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Effects of clonidine and yohimbine on the pupillary light reflex and carbachol-evoked sweating in healthy volunteers

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The effects of single doses of clonidine hydrochloride (200 μg), yohimbine hydrochloride (22 mg), a combination of the two treatments, and placebo, on some autonomic functions were studied in healthy volunteers using a double-blind crossover design. Clonidine prolonged the recovery time of the light reflex, lowered systolic blood pressure and reduced subjectively rated alertness; these effects were reversed by yohimbine. Responsiveness of sweat glands to carbachol was not affected by the treatments.

Keywords clonidine yohimbine pupillary light reflex carbachol-evoked sweating α_2-adrenoceptors

Introduction

It has been hypothesized that central α_2-adrenoceptors may play an important role in regulating central sympathetic outflow (Charney & Heninger, 1985). This hypothesis has been invoked to explain the antihypertensive action of clonidine (Kobinger, 1978), and the finding that yohimbine can evoke subjective experiences which may be attributable to sympathetic activation (Charney & Heninger, 1985). We report here the effects of clonidine and yohimbine on two indices of sympathetic activation, the recovery time of the pupillary light reflex (Smith, 1988) and the sensitivity of eccrine sweat glands to carbachol (Banjar *et al.*, 1987). We also measured changes in cardiovascular parameters and subjectively rated mood, which are known to be influenced by clonidine and yohimbine (Charney & Heninger, 1985).

Methods

Eight drug-free healthy male volunteers (19–23 years) participated in four sessions at weekly intervals, in which they received the following treatments according to a double-blind balanced crossover design: (1) yohimbine hydrochloride (22 mg); (2) clonidine hydrochloride (200 μg); (3) yohimbine hydrochloride (22 mg) + clonidine hydrochloride (200 μg); (4) lactose placebo. Subjects gave written informed consent; the study was approved by the local Ethics Committee.

Tests

Pupil diameter was monitored in the dark using infrared television pupillometry; miotic responses to twelve 200 ms light stimuli (peak wavelength 565 nm; intensity range 0.09–180 mcd) were measured (Bakes *et al.*, 1990). Sweat gland activity evoked by eight intradermal injections of carbachol chloride (0.05 ml; concentration range 0.3–1000 μM) were measured on the volar surfaces of the forearms using the plastic paint impression method (Banjar *et al.*, 1987). Systolic and diastolic blood pressures were measured in the supine position with an electro-aneroid sphygmomanometer; pulse rate was measured conventionally. Sixteen visual analogue scales (Bond & Lader, 1974) were used to assess subjective mood.

Procedure

At the beginning of each session, the subjects rested for 30 min, followed by measurement of blood pressure, heart rate and mood state. They then ingested the capsules, and rested for 150 min. Then, following accommodation to red light (30 min), pupillometric measurements and assessment of sweat gland responsiveness to carbachol were carried out. Finally, blood pressure, heart rate, and subjective ratings were repeated.

Analysis

The following data were analyzed: resting pupil diameter; latency, amplitude and 75% recovery time of response to the light stimuli (Bakes *et al.*, 1990); number of sweat glands activated by each concentration of carbachol (Banjar *et al.*, 1987); pre-/post-treatment changes in heart rate, systolic and diastolic blood pressures and scores on the 'alertness', 'contentedness' and 'calmness' factors derived from the sixteen visual-analogue scales

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(Bond & Lader, 1974). Each measure was subjected to repeated-measures analysis of variance, followed, in the case of a significant main effect of treatment, by individual comparisons using Dunnett's test.

Results

Pupillometry

Yohimbine produced a small increase in resting pupil diameter (Table 1).

The latency, amplitude and 75% recovery time of the miotic responses were linearly related to the logarithm of the stimulus intensity (placebo condition: $r = -0.94$ [latency], +0.99 [amplitude], +0.95 [75% recovery time]) (Figure 1). There was a significant main effect of light intensity on each parameter ($P < 0.001$). Latency and amplitude showed no significant main effect of treatment condition and no significant interaction effect ($P > 0.1$). 75% recovery time showed a significant main effect of treatment condition ($P < 0.05$) and a significant interaction effect ($P < 0.05$). Individual comparisons between treatments showed that clonidine significantly increased 75% recovery time ($P < 0.05$); co-administration of yohimbine and clonidine reversed this effect ($P < 0.05$).

Responsiveness of sweat glands (Table 1)

There was a significant main effect of the concentration of carbachol ($P < 0.001$), but no significant main effect of treatment and no significant interaction effect ($P > 0.1$).

Cardiovascular measures (Table 1)

Systolic blood pressure showed a significant main effect of treatment ($P < 0.02$); individual comparisons indicated

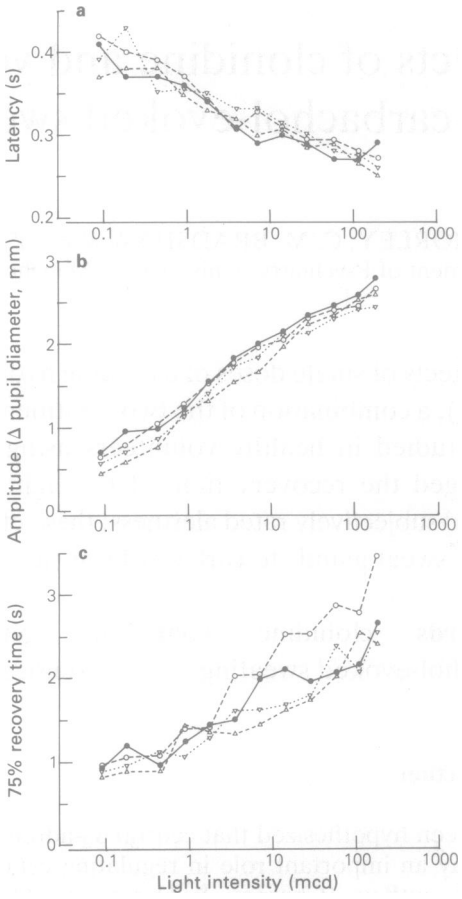

Figure 1 Relationship between parameters of the light reflex and the intensity of the light stimulus. *Ordinates*: a, latency (s); b, amplitude (change in pupil diameter, mm); c, 75% recovery time (s). *Abscissa*: light intensity (mcd, logarithmic scale). Points are mean data ($n = 8$); symbols indicate treatment conditions: • placebo; △ yohimbine hydrochloride (22 mg); ○ clonidine hydrochloride (200 μg); ▽ yohimbine hydrochloride (22 mg) + clonidine hydrochloride (200 μg).

Table 1 Resting pupil diameter, heart rate (HR), systolic and diastolic blood pressures (BP), subjective mood ratings and sweat gland responses to intradermally injected carbachol (mean ± s.e. mean ($n = 8$)).

	Placebo	*Yohimbine*	*Clonidine*	*Yohimbine + clonidine*
Resting pupil diameter (mm)	7.1 ± 0.2	7.4 ± 0.2*	6.9 ± 0.2	7.2 ± 0.2
Change in HR (beats min^{-1})	−12.5 ± 3.0	−4.3 ± 3.8	−11.0 ± 1.7	−7.3 ± 1.7
Change in systolic BP (mm Hg)	−2.3 ± 3.3	+6.5 ± 4.4	−14.0 ± 4.0**	−3.0 ± 5.0
Change in diastolic BP (mm Hg)	+2.3 ± 3.3	+6.3 ± 3.9	−0.3 ± 3.9	+1.0 ± 2.7
Change in 'alertness' (cm)	+0.3 ± 0.2	+0.6 ± 0.3	−1.1 ± 0.3***	+0.1 ± 0.3
Change in 'contentedness' (cm)	−0.1 ± 0.1	+0.1 ± 0.2	+0.6 ± 0.2	+0.1 ± 0.2
Change in 'calmness' (cm)	−0.4 ± 0.4	+0.3 ± 0.2	+0.6 ± 0.3	−0.2 ± 0.1
Response of sweat glands to carbachol (number of glands activated)				
0.3 μM	41.3 ± 12.7	36.0 ± 8.2	24.5 ± 4.9	43.0 ± 5.1
1 μM	48.5 ± 14.6	42.9 ± 8.0	54.1 ± 14.0	49.1 ± 4.8
3 μM	66.6 ± 17.6	51.5 ± 13.4	50.1 ± 14.2	62.6 ± 11.4
10 μM	71.9 ± 23.9	73.9 ± 14.4	69.0 ± 21.1	94.5 ± 20.5
30 μM	87.6 ± 30.6	80.8 ± 13.9	105.6 ± 34.1	111.0 ± 21.9
100 μM	145.5 ± 57.3	143.4 ± 28.0	142.3 ± 45.5	140.5 ± 33.9
300 μM	144.1 ± 37.4	157.4 ± 27.1	172.4 ± 45.1	183.9 ± 27.3
1000 μM	291.6 ± 57.4	225.6 ± 40.4	192.0 ± 48.4	222.6 ± 40.1

Significance of difference from placebo: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

that clonidine significantly reduced systolic blood pressure ($P < 0.01$), none of the other treatments having significant effects. There were no significant effects of treatment on diastolic blood pressure or heart rate ($P > 0.1$).

Subjective ratings (Table 1)

There was a significant effect of treatment on the 'alertness' factor ($P < 0.001$); individual comparisons revealed significant differences between the placebo and clonidine conditions ($P < 0.001$) and between the yohimbine + clonidine and the clonidine conditions ($P < 0.05$). There were no significant treatment effects on the 'calmness' and 'contentedness' factors.

Discussion

The latency and amplitude of the miotic response to light are determined primarily by activity in the parasympathetic reflex arc, whereas the recovery time of the response is determined partly by cessation of parasympathetic reflex activity, and partly by active redilatation brought about by the sympathetic innervation of the radial muscle (Smith, 1988). The effects of clonidine and yohimbine on the light reflex seen here are thus consistent with an interaction with the sympathetic drive to the iris: clonidine prolonged the 75% recovery time, and this effect was reversed by yohimbine.

Eccrine sweat glands are innervated by cholinergic sympathetic nerves, and their responsiveness to intradermally injected cholinomimetics is dependent upon the level of activity in the innervating fibres (see Banjar *et al.*, 1987). Since responsiveness of sweat glands to carbachol was not affected by either clonidine or yohimbine, the present results do not provide evidence for a role of central α_2-adrenoceptors in regulating sudomotor sympathetic outflow.

The significant reduction of systolic blood pressure by clonidine is consistent with previous reports (e.g. Kooner *et al.*, 1989), and is compatible with an interaction of clonidine with central and/or peripheral α_2-adrenoceptors (Kobinger, 1978).

The reduction of subjectively-rated 'alertness' by clonidine is consistent with its known sedative properties (Kooner *et al.*, 1989). The attenuation of this effect by yohimbine is consistent with an involvement of central α_2-adrenoceptors in clonidine's sedative action (Kooner *et al.*, 1989).

It has been proposed that yohimbine acts via blockade of central α_2-adrenoceptors to promote sympathetic outflow; this has led to the suggestion that acute treatment with yohimbine might serve as a model anxiety state and that clonidine might have clinically useful anxiolytic properties (see Charney & Heninger, 1985). However, anxious patients show some features that do not fit readily with this model. Firstly, we have observed that the amplitude of the light reflex is reduced in anxious patients, whereas the recovery time does not differ between the anxious and normal subjects (Bakes *et al.*, 1990). Secondly, the responsiveness of sweat glands to carbachol is enhanced in anxious patients (Maple *et al.*, 1982). These effects were not mimicked by yohimbine in the present experiment; moreover the changes in autonomic functions produced by clonidine were not simply the reverse of those seen in anxiety states: clonidine had no effect on miotic response amplitude, but did prolong the recovery time, and it failed to suppress the responsiveness of sweat glands to carbachol. It therefore seems unlikely that the autonomic changes seen in anxiety states simply reflect an alteration of central α_2-adrenoceptor function (cf. Charney & Heninger, 1985).

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