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THE EFFECTS OF β-ADRENOCEPTOR BLOCKADE ON RENIN, ANGIOTENSIN, ALDOSTERONE AND CATECHOLAMINES AT REST AND DURING EXERCISE

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1 β-adrenoceptor blockade with metoprolol provoked, both at rest and during exercise, a decrease of 'active' renin and angiotensin II together with an increase of 'inactive' renin and unchanged 'total' renin. The significant exercise-provoked increases in angiotensin II, plasma renin activity and 'active', 'inactive' and 'total' renin when on placebo, were reduced by metoprolol.

2 No significant change in serum sodium and potassium and in plasma aldosterone was found during β-adrenoceptor blockade at rest. During exercise plasma aldosterone dropped significantly without any change in serum sodium or potassium.

3 Plasma noradrenaline increased significantly at rest on metoprolol. The increase in plasma noradrenaline and adrenaline during exercise was similar on placebo and on metoprolol.

Introduction

At rest a marked and significant reduction of plasma renin activity (PRA) has been reported with the non-selective β-adrenoceptor blocker propranolol (Winer, Chokshi & Yoon, 1969; Michelakis & McAllister, 1972; Bühler, Laragh & Bear, 1972) and with the more cardioselective agents atenolol (Åberg, 1974; Sassard, Pozet & Vincent, 1976) and metoprolol (Attman, Aurell & Johnson, 1975). In contrast, the decrease of plasma renin concentration (PRC) during treatment with atenolol (Amery, Billiet & Fagard, 1974), propranolol (Birkenhäger, Krauss & Schalekamp, 1971; Lijnen, Amery, De Plaen, Fagard & Reybrouck, 1976) and sotalol (Verniory, Staroukine, Delwiche & Telerman, 1976) was small or even not significant. These differences can at least be partly explained by differences in methodology. Indeed, PRA is considered as a measure of 'active' renin and PRC of 'total' renin and it has been shown that treatment with propranolol and metoprolol produces a decrease of 'active' renin and an increase of 'inactive' renin, resulting in unchanged 'total' renin concentration (Amery, Lijnen, Fagard & Reybrouck, 1977; Derckx, Wenting & Man in 'T Veld, 1976).

In subjects treated with propranolol PRC did not increase at exercise while the rise in plasma aldosterone was not completely suppressed; propranolol had no influence on the exercise-induced increase in urinary catecholamine excretion (Bonelli, Waldhausl, Magametschnigg, Schwarzmeier, Korn & Hitzenberger, 1977).

To study further these problems we have investigated the influence of metoprolol both at rest and during exercise, not only on plasma renin activity, 'total', 'active' and 'inactive' plasma renin concentration but also on plasma aldosterone, angiotensin II, noradrenaline and adrenaline concentrations.

Methods

Eight patients with essential hypertension ($n=6$) or hypertension with renal arterial ($n=1$) or parenchymal disease ($n=1$) were selected for this supply. The diagnosis was based on history, physical examination, appropriate laboratory tests, rapid sequence intravenous pyelography and renal arteriography. No patient had a history of cerebrovascular or coronary accident.

In patients receiving antihypertensive treatment, all therapy had been interrupted for at least 3 weeks before they entered in the study. The patients were given tablets containing either placebo or 100 mg metoprolol three times daily for a period of 4 weeks. The tablets were identical in taste, shape and colour.

At the end of each period the patients came to the hospital and performed at 11.00 h and at 15.00 h an uninterrupted, graded exercise test on the bicycle ergometer starting at 20 Watt with increments of 30 Watt every 4 min until exhaustion (Reybrouck, Amery & Billiet, 1977). Blood was withdrawn from an antecubital vein at rest recumbent before and at the end of the exercise test for the determination of plasma renin, aldosterone, angiotensin II, noradrenaline and adrenaline concentration and of plasma renin activity.

Plasma renin activity (PRA) was measured by radioimmunoassay of the angiotensin I generated during a 1 h incubation of the plasma samples with the endogenous renin substrate at pH 5.5 and at 37°C, according to the method of Vallotton (1971). The results of the plasma renin determinations are expressed as ng angiotensin I generated per ml plasma and per hour (ng ml^{-1} h^{-1}).

The '*total*' plasma renin concentration (TPRC) was measured in plasma as the rate of angiotensin I generated during 1 h incubation with renin substrate, obtained from nephrectomized sheep at pH 7.4 under zero order kinetic conditions, according to the method of Skinner (1967) adapted for radioimmunoassay (Lijnen, Amery & Fagard, 1976). This assay includes denaturation of endogenous renin substrate by dialysis against a 0.05M aminoacetic acid buffer pH 3.3, and inactivation of the angiotensinases by heating at 32°C during 1 h.

The '*active*' plasma renin concentration (APRC) was determined after dialysing the plasma against a 0.05M aminoacetic buffer pH 4.5 and after heating the dialysate at 32°C during 1 h to perform an adequate inhibition of the angiotensinases. Incubation was performed with an excess sheep renin substrate at pH 7.4 during 1 h. The difference (TPRC−APRC) will be presented as the '*inactive*' plasma renin concentration (IPRC).

A radioimmunoassay method was used for the measurement of plasma angiotensin II concentration (PAII) and of the plasma aldosterone concentration (PAC), as previously described by Lijnen, Amery & Fagard (1977) and Lijnen, Amery, Fagard & Reybrouck (1978). Plasma noradrenaline (PNA) and noradrenaline (PA) were measured by a radiometric assay (Moerman, Bogaert & de Schaepdryver, 1976).

The 24 h urine was collected starting the day prior to the study for measurements of creatinine and sodium.

Results

The general characteristics of the patients are given in Table 1. Their 24 h urinary sodium excretion averaged 142.0 ± 18.8 mEq during the placebo period and 130.8 ± 19.7 mEq during the active treatment period ($P > 0.10$). Plasma renin activity; 'active', 'inactive' and 'total' plasma renin concentration; plasma aldosterone, angiotensin II, adrenaline and noradrenaline concentration were similar in the late morning and early afternoon and therefore the results were pooled. Maximal exercise capacity averaged 144.4 ± 22.0 Watt in the late morning and 140.0 ± 23.6 Watt in the early afternoon ($P > 0.10$) in the placebo period and was not different ($P > 0.10$) in the metoprolol period.

'Active', 'inactive' and 'total' renin

Compared to the placebo period plasma renin activity and 'active' plasma renin concentration decreased significantly ($P < 0.01$) during metoprolol treatment at

Table 1 General characteristics of the patients

Total number (*n*)	8
Number of males/females (*n*)	4/4
Age (years)	42.8 ± 3.1*
Weight (kg)	72.3 ± 4.6
Classification according to WHO	
stage 1 (*n*)	2
stage 2 (*n*)	5
stage 3 (*n*)	1
Creatinine clearance (ml min^{-1} 1.73 m^2)	93.6 ± 14.9
Hypertensive retinopathy (according to Keith-Wagener)	
grade 1 (*n*)	3
grade 2 (*n*)	5
Recumbent systolic and diastolic blood pressure (mmHg) during the placebo period at 11.00 h	187.1 ± 13.1 118.9 ± 6.8
ECG: normal (*n*)	5
left ventricular hypertrophy (*n*)	3

* For quantitative data the mean ± s.e. mean are given.

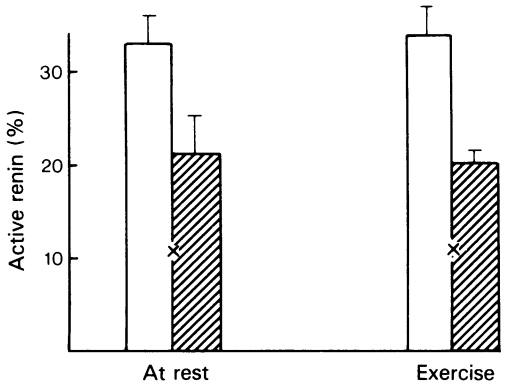

Figure 1 Proportion of active to total renin (mean ± s.e. mean) at rest and during exercise in hypertensive patients on placebo (□) or on metoprolol (■, 3 × 100 mg).

rest, while an increase in 'inactive' plasma renin concentration ($P<0.05$) and no significant change in 'total' plasma renin concentration were observed (Table 2). At exercise significant reductions ($P<0.001$) of plasma renin activity and 'active' plasma renin concentration were found during treatment with metoprolol (Table 2). However no changes were observed for 'total' and 'inactive' plasma renin concentrations.

At rest the proportion of 'active' to 'total' plasma renin concentration averaged 33% on placebo which was significantly ($P<0.05$) higher than the 21% obtained on metoprolol. At exercise the proportions were similar (Figure 1). Compared to the resting levels 'active' renin (APRC and PRA), 'total' and 'inactive' plasma renin concentration increased significantly at exercise on placebo (Figure 2).

During metoprolol treatment the increases in plasma renin activity, 'active', 'inactive' and 'total' plasma renin concentration during exercise were not significant and were reduced when compared to the observations during placebo (Figure 2).

Angiotensin II

Metoprolol reduced plasma angiotensin II ($P<0.05$) both at rest and at exercise (Table 2). The exercise induced rise in plasma angiotensin II was significant during placebo but not on metoprolol (Figure 2).

Table 2 Biochemical parameters (mean ± s.e. mean) on placebo and on metoprolol

	At rest		*At exercise*	
Biochemical parameter	*Placebo*	*Metoprolol (300 mg)*	*Placebo*	*Metoprolol (300 mg)*
PRA (ng ml^{-1} h^{-1})	1.42 ± 0.35 (15)	0.30 ± 0.06** (16)	2.66 ± 0.53 (15)	0.60 ± 0.18*** (16)
TPRC (ng ml^{-1} h^{-1})	22.96 ± 2.16 (16)	27.46 ± 3.95 (16)	31.18 ± 3.85 (14)	29.32 ± 3.73 (16)
APRC (ng ml^{-1} h^{-1})	7.24 ± 0.58 (16)	4.48 ± 0.55*** (16)	10.0 ± 1.17 (14)	5.65 ± 0.79*** (15)
IPRC (ng ml^{-1} h^{-1})	15.72 ± 1.87 (16)	23.11 ± 3.67* (16)	21.32 ± 3.09 (14)	23.44 ± 3.23 (15)
PAII (pg/ml)	49.44 ± 11.47 (12)	16.19 ± 2.86* (10)	87.97 ± 29.17 (9)	23.57 ± 3.42* (10)
PAC (ng/100 ml)	9.51 ± 1.33 (16)	7.94 ± 1.52 (15)	40.76 ± 8.06 (14)	25.63 ± 7.90* (16)
PNA (ng/ml)	0.36 ± 0.07 (16)	0.51 ± 0.08* (16)	3.10 ± 0.37 (14)	3.24 ± 0.25 (16)
PA (ng/ml)	0.08 ± 0.02 (16)	0.14 ± 0.07 (16)	0.76 ± 0.33 (14)	0.57 ± 0.09 (16)
Serum potassium (mEq/l) concentration	4.25 ± 0.05 (14)	4.21 ± 0.13 (15)	4.54 ± 0.08 (11)	4.57 ± 0.09 (15)
Serum sodium (mEq/l) concentration	142.0 ± 0.3 (13)	142.1 ± 0.5 (15)	143.7 ± 0.6 (11)	142.9 ± 0.6 (15)

Number of observations in brackets.
* $0.01<P<0.05$
** $0.001<P<0.01$
*** $P<0.001$ compared with placebo
PRA: plasma renin activity; TPRC: total plasma renin concentration; APRC: active plasma renin concentration; IPRC: inactive plasma renin concentration; PAII; plasma angiotensin II; PAC: plasma aldosterone concentration;
PNA: plasma noradrenaline; PA: plasma adrenaline.

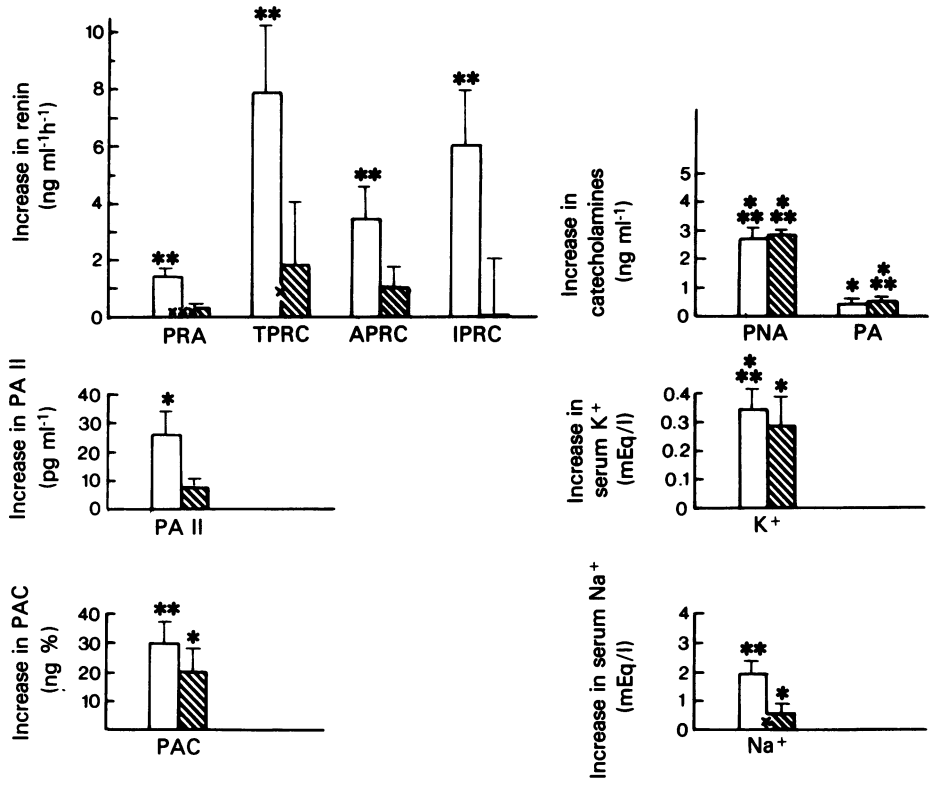

Figure 2 Increase (mean ± s.e. mean) in renin, angiotensin, aldosterone and catecholamines during exercise in hypertensive patients on placebo (□) or on metoprolol (■ 3 × 100 mg). * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$. PRA: plasma renin activity; TPRC: total plasma renin concentration; APRC: active plasma renin concentration; IPRC: inactive plasma renin concentration; PAII: plasma angiotensin II; PAC: plasma aldosterone concentration; PNA: plasma noradrenaline: PA: plasma adrenaline.

Aldosterone

No significant change in plasma aldosterone concentration or serum potassium or sodium concentration was found during metoprolol treatment at rest. However at exercise a significant reduction in plasma aldosterone concentration was observed during treatment with metoprolol. This reduction in plasma aldosterone concentration was accompanied by a change in serum potassium or sodium concentration (Table 2).

Compared with the resting recumbent value, on placebo a significant increase in plasma aldosterone concentration and in serum potassium and sodium concentration was observed at exercise (Figure 2). On metoprolol these increases were not completely suppressed.

Noradrenaline and adrenaline

During β-adrenoceptor blockade with metoprolol rest PNA increased ($P < 0.05$) and PA tended to increase; but the latter rise was not statistically significant. Exercise provoked increases of PNA and PA (Table 2) which were similar on placebo and on metoprolol (Figure 2).

Discussion

The renin concentration in pH 3.3 treated plasma, referred to as 'total' renin, is significantly higher than the renin concentration determined in pH 4.5 treated plasma, referred to as 'active' renin. During acidification of plasma to pH 3.3 an inactive form of renin seems to be activated (Lumbers, 1971; Morris & Lumbers, 1972; Skinner, Cran & Gibson, 1975; Leckie & McConnell, 1975a; Derckx *et al.*, 1976; Boyd, 1977; Lijnen & Amery, 1977). The difference between the results obtained with pH 3.3 and 4.5 treated plasma is taken as the concentration of 'inactive' renin which could be similar to prorenin (Derckx *et al.*, 1976) or to big renin (Leckie & McConnell, 1975b).

In this study we found one third of 'total' renin to be present as 'active' renin and two thirds as 'inactive' renin both at rest and at exercise in hypertensive patients on placebo treatment. These data correspond to the findings of Skinner *et al.* (1975) who reported that approximately two-thirds of the 'total' renin in normal human plasma constitutes an 'inactive', acid-activable form. Boyd (1977) reported also that 'active' renin represents about 50% of the 'total' renin concentration and that 'inactive' renin is present in all subjects. Day & Luetscher (1975) and Day, Luetscher & Gonzales (1975) however described an 'inactive' form of renin only in the plasma of patients with Wilm's tumour, in diabetic patients and in hypertensive patients with proteinuria. They found no 'inactive' renin in normal subjects or in the plasma of hypertensive patients without proteinuria. The discrepancy may be due to the diuretics given prior to blood sampling to increase the renin concentration in their normal subjects. The data of Boyd (1977) clearly indicate that diuretics greatly reduce the proportion of 'inactive' renin in the acute stage.

Metoprolol, one of the more cardioselective beta blockers, reduces 'active' renin (PRA and APRC) and increases 'inactive' renin (IPRC) without changes in 'total' renin (TPRC). Derckx *et al.* (1976) reported similar responses of 'active', 'inactive' and 'total' renin using the β-adrenoceptor blocker propranolol. According to Atlas, Sealey, Laragh & Moon (1977) propranolol lowered plasma renin activity by 55% in patients with uncomplicated, essential hypertension. In fifteen patients with a fall in blood pressure, the drug increased 'prorenin', defined by Sealey, Moon, Laragh & Alderman (1976) as cryoactivable renin, reduced 'active' renin with unchanged 'total' renin; in the remaining five patients with unchanged blood pressure β-adrenoceptor blockade produced falls in 'total' and 'active' renin while 'prorenin' was unchanged.

Metoprolol caused a significant fall in plasma angiotensin II of 68% at rest and of 73% during exercise. The concomitant falls in 'active' renin and plasma angiotensin II during metoprolol with even increases in 'inactive' renin may suggest that 'inactive' renin *per se* is indeed biologically inactive *in vivo*.

The data of Verniory *et al.* (1976) also demonstrate a reduction in plasma angiotensin II (average: 46%) in hypertensive patients treated with sotalol. They also found a fall in urinary excretion of aldosterone accompanied by a rise in plasma potassium concentration.

The rest recumbent plasma aldosterone concentration (PAC) did not change significantly during metoprolol while the exercise PAC dropped significantly. On the contrary at rest and at exercise a significant decrease in plasma renin activity and no changes in serum potassium or sodium concentration were observed. Also Klumpp, Braun, Klaus, Lemke & Zehner (1976) reported no changes in basal plasma aldosterone concentration during treatment with propranolol in 31 patients with essential hypertension. However, the stimulated PAC, measured 2 h after frusemide administration, decreased ($P<0.01$) on propranolol, while the basal and stimulated plasma renin activity dropped both significantly. On the contrary acute oral administration of metoprolol or propranolol to ten normal males resulted in a reduction in supine and tilted plasma renin activity, while no effect was observed on supine or tilted PAC (Viol, Smith & Fitzgerald, 1976). Thus during β-adrenoceptor blockade some discrepancies can be found between the renin and the aldosterone response, which is not surprising as renin is not the only parameter influencing the aldosterone secretion. In a previous paper (Lijnen, Amery, Fagard & Reybrouck, 1978) we found a significant but poor correlation between plasma aldosterone and 'active' or 'total' renin.

The plasma concentration of noradrenaline has been proposed as an index of peripheral sympathetic nervous activity. During chronic treatment with 3×80 mg propranolol in male hypertensives, previously 'responsive' ($n=4$) or 'non-responsive' ($n=4$) to propranolol therapy, Bühler & Lütold (1977) found an equal reduction in plasma renin activity in both groups and a concomitant fall of plasma noradrenaline and plasma adrenaline in the 'responders' and a reactive increase in plasma noradrenaline and plasma adrenaline in the 'non-responders' at exercise. At rest no significant changes in plasma noradrenaline and adrenaline were observed. The decrease in plasma noradrenaline and adrenaline during β-adrenoceptor blockade at exercise in the 'responders' reflects probably an adaptation of the autonomic system and the increase in plasma noradrenaline and adrenaline in the 'non-responders' reflects an activated adrenergic and adrenomedullary mechanism(s) during β-adrenoceptor blockade.

At rest and at exercise we found no significant changes in plasma adrenaline during metoprolol treatment (Table 2). Similar results were reported for propranolol by Polak, Jones, Reid, Hamilton & Dollery (1977). However, Distler, Philipp & Cordes (1977) found an increase in exercise plasma noradrenaline during 5 weeks treatment of 16 patients with essential hypertension with 200 mg atenolol. On the contrary Brecht, Banthien, Ernst & Schoeppe (1976) reported during long-term treatment with pindolol in 59 patients with essential hypertension a significant reduction in plasma noradrenaline under basal and orthostatic conditions.

The conflicting data in the literature suggest that actions of β-adrenoceptor blockers are probably not associated with marked reductions in peripheral sympathetic activity.

During exercise a significant increase in 'active', 'inactive' and 'total' renin concentration, renin activity, angiotensin II, aldosterone and noradrenaline and adrenaline was found in the hypertensive patients

on placebo (Figure 2). However, during metoprolol treatment this increase remained only significant for aldosterone, angiotensin II and noradrenaline and adrenaline, while the other parameters did not change significantly during exercise in hypertensive patients on metoprolol (Figure 2).

The more pronounced increase in plasma aldosterone concentration at exercise, compared to the renin-angiotensin response, can be attributed to different factors such as reduction of hepatic blood flow and consequently a decreased metabolism (Rowell, Blackman & Bruce, 1964), a rise in ACTH (Sundsfjord, Stromme & Aakvaag, 1975; present study), and variations in prostaglandin A_1 concentration (Golub, Speckart, Zia & Horton, 1976).

Parallel increases in blood catecholamines and plasma renin activity in response to graded exercise have been reported in healthy subjects (Kotchen, Hartley, Rice, Mougey, Johnes & Mason, 1971). Also the increase in plasma renin activity in control subjects and in patients with essential hypertension was similar. However the increase in plasma noradrenaline and plasma adrenaline was significantly different in both groups at all levels of exercise (Chodakowska, Nazar, Wocial, Jarecki & Skorka, 1975).

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