The haemodynamic and hormonal responses after clonidine occur independently of sedation in essential hypertension

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1 To investigate the contribution of sedation to the cardiovascular effects induced by clonidine, we studied patients with essential hypertension on two separate occasions when similar levels of sedation were induced by clonidine and nitrazepam.

2 After clonidine, there was a fall in blood pressure, heart rate, digital skin vascular resistance and plasma noradrenaline which is consistent with its ability to reduce sympathetic outflow. Dissociation of the circulatory/neurohormonal and sedative responses after clonidine indicated that sedation alone is not an important factor in the blood pressure lowering effect of clonidine.

3 The absence of a fall in blood pressure, heart rate, digital skin vascular resistance and plasma noradrenaline after nitrazepam further suggest that sedation did not influence the hypotensive response in essential hypertension.

Keywords clonidine sedation haemodynamic hormonal sympatholytic nitrazepam

Introduction

Evidence from previous studies suggests that sleep lowers blood pressure by a reduction in peripheral vascular resistance due to a reduction in central sympathetic nervous activity (Bristow *et al.*, 1969; Khatri & Ferris, 1969). Since clonidine, a centrally acting sympatholytic agent, induces sedation (Kleinlogel *et al.*, 1975; Putkonen *et al.*, 1977) and lowers blood pressure and heart rate, it has been suggested that sedation alone may contribute to its hypotensive action. To investigate this, we studied the haemodynamic, hormonal and sedative responses to clonidine in patients with essential hypertension. To determine the significance and specificity of these responses to clonidine, additional studies were performed on the same patients after inducing a similar level of sedation using the benzodiazepine, nitrazepam.

Methods

Six patients with essential hypertension were studied (mean age 54 years, range 34 to 64 years, two male and four females). Patients were studied after an overnight fast, and all medication was withdrawn at least 3 days before investigation; previous medication usually consisted of β -adrenoceptor blockers and diuretics. Studies were performed in a quiet, well lit temperature controlled room, with patients supine; reading was not permitted. Studies with clonidine and nitrazepam were performed at least 3 days apart.

Blood pressure and heart rate

Non-invasive measurements of blood pressure and heart rate were made at 5 min intervals using an automated sphygmomanometer (Sentron).

Digital skin blood flow

Digital (index finger) skin blood flow was measured continuously with a laser Doppler flow meter (Periflux Model PF2b, Perimed Ltd., Sweden). A red laser light produced by the instrument is led to the skin surface by an optical fibre to the probe held in place by a plastic probe holder. The light penetrates the skin and is repeatedly reflected and refracted and gradually absorbed. The multiple scattering produces a volume of isotopic illumination in front of the probe head (hemisphere with a radius of approximately 1 mm) and the red cells traversing this volume are struck by light partly reflecting it. Some of the light is altered in frequency (Doppler shift) by the red cells moving in the microvasculature and returned to the instrument by efferent optical fibres arranged in parallel with the afferent fibre carrying the light of the instrument. Dual efferent light guides are used to reduce noise resulting from variations in the light signal. The frequency shift of the light is detected by photodetectors, which produces a voltage signal directly proportional to the quantity of blood flow (velocity and number of red cells) in the microvasculature of superficial skin (Nilsson *et al.*, 1980; Tooke *et al.*, 1983). The signal was recorded on a chart recorder (BBC SE120, Coventry, U.K.).

Skin temperatures

Skin temperatures were measured by thermistors (Panlab) at forehead, chest, abdomen, palmar aspect of left index finger, and the plantar surface of the first toe.

Sedation

Sedation was scored on a 0 to 100 mm visual bipolar analogue scale, ranging from 'wide awake' to 'fully asleep', at 15 min intervals.

Blood collection and analysis

Blood samples were collected from an indwelling venous canula at the antecubital fossa before and at 15 min intervals after clonidine and 30 min after nitrazepam. Tubes were stored on ice until centrifugation at 4°C for separation of plasma.

Plasma renin activity Blood (5 ml) was collected in chilled tubes containing ethylene-diaminetetraacetate (potassium salt) and plasma stored at -20°C until assay. Plasma renin activity was measured by radioimmunoassay of angiotensin I generated from its endogenous substrate in the

presence of angiotensinase and converting enzyme inhibitors. Angiotensin I was measured by the modified method of Boyd *et al.* (1969). The intra-assay coefficient of variation was 4% and the inter-assay coefficient of variation was 7%.

Plasma aldosterone Aldosterone was extracted from plasma into DCM (dichloromethane, Fisons SLR grade, redistilled). The extracts were decanted and evaporated to dryness. Residues were taken up in 400 µl 0.1% BSA (bovine serum albumin, Sigma) dissolved in a phosphate buffer (50 mmol l⁻¹, pH 7.4). Duplicate 100 µl aliquots were dispensed into radioimmunoassay tubes. Standard curves were prepared in duplicate over the range 5–100 pg/tube of aldosterone. Two standard 200 µl aldosterone antibody and of [¹²⁵I]-aldosterone label, containing about 4000 counts min⁻¹ were added to all tubes. The final antibody dilution was 1:140 000. After overnight incubation at 4°C, separation of bound from free steroid was achieved by adding 200 µl dextran coated charcoal suspension in phosphate buffer comprising 0.6% activated neutralised charcoal, 0.1% each of swine skin gelatin and Dextran C (MW 60,000 to 90,000) and 0.01% sodium azide (all Sigma). After centrifugation at 2000 g for 15 min at 4°C the supernatants were discarded and radioactivity in the charcoal pellets was counted in a Packard Multi Prias 2 gamma counter with an efficiency of 70%.

Plasma noradrenaline and adrenaline Blood (5 ml) was collected in chilled tubes containing 20 µl ethyleneglycol bis(aminoethylester)tetraacetate (0.095% w/v), and plasma stored at -70°C until the assay. Catecholamines were extracted from plasma by the solvent extraction using the technique employed by Smedes *et al.* (1982). Separation was achieved by h.p.l.c. on a 5 µm 22 cm by 0.46 cm ODS column (RP 18, Brownlee labs) using 15% (v/v) acetonitrile in 0.05 M phosphate acetate buffer pH 3.0, containing sodium dodecyl sulphate (120 mg l⁻¹) as mobile phase. Catecholamines were detected by electrochemical detection (ESA Coulochem 5100 A) with the conditioning cell (5021) set at +0.40 V and the analytical cell (5011) at -0.10 V for detector 1 and -0.35 V for detector 2. The interassay coefficient of variation for noradrenaline was 6.6% and for adrenaline was 11.0%. The detection limit when using 1 ml of plasma was 0.1 nmol l⁻¹.

Drugs

Clonidine was administered intravenously (2 µg kg⁻¹) over 10 min with an infusion pump.

Single oral dose of nitrazepam (10 mg) was administered with 20 ml of water. This dose was selected following pilot studies to induce a level of sedation comparable with that after clonidine.

Data analysis

Results are presented as mean values $\pm$ s.e. mean. Repeated measures analysis of variance (GLM procedure, SAS Institute, Carey, NC, USA) was used in the analysis of data. If the null hypothesis was rejected, Scheffe's comparisons were performed on means before and after each drug to characterise further significant differences. $P < 0.05$ was considered significant.

Results

Sedation (Figure 1)

Sedation score was increased after both clonidine and nitrazepam. Maximum sedation occurred at 30 min after clonidine (3 ± 3 to 67 ± 7 mm, $P < 0.05$) and reverted to 8 ± 6 mm at 120 min (NS). A higher level of sedation occurred after nitrazepam ($P < 0.05$). Maximum sedation occurred at 30 min (14 ± 6 mm to 88 ± 5 mm at 30 min, $P < 0.05$) after which it reverted slowly to 40 ± 9 mm at 120 min ($P < 0.05$).

Blood pressure (Figure 1)

There was a rapid fall in blood pressure after clonidine reaching near maximal reduction at 20 min ($174 \pm 8/94 \pm 3$ to $134 \pm 7/77 \pm 4$ mmHg, $P < 0.05$), and was sustained for at least 2 h of observation ($P < 0.05$). Blood pressure did not fall after nitrazepam (NS).

Heart rate

Heart rate fell during initial recumbency on both study days. After clonidine there was a further fall in heart rate (70 ± 4 to 61 ± 4 beats min^{-1} , $P < 0.05$) which did not occur after nitrazepam (65 ± 4 to 63 ± 4 beats min^{-1} , NS).

Digital skin blood flow and vascular resistance (Figure 2)

Digital skin (index finger) blood flow increased after clonidine ($P < 0.05$ from 10 min to 120 min) and was accompanied by a reciprocal fall in digital vascular resistance ($P < 0.05$, from 10 min to 120 min). After nitrazepam digital skin blood flow and vascular resistance were unchanged.

Figure 1 Sedation score and blood pressure responses to clonidine ($\square$) and nitrazepam ($\blacksquare$) in patients with essential hypertension.

Skin temperatures (Figure 3)

After clonidine there was a rise in digital skin (index finger) and toe temperatures (both $P < 0.05$, from 20 min to 120 min). After nitrazepam digital skin and toe temperatures were unchanged. Skin temperatures were unchanged after both drugs at all other measured sites.

Plasma noradrenaline (Figure 4)

Plasma noradrenaline fell after clonidine ($P < 0.05$, from 15 min to 120 min) but not significantly after nitrazepam (NS).

Plasma renin activity (Figure 5)

There was no significant change in plasma renin activity after clonidine or nitrazepam.

Figure 2 Effects of clonidine (□) and nitrazepam (■) on digital skin blood flow (laser Doppler) and digital skin vascular resistance in patients with essential hypertension.

Plasma aldosterone (Figure 5)

Plasma aldosterone was unchanged after clonidine and nitrazepam (both NS).

Discussion

Sedation was increased after both clonidine and nitrazepam. Although time to peak sedation was similar after both agents, sedation was more marked after nitrazepam compared to clonidine. The increase in sedation after clonidine (Kleinlogel *et al.*, 1975; Putkonen *et al.*, 1977) and benzodiazepenes (Oswald *et al.*, 1975; Whitsett *et al.*, 1974) is thought to be exerted by an action of both agents on the noradrenergic cell system of the locus coeruleus complex, and may account

Figure 3 Digital skin and toe temperature responses to clonidine (□) and nitrazepam (■) in patients with essential hypertension.

Figure 4 Effects of clonidine (□) and nitrazepam (■) on plasma noradrenaline in patients with essential hypertension.

Figure 5 Plasma renin and aldosterone responses to clonidine (□) and nitrazepam (■) in patients with essential hypertension.

for clonidine induced reduction of REM (paradoxical) sleep time in normal subjects (Maling *et al.*, 1979). In this study a pronounced fall in blood pressure occurred after clonidine. The reduction in blood pressure was sustained for at least 2 h during observation, although sedation had returned to basal levels. The dissociation between the hypotensive and the sedative effects of clonidine in this study suggests that sedation alone is not an important factor in the ability of clonidine to lower blood pressure. There was no significant fall in blood pressure after nitrazepam, despite a higher level of sedation compared with clonidine. Although our results are at variance with the findings of Hossman *et al.* (1981), who reported a fall in blood pressure and noradrenaline after 20 mg of nitrazepam in normotensive subjects, there are two important differences between the studies. In our study, subjects with established hypertension were investigated, and

may respond differently to various pressor and depressor stimuli compared with normal subjects for a variety of reasons, including increased sympathetic nervous activity and/or vascular reactivity. Secondly, the dose of nitrazepam selected in our study was half that used by Hossman *et al.* (1981). Data from previous studies suggests that the effects of benzodiazepines are dose related. Blood pressure does not fall after oral (Knapp *et al.*, 1977; Elliot *et al.*, 1970; Zweifler *et al.*, 1983) and intravenous (Katz *et al.*, 1967; McClish, 1966) doses (5–10 mg diazepam) of benzodiazepines causing sedation alone. At higher doses (30–120 mg diazepam) of benzodiazepines however, a variable decrease in blood pressure has been reported (Rao *et al.*, 1972; Elliot *et al.*, 1970). It should be noted however that even severe intoxication with benzodiazepines does not result in a major fall in blood pressure (Greenblatt & Shader, 1974; Matthew *et al.*, 1972).

There was a fall in heart rate after clonidine but not after nitrazepam. The decrease in heart rate after clonidine is likely to be related to an effect on medullary centres in the brain stem increasing vagal reflex activity (Robson *et al.*, 1969). The lack of change in heart rate after nitrazepam is in keeping with the inability of benzodiazepines to alter cardiovascular reflexes at lower doses (Antonaccio *et al.*, 1978).

Measurements of digital skin blood flow were made in our study as it provides a sensitive index of sympathetic vasoconstrictor tone (Pickering, 1933). After clonidine, there was an increase in digital skin blood flow and a reciprocal fall in digital skin vascular resistance in patients with essential hypertension. Skin temperatures increased after clonidine in the finger and toe alone. Vasodilatation in digital skin vessels after clonidine is due to the withdrawal of sympathetic activity (Kooner *et al.*, 1988) and in our patients was accompanied by a fall in plasma noradrenaline. There was a highly significant correlation between the reduction in digital skin vascular resistance and mean blood pressure ($r = 0.78$, $P < 0.01$) (Figure 6) after clonidine in our patients, which is further consistent with its ability to withdraw central sympathetic outflow. The dissociation of sedation from the circulatory and plasma noradrenaline responses after clonidine suggest that these effects are exerted independently of each other. There was no fall in digital skin vascular resistance or plasma noradrenaline after nitrazepam despite a higher level of sedation, and is further consistent with its inability to reduce sympathetic nervous activity in our patients. Renin release is dependent on a number of factors which include sympathetic

Figure 6 Correlation of percentage change in mean blood pressure and percentage change in laser Doppler (digital skin) resistance after a) clonidine ($r = 0.78$, $P < 0.05$) and b) nitrazepam ($r = 0.34$, $P < 0.2$) in patients with essential hypertension.

activation through β -adrenoceptors and afferent arteriolar dilatation secondary to reduced renal perfusion pressure (Keeton & Cambell, 1981). After clonidine, despite a reduction in sympathetic activity, there was no change in plasma renin activity. Whether the reduction in plasma renin activity mediated by reduced sympathetic activity after clonidine was counteracted by renin release secondary to afferent arteriolar dilatation following hypotension and presumably decreased renal perfusion pressure is not certain. However, suppression of the renin/angiotensin system does not appear to participate in the blood pressure lowering effect of clonidine in our patients. Plasma aldosterone also did not change significantly after clonidine or nitrazepam.

We conclude that in our patients with essential hypertension, clonidine lowered blood pressure, heart rate, digital skin vascular resistance and plasma noradrenaline due to a reduction in central sympathetic nervous activity. Dissociation of these responses from sedation indicate that sedation alone is not important in the blood pressure lowering effect of clonidine. The absence of a fall in blood pressure, heart rate, digital skin vascular resistance and plasma noradrenaline after nitrazepam further suggest that sedation does not influence the hypotensive response in essential hypertension.

CJM is a Wellcome Senior Lecturer in Medicine.

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