors, and recent evidence suggests that some of its effects may be mediated via imidazoline receptors (Szabadi and Bradshaw, 1996).

Since the central noradrenergic system has been implicated in anxiety (Redmond, 1981), it was predicted that clonidine will have some anxiolytic effect. Indeed, it has been shown that clonidine has some therapeutic potential both in panic disorder (Hoehn-Saric *et al.*, 1981; Uhde *et al.*, 1989) and generalized anxiety disorder (Hoehn-Saric *et al.*, 1981). However, the use of clonidine has not become established for the treatment of anxiety disorders, partly due to sedation complicating any relief of anxiety, and partly due to difficulties in sustaining an anxiolytic effect in the course of long-term medication.

In an attempt to investigate the anxiolytic effect of clonidine, in the present study we examined its effect on the fear-inhibited light reflex, a potential laboratory model of human anxiety. Clonidine had effects on all the pupillary measures under both the SAFE and THREAT conditions. Clonidine reduced systolic and diastolic blood pressure and heart rate 2 h after oral administration, and these effects were

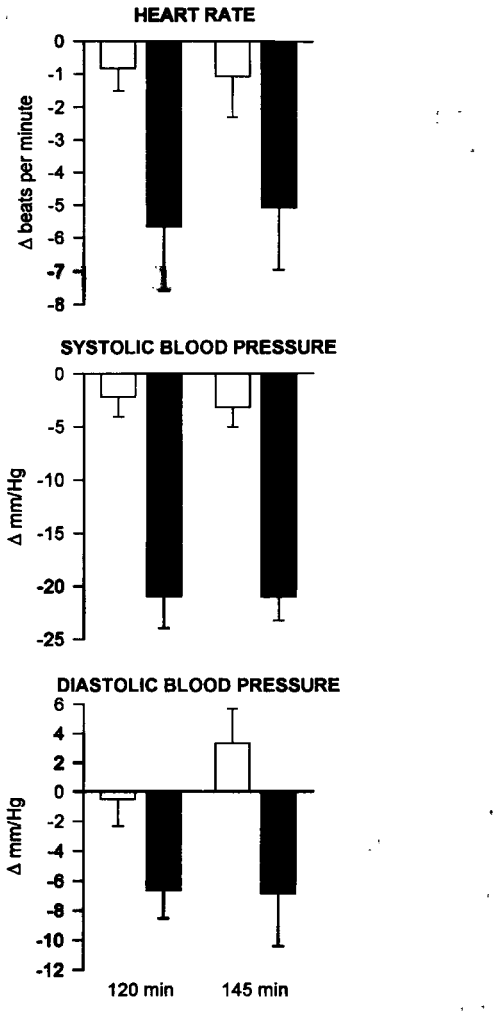

Figure 4 Change in heart rate (beats min), systolic and diastolic blood pressure (mm Hg), 120 min after treatment and at the end of the experimental session (145 min after treatment); compared to pre-treatment. Each bar represents the mean scores (±SEM) obtained in the group of 12 subjects. Treatment with clonidine reduced all cardiovascular measures 2 h after treatment ($p<0.001$) and this reduction was still present at the end of the session ($p<0.001$)

still present at the end of the session. The observation of characteristic cardiovascular effects of clonidine (Reid, 1981; Szabadi and Bradshaw, 1996) in our experiment indicates that the single oral dose used resulted in pharmacodynamically effective tissue levels of the drug.

Clonidine reduced resting (initial) pupil diameter, and prolonged the recovery time and enhanced the amplitude of the light reflex response. This effect was apparent both in the SAFE and the THREAT conditions. Some of these effects of clonidine on the pupil have been reported previously (miotic effect: Clifford *et al.*, 1982, 1989; Fanciullacci *et al.*, 1988; Bitsios *et al.*, 1996b; prolongation of the recovery time of the light reflex response: Morley *et al.*, 1991). The prolongation of the recovery time is likely to reflect the decrease in the sympathetic influence on the iris, whereas the increase of light reflex amplitude would be consistent with enhanced parasympathetic activity (Smith, 1992; Loewenfeld, 1993a). Indeed, the reduction in resting pupil diameter may reflect both sympathetic inhibition and parasympathetic activation.

The prolongation of the recovery time of the light reflex response is the strongest indicator of a sympatholytic effect of clonidine. However, it should be noted that recovery time is closely linked to amplitude: larger responses take longer to recover (Theofilopoulos *et al.*, 1995). Indeed, in the present study, clonidine both increased amplitude and prolonged recovery time. It is, however, unlikely that the prolongation of the recovery time is merely a reflection of the increase in amplitude since the analysis of covariance indicated the independence of the changes in the two measures. Furthermore, in a previous study, clonidine prolonged recovery time without causing much change in light reflex amplitude (Morley *et al.*, 1991). The decrease in resting pupil diameter in the presence of clonidine is also consistent with a sympatholytic effect, although it could equally reflect a parasympathomimetic effect. However, a parasympathomimetic effect of clonidine on the pupillary light reflex is likely to be secondary to the central sympatholytic effect of the drug (see later).

The decrease in the sympathetic influence on the iris, in the presence of clonidine, is consistent with the well-documented central sympatholytic effect of the drug which is generally attributed to the stimulation of inhibitory α_2-adrenoceptors on the cell bodies of noradrenergic neurones in the brain stem (Szabadi and Bradshaw, 1996). Noradrenergic neurones in the A1 and A5 areas of the brainstem project to the preganglionic sympathetic neurones in the intermedio-lateral columns of the spinal cord (Nieuwenhuys, 1985). Furthermore, the hypothalamus, a major sympathetic relay station, is influenced by noradrenergic neurones, both directly from the A1 and A5 areas (Nieuwenhuys, 1985) and indirectly from the locus coeruleus via the cerebral cortex (Foote and Aston-Jones, 1995). Thus, the stimulation of inhibitory somatodendritic autoreceptors on central noradrenergic neurones by clonidine 'switches off' the noradrenergic influence on sympathetic activity, leading to a sympatholytic effect. The role of α_2-adrenoceptors in the pupillary effects of clonidine is confirmed by observations that these effects can be reversed by α_2-adrenoceptor antagonists (i.e. miosis by idazoxan: Clifford *et al.*, 1982; prolongation of the recovery time of the light reflex response by yohimbine: Morley *et al.*, 1991). It is of interest that MK-467, a peripherally acting α_2-adrenoceptor antagonist, failed to reverse clonidine-induced miosis (Warren *et al.*, 1991), indicating that the pupillary effects of clonidine are mediated by central α_2-adrenoceptors rather than by peripheral release-modulating α_2-adrenoceptors located on sympathetic nerve terminals in the iris.

Clonidine also had an apparent parasympathomimetic effect on the pupil indicated by the enhancement of light reflex amplitude. Furthermore, the reduction in resting pupil size would also be consistent with an increase in the parasympathetic influence on the iris. We would like to propose that the increase in parasympathetic activity may be secondary to the inhibition of central noradrenergic neurones via the activation of inhibitory α_2-adrenoceptors (somato-dendritic autoreceptors) on these neurones which exert a tonic inhibitory influence on the preganglionic parasympathetic pupillomotor neurones of the Edinger–Westphal nucleus (for details see Szabadi and

Bradshaw, 1996). Thus, the suppression of activity of central noradrenergic neurones results in both sympathetic inhibition (see earlier) and parasympathetic disinhibition (i.e. a parasympathomimetic effect) in the iris. Futhermore, the suppression of the activity of locus coeruleus neurones due to α_2-adrenoceptor stimulation is likely to underlie the sedation caused by clonidine (Heal, 1990; Foote and Aston-Jones, 1995).

As sedation due to a variety of causes (e.g. physiological somnolence, narcolepsy, anaesthesia) may be accompanied by miosis (Loewenfeld, 1993b), the possibility arises that the miosis evoked by clonidine was secondary to sedation. Indeed, there was evidence for a reduction in alertness following the administration of clonidine in the present experiment (see later). It should be noted, however, that sedation and miosis do not always occur together, and thus the mechanisms underlying the two phenomena can be dissociated. Indeed, the independence of the sedative and pupillary effects of clonidine can be demonstrated in experimental animals (i.e. rats and cats) in whom clonidine-evoked sedation is accompanied by mydriasis, rather than miosis (see Szabadi and Bradshaw, 1996). Furthermore, sedation and miosis can be dissociated in the case of diazepam in human subjects in whom the drug causes sedation but no change in resting pupil size (Bitsios *et al.*, 1996c).

In agreement with our previous report (Bitsios *et al.*, 1996b), the threat of an electric shock increased resting pupil diameter and decreased the amplitude of the pupillary light reflex response. Thus, the effects of threat on the two pupillary measures were opposite to those of clonidine. When threat was applied in the presence of clonidine, the effects of the two variables were mutually antagonistic, conforming to the model of 'physiological antagonism' (Ariëns, 1970). It is of interest that this physiological antagonism also operated in the case of the recovery time of the light reflex response, threat antagonizing the prolongation of the recovery time by clonidine. Threat, on its own, i.e. in the absence of clonidine, had no effect on recovery time. This may reflect the fact that recovery time cannot be shortened below a minimal period of time required for redilatation. However, it should be noted that high ambient temperature, which is known to be associated with sympathetic activation (Bini *et al.*, 1980), has been reported to lead to a shortening of the recovery time of the light reflex response (Leung *et al.*, 1992; Mortlock *et al.*, 1996), suggesting that recovery time can be shortened in untreated subjects provided that the sympathetic activation is strong enough.

The physiological antagonism between threat and clonidine raises the possibility that the two variables, although they may affect different mechanisms in the brain, are likely to affect pupillary control via common final neuronal pathways. There is evidence that the amygdala plays a crucial role in mediating the effect of threat in another experimental paradigm of anxiety, the fear-potentiated acoustic startle reflex response (Davis *et al.*, 1979, 1993), and it is likely that the amygdala also plays a pivotal role in the 'fear-inhibited light reflex' response (Bitsios *et al.*, 1996b). The amygdala has well-established outputs to the hypothalamus (LeDoux, 1988; Davis, 1992). The activation of the hypothalamus could result in increased sympathetic output, leading to an increase in resting pupil size and shortening of the recovery time of the light reflex response. In addition, hypothalamic activation could result in the enhancement of the supranuclear inhibition of the Edinger–Westphal nucleus, leading to a reduction in the amplitude of the light reflex response. As discussed above, clonidine leads to a decrease in sympathetic, and an increase in parasympathetic, outflow to the iris, by 'switching off' the noradrenergic neurones of the brainstem.

The threat of an electric shock, in agreement with our previous report (Bitsios *et al.*, 1996b), increased both subjectively rated anxiety and alertness. Clonidine reduced alertness, and there was mutual antagonism between the effects of threat and clonidine on this measure. The effect of clonidine on alertness is in agreement with previous reports (Glue and Nutt, 1988; Morley *et al.*, 1991). The sedative effect of clonidine is likely to reflect the 'switching off' of the alerting effect of the ascending input from the locus coeruleus to the cerebral cortex (Foote and Aston-Jones, 1995) via the activation of inhibitory α_2-adrenoceptors on the cell bodies of noradrenergic neurones (Heal, 1990). Thus, the sedative and sympatholytic effects of clonidine are brought about by the same mechanism, i.e. the activation of α_2-adrenoceptors on central noradrenergic neurones.

In contrast to the reduction in alertness ratings, clonidine had no significant effect on subjectively rated anxiety, and it also failed to antagonize the increase in anxiety evoked by the threat of an electric shock. Thus, while clonidine and threat had symmetrically opposite effects on the pupillary measures, this symmetry did not extend to subjective anxiety, indicating a possible dissociation between the antagonism of the inhibition of the light reflex response by threat and an anxiolytic effect. In this context, it is of interest to compare the effects of clonidine with those of the classical anxiolytic drug diazepam, both on pupillary measures and the ratings of alertness and anxiety. We have found that single doses (5 and 10 mg) of diazepam effectively antagonized both the inhibition of the light reflex response by threat and the threat-evoked increase in subjective anxiety, without affecting either resting (initial) pupil diameter or subjectively rated alertness (Bitsios *et al.*, 1998). It is an interesting possibility that diazepam and clonidine may act at different sites in the neuronal pathways mediating the effect of threat: diazepam at the level of the amygdala and clonidine at the level of central sympathetic regulation. Indeed, it has been shown that the profile of action of clonidine is different from that of diazepam in an animal test of anxiolytic activity (Handley and Mithani, 1984).

The findings of the present experiment may have bearing on the interpretation of the mode of action of clonidine in pathological anxiety states. As clonidine failed to affect subjective anxiety in the present study, it is possible that the sedative and sympatholytic effects of the drug may contribute to its limited clinical effectiveness in anxiety disorders. Indeed, these effects may also partly explain the effectiveness of clonidine in the treatment of the opiate-withdrawal syndrome which is characterized by high arousal and autonomic overactivity (Charney *et al.*, 1981).

In conclusion, the present results confirm the sedative effect of clonidine which was reflected both in a decrease in subjectively rated alertness and resting pupil size. Although the effects of threat and clonidine were mutually antagonistic on the amplitude of the pupillary light reflex response, this

physiological interaction was not accompanied by a reduction in subjectively rated anxiety. These findings suggest that the mutual antagonism between clonidine and threat is likely to reflect the opposite effects of the two variables on the central noradrenergic control of pupillary functions, rather than a specific anxiolytic effect of clonidine. Therefore, caution is needed when interpreting the antagonism of the fear-inhibited light reflex by a drug as evidence for anxiolysis if the drug itself has some intrinsic agonistic activity in the test system.

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