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EFFECT OF β-ADRENERGIC RECEPTOR BLOCKADE WITH PROPRANOLOL ON THE RESPONSE OF PLASMA CATECHOLAMINES AND RENIN ACTIVITY TO UPRIGHT TILTING IN NORMAL SUBJECTS

R. VANDONGEN, L. DAVIDSON, L.J. BEILIN & A.E. BARDEN

University Department of Medicine,
Royal Perth Hospital, Perth, Western Australia 6001

1 Relationships between plasma catecholamines (measured as noradrenaline and adrenaline) and plasma renin activity (PRA) were examined at rest and during passive head-up tilting for 30 min in nine normal subjects, before and after treatment with propranolol 160 mg daily for 7 days.

2 Noradrenaline (NA) and adrenaline (A) increased substantially after tilting for 15 min. There were no changes in PRA. After 30 min tilting, NA remained elevated, whereas A had returned to resting levels. A significant increase in PRA was apparent at 30 min. Pulse rate and diastolic blood pressure increased progressively during tilting. Systolic pressure did not change.

3 Treatment with propranolol reduced pulse rate and systolic blood pressure at rest and during tilting. Resting catecholamine concentrations and the response of NA to tilting were unaffected. In contrast, treatment prolonged the A response leading to significantly higher levels after 30 min tilting. Propranolol reduced PRA in six of the nine subjects and prevented the increase with tilting observed before treatment.

Introduction

Adaptive responses to orthostasis are characterized by increased activity of the sympathetic nervous (Mathias *et al.*, 1975; Hörtnagl *et al.*, 1977; Robertson *et al.*, 1979) and renin-angiotensin system (Oparil *et al.*, 1970; Esler & Nestel, 1973; Davies & Slater, 1976; Robertson *et al.*, 1979), which contribute to the maintenance of systemic blood pressure. The increase in plasma renin levels may be secondary to stimulation of renal β-adrenoreceptors by circulating catecholamines (Vandongen, Peart & Boyd, 1973; Johnson, Shier & Barger, 1979) and is substantially reduced by β-adrenoreceptor blocking drugs (Davies & Slater, 1976).

Several reports have indicated that the level of catecholamines in plasma is influenced by concurrent administration of β-adrenoceptor blocking drugs (Irving *et al.*, 1974; Brecht *et al.*, 1976; Rahn *et al.*, 1978; Distler *et al.*, 1978). Consequences of β-adrenoceptor blockade which may affect plasma catecholamine concentration include alterations in central and baroreceptor mediated sympathetic discharge, or interference with the neuronal release and disposal of catecholamines. It is conceivable, therefore, that the attenuation of haemodynamic and renin responses to upright tilting by β-adrenoceptor blocking drugs is due to a net reduction in plasma catecholamine concentration. In this study we have examined the cardiovascular, catecholamine and renin responses to tilting in normal subjects before and after treatment with propranolol given orally for 7 days.

Methods

Nine healthy males aged 21–38 years from the laboratory and clinical staff participated in these studies. The subjects were fully informed as to the nature of the investigation and the protocol was approved by the Hospital's Ethics Committee on Human Experimentations. No attempt was made to standardize sodium intake. The subjects were non-smokers, took no drugs, and were asked to eat their customary breakfast but avoid coffee before reporting to the laboratory between 09.00–10.00 h. An indwelling scalp vein needle was inserted into a forearm vein and the subjects rested horizontally on a

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manually adjustable tilt table. Thirty minutes later, pulse rate was recorded and blood pressure measured with a standard mercury sphygmomanometer. Blood samples were drawn into tubes kept on ice for assay of PRA and catecholamines. The tubes were immediately centrifuged at 4°C and the plasma stored at −20°C until assay within 4 weeks. A recent report has indicated that forearm venous catecholamine concentrations are representative of those elsewhere in the vascular system (Watson *et al.*, 1979). The subjects were then tilted head up within 1 min to 75°. Three subjects experienced immediate faintness and the tilt was reduced to 50–60° with alleviation of their symptoms. The data obtained from these subjects was included in the study. Pulse rate and blood pressure were measured again and further blood samples taken after 15 and 30 min of continuous tilting. The subjects were then instructed to take 160 mg of propranolol daily in four divided doses for 7 days. The above protocol was repeated with an additional blood sample for measurement of propranolol levels.

PRA was measured by radioimmunoassay of angiotensin 1 generated from endogenous substrate at 37°C and pH 7.4 in the presence of EDTA (0.003M) dimercaprol (0.0014mM) and 8-hydroxy quinoline (0.0035M). The interassay coefficient of variation of this assay was 9.8% with a lower limit of sensitivity of 0.04 pmol/ml.

Plasma NA and A were measured by the single isotope radioenzymatic method described by Peuler & Johnson (1977). This method was modified by incorporating into the assay a calibration curve (range 0–100 pg) for NA and A, in place of internal standards added to each plasma sample. A further modification was the use of chloroform : ethanol : ethylamine as the solvent system for thin-layer chromatography. The interassay coefficient of variation is 10.9% for NA and 16.9% for A, based on eight aliquots of the same plasma sample assayed over a period of 5 weeks. The lower limit of sensitivity of the assay is 0.10 nmol/l for NA and A. Plasma propranolol was measured in eight subjects by high performance liquid chromatography using the method of Dusei & Hackett (1979). Values given are means ± s.e.mean and statistical analysis was performed by Student's *t*-test for paired data.

Results

Heart rate increased substantially during head-up tilting at 15 and 30 min in the nine normal subjects tested ($P<0.001$) (Figure 1). Although treatment with propranolol 160 mg daily for 7 days lowered heart rate, a smaller but nevertheless significant increase was observed during tilting at 15 and at 30 min ($P<0.05$). Resting systolic blood pressure was reduced from 120±3 to 110±1.7 mmHg (mean ± s.e.mean) after propranolol treatment (Figure 1). No change in diastolic pressure was observed. Following tilting, systolic pressure remained constant whereas diastolic pressure increased at 15 ($P<0.05$) and 30 min ($P<0.01$). Similar increases in diastolic pressure occurred after propranolol treatment ($P<0.01$).

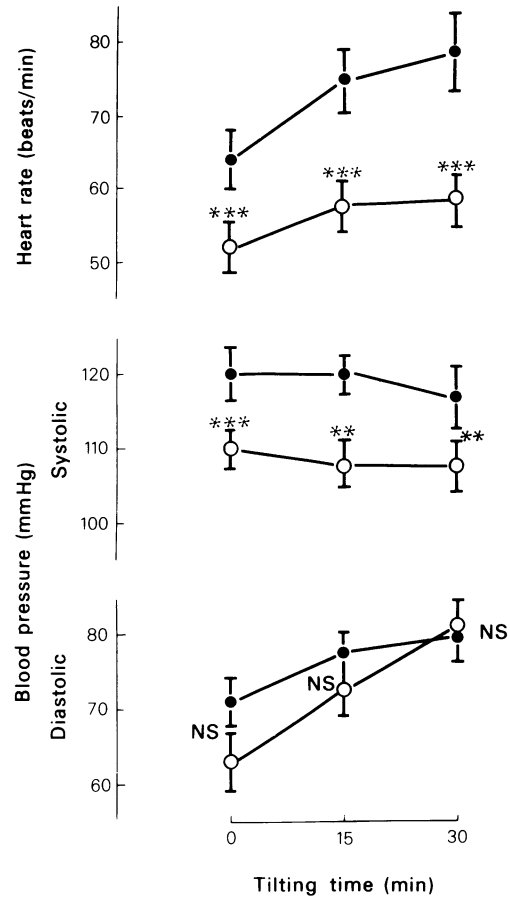

Figure 1 Heart rate and blood pressure at rest (0 min) and after head-up tilting for 15 and 30 min in normal subjects, before (●) and after (○) treatment with propranolol 160 mg daily for 7 days. Values shown are means ± s.e.mean (n=9). For significance of differences between untreated and treated values in this and subsequent figures: NS $P>0.05$; $*P<0.05$; $**P<0.01$; $***P<0.001$.

The mean plasma propranolol concentration in eight subjects was 181±25 nmol/l which generally provides adequate β-adrenoceptor blockade of exercise induced tachycardia (Coltart & Shand, 1970).

As shown in Figures 2 and 3, resting plasma NA and A levels were not altered by propranolol treat-

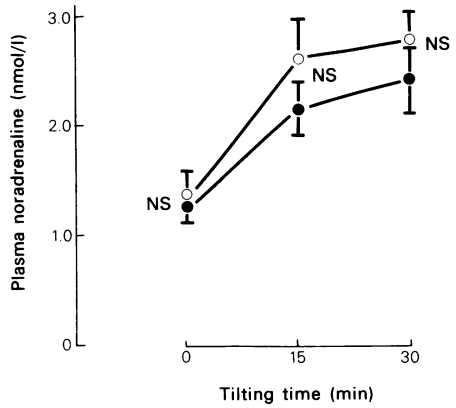

Figure 2 Plasma noradrenaline concentration at rest (0 min) and during 15 and 30 min tilting in normal subjects, before (●) and after (○) treatment with propranolol.

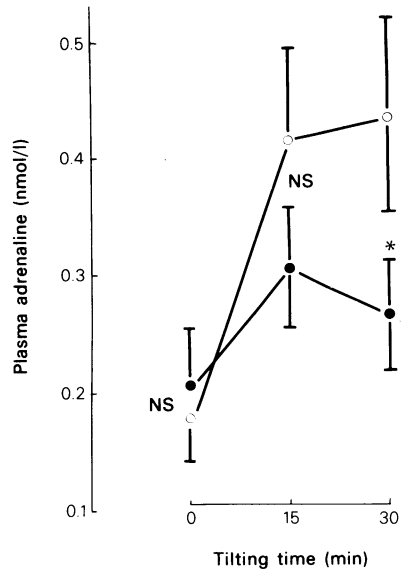

Figure 3 Plasma adrenaline concentration at rest (0 min) and during 15 and 30 min tilting in normal subjects, before (●) and after (○) propranolol treatment.

ment. Treatment also did not affect the NA response to tilting, with similar increases at 15 and 30 min before ($P<0.001$) and after propranolol ($P<0.01$ at 15 and $P<0.001$ at 30 min). Compared with resting levels, plasma A was increased at 15 ($P<0.01$) but not at 30 min ($P>0.05$) in untreated subjects (Figure 3). After treatment with propranolol, plasma A was elevated at 15 ($P<0.01$) and also at 30 min ($P<0.001$). These levels were higher than before treatment, but this was significant only at 30 min (Figure 3).

Pretreatment PRA showed considerable variability in resting levels (1.51 ± 0.35 nmol h^{-1} l^{-1}) and in response to tilting, with no change at 15 min (1.81 ± 0.27 nmol h^{-1} l^{-1}, $P>0.05$) but a significant increase at 30 min (2.5 ± 0.51 nmol h^{-1} l^{-1}, $P<0.01$).

In six of the nine subjects resting PRA was reduced from 1.32 ± 0.35 to 0.51 ± 0.24 nmol h^{-1} l^{-1} ($P<0.05$) after propranolol and did not change significantly during tilting (Table 1). In the remaining three subjects PRA was either unchanged (1) or increased (2). Resting NA and A levels were unaffected by propranolol in these subjects. As observed for the group as a whole, the increase in A was more marked and prolonged during tilting after propranolol administration.

Discussion

The haemodynamic responses to tilting are predictable and mediated by rapidly activated neural mechanisms which are responsible for maintaining blood pressure. Evidence for enhanced sympathetic activity is provided by the increase in circulating NA levels, occurring in parallel with the increase in pulse rate and diastolic blood pressure, which are sustained over the tilting period. In contrast to plasma NA the increase in A concentration was not maintained and returned to resting levels at the end of tilting. Persistent elevation of plasma catecholamines in the upright position has recently been reported (Saar &

Table 1 Plasma renin activity (nmol h^{-1} l^{-1}), noradrenaline and adrenaline concentration (nmol/l) in six subjects, at rest (0 min) and during tilting (15 and 30 min), before and after treatment with propranolol 160 mg daily for 7 days. Values are mean ± s.e.mean.

	Plasma renin activity			*Noradrenaline*			*Adrenaline*		
Time (min)	0	15	30	0	15	30	0	15	30
Before propranolol	1.32 ±0.35	1.98 ±0.45NS	2.12 ±0.45*	1.42 ±0.12	2.43 ±0.24*	2.66 ±0.36*	0.25 ±0.08	0.36 ±0.07*	0.31 ±0.08NS
After propranolol	0.61 ±0.24	0.71 ±0.18NS	1.0 ±0.45NS	1.42 ±0.18	2.84 ±0.47**	2.84 ±0.24**	0.22 ±0.07	0.45 ±0.11**	0.46 ±0.11**

For significance of difference from 0 min values, NS $P>0.05$; * $P<0.05$; ** $P<0.01$

Gordon, 1979), although NA and A were not measured separately. Although the peripheral sympathetic nerves are probably the main source of the increased plasma NA, reduction in hepatic and renal blood flow during tilting could delay clearance of catecholamines and may contribute to the increase in plasma levels.

It is readily apparent that these changes in catecholamines occur before there is a rise in PRA. This is consistent with the view that the renin-angiotensin system responds slowly to transient stimulation and functions more in relation to long-term circulatory adjustments (Guyton *et al.*, 1972).

Our finding that the renin response to tilting was considerably reduced by oral propranolol treatment confirms published observations (Davies & Slater, 1976; Morganti *et al.*, 1979), although the degree of suppression was more variable and less complete even in subjects where basal resting levels were significantly lower. This may be related to the administration of propranolol by the oral rather than the intravenous route employed in these other studies. Substantial falls in systolic blood pressure occurred in our normotensive subjects, which was not recorded in hypertensive patients given intravenous propranolol (Morganti *et al.*, 1979), and may have provided an additional stimulus to renin release mediated by a local baroreceptor mechanism. However, the findings clearly demonstrate that when basal PRA is suppressed no significant response to tilting is seen.

The increase in plasma NA consequent to head-up tilting is similar to that reported previously with this manoeuvre (Hortnagl *et al.*, 1977), and upon standing (Jones, Hamilton & Reid, 1979; Saar & Gordon, 1979). Changes in plasma A levels under these conditions are less well documented, largely because accurate determination has only recently been possible. We observed a definite increase at 15 but not at 30 min after commencing tilting. Using a similar assay for measuring plasma catecholamines, Hortnagl and co-workers (1977) were unable to demonstrate an increase in plasma A concentration after tilting normal subjects to 40° for 5 min. The smaller degree and shorter duration of tilt may account for this discrepancy. Also the values quoted indicate considerably less sensitivity compared with the modified technique employed in our study and the changes may not have been detectable.

Despite the fall in systolic blood pressure after propranolol treatment there was no increase in resting plasma NA or A concentrations in our subjects. It is conceivable that administration of propranolol for one week results in baroreceptor adaptation and resetting, a situation that may not occur when blood pressure is lowered abruptly by acute administration. It is of interest that treatment with propranolol extends the increase in A during tilting to 30 min. This difference between NA and A levels after propranolol was also observed during severe exercise (Galbo *et al.*, 1976). Possible explanations include an exaggerated adrenal medullary response induced by propranolol administration or reduced uptake into tissues or nerves due to receptor occupancy. Since plasma A increased during tilting before PRA, it is unlikely that an effect of angiotensin II on the adrenal medulla (Reit, 1972) is involved.

Although there are a number of reasons why changes in plasma catecholamines may follow administration of β-adrenoceptor blocking drugs, no firm agreement on the direction of these changes, if any, has ensued. Perusal of published reports indicates that noradrenaline levels have been found increased (Galbo *et al.*, 1976; Philipp, Cordes & Distler, 1977; Distler *et al.*, 1978; Hansen, Hesse & Christensen, 1978; Rahn *et al.*, 1978; McGrath *et al.*, 1979; Lijnen *et al.*, 1979), unchanged (Irving *et al.*, 1974; de Leeuw *et al.*, 1977) or even reduced (Brecht *et al.*, 1976; Mueller & Ayres, 1980) after treatment with β-adrenoceptor blocking drugs in normotensive or hypertensive subjects.

It is not possible at this stage to reconcile the conflicting evidence of the influence of β-adrenoceptor blocking drugs on plasma catecholamines. Some of the differences could be attributable to the variable conditions under which blood samples were drawn, the sensitivity and specificity of the catecholamine assays employed, and the pharmacological properties of the individual β-adrenoceptor blocking drugs. The effect of age and duration of treatment should be considered (Watson, Stallard & Littler, 1979) and important differences may also exist in baroreceptor sensitivity, and therefore sympathetic activity, in response to blood pressure changes in normal and hypertensive subjects (Mancia *et al.*, 1978).

Our findings clearly demonstrate parallel increases in NA and A, with a delayed rise in PRA, during head-up tilting in man. Basal catecholamine concentrations were not affected by propranolol treatment despite a substantial reduction in systolic blood pressure. Although the NA response to tilting was unaltered, A levels during tilting were higher after propranolol. We have also shown that suppression of PRA by propranolol treatment is not due to a reduction in circulating catecholamines, but most probably to blockade of intrarenal receptors mediating renin release (Vandongen *et al.*, 1973).

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