Sex and ageing differences in resting arterial pressure regulation: the role of the β-adrenergic receptors

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The Journal of Physiology

Non-technical summary In young men, sympathetic nerve activity is directly related to the level of vasoconstrictor tone in the peripheral vasculature. However, in young women this relationship does not exist, suggesting that certain factors (potentially related to the female sex hormones) offset the transfer of sympathetic nerve activity into vasoconstrictor tone in this population. In the present study we show that, in young women, the β-adrenergic receptors (which cause vaso-dilatation in response to noradrenaline) blunt the vasoconstrictor effect of resting sympathetic nerve activity in young women. This mechanism does not occur in young men or postmenopausal women. It is possible that the β-adrenergic receptors may partially protect young women against the sometimes harmful effects of high sympathetic nerve activity. This may explain why the risk of developing hypertension is greater in young men and postmenopausal women (who have very high sympathetic nerve activity) compared to young women.

Abstract In men, muscle sympathetic nerve activity (MSNA) is positively related to total peripheral resistance (TPR) and inversely related to cardiac output (CO). However, this relationship was not observed in young women. We aimed to investigate whether simultaneous β-adrenergic stimulation offsets this balance in young women. Furthermore, we aimed to examine whether the ability of the β-adrenergic receptors to offset the transduction of MSNA into vasoconstrictor tone was lost in postmenopausal women. We measured MSNA (peroneal microneurography), arterial pressure (brachial line), CO (Modelflow), TPR and changes in forearm vascular conductance (FVC) to increasing doses of noradrenaline (NA; 2, 4 and 8 ng $(100\,ml)^{-1}\,min^{-1}$) before and after systemic β-blockade with propranolol in 17 young men, 17 young women and 15 post-menopausal (PM) women. The percentage and absolute change in FVC to the last two doses of NA were greater during β-blockade in young women ($P < 0.05$), whereas the change in FVC was similar before and during β-blockade in young men and PM women ($P > 0.05$). Before β-blockade there was no relationship of MSNA to TPR or mean arterial pressure (MAP) in young women. Following β-blockade, MSNA became positively related to TPR ($r = 0.59$, $P < 0.05$) and MAP ($r = 0.58$, $P < 0.05$). In the PM women and young men, MSNA was positively associated with TPR. β-Blockade had no effect on this relationship. Our data suggest that the β-adrenergic receptors offset α-adrenergic vasoconstriction in young women but not young men or PM women. These findings may explain in part the tendency for blood pressure to rise after menopause in women.

Abbreviations CO, cardiac output; FVC, forearm vascular conductance; MSNA, muscle sympathetic nerve activity; NA, noradrenaline; PM, postmenopausal; TPR, total peripheral resistance.

Introduction

It is well known that young women are at less risk of developing hypertension and other cardiovascular diseases compared to men of a similar age (Burt *et al.* 1995; Wiinberg *et al.* 1995). However, as women age the risk of developing hypertension increases (Burt *et al.* 1995; Wiinberg *et al.* 1995). This is particularly apparent around the onset of menopause, where the incidence of hypertension becomes similar to, or even exceeds, that observed in men of the same age (Burt *et al.* 1995). Therefore, the female sex hormones have long been associated with a protective effect on the cardiovascular system (Wuest *et al.* 1953; Miller & Duckles, 2008). Understanding how the female sex hormones exert their cardioprotective effect is becoming increasingly important, since the post-menopausal world population is projected to rise 2- to 5-fold by 2050 (US Census Bureau on World Population; Meyer *et al.* 2008).

Recently, we and others have demonstrated fundamental sex differences in arterial pressure regulation (Shoemaker *et al.* 2001; Charkoudian *et al.* 2005; Carter & Ray, 2009; Hart *et al.* 2009*a*). It appears that a reciprocal balance between cardiac output and sympathetic control of peripheral vascular resistance (TPR) is a key factor in normal arterial pressure regulation in young men (Charkoudian *et al.* 2005; Hart *et al.* 2009*a*). This balance minimizes the effect of high muscle sympathetic nerve activity (MSNA) on arterial pressure. However, data from our lab demonstrate that in complete contrast to men, there is no relationship of MSNA to TPR or cardiac output in women (Hart *et al.* 2009*a*). The lack of relationship between MSNA and TPR suggests that factors in addition to sympathetic neural activity modulate whole body 'net' vasoconstrictor tone in young women.

Previous work indicates that β-adrenergic receptor sensitivity is enhanced in young women compared to men of a similar age (Kneale *et al.* 2000). Consequently, forearm vasoconstrictor responses to noradrenaline are blunted in young women due to concurrent β-adrenergic-mediated vasodilatation. Thus, it is possible that β_2-adrenergic receptor-mediated vasodilatation is enhanced in young women and offsets the vasoconstrictor effects of high levels of MSNA. Therefore, our first aim in the present study was to examine the role of β-adrenergic receptors in the balance among MSNA, TPR and cardiac output in young normotensive men and women.

As men and women age the relationship between MSNA and blood pressure becomes positive: older men and women with higher MSNA have higher blood pressure. In contrast, in younger people at rest, MSNA is unrelated to arterial pressure (Sundlof & Wallin, 1978*a*; Charkoudian *et al.* 2005; Narkiewicz *et al.* 2005). The MSNA–arterial pressure relationship is particularly robust in older women (Matsukawa *et al.* 1998; Narkiewicz *et al.* 2005). In the present study, our second aim was to examine whether the decrease in oestrogens and progesterone after menopause modulates the role of the β-adrenergic receptors in counterbalancing sympathetic vasoconstrictor activity. Changes in the ability of the β-adrenergic receptors to counterbalance the effects of MSNA on the peripheral vasculature might explain (in part) the steeper MSNA–blood pressure relationship in older women (Narkiewicz *et al.* 2005).

We hypothesized that (a) systemic non-selective β-blockade would augment the forearm vasoconstrictor response to noradrenaline in young women, but not in young men or postmenopausal women, (b) systemic β-blockade in young women would cause the relationship between MSNA and TPR to become positive (similar to that seen in men) and (c) systemic β-blockade would not affect any relationship observed among MSNA, TPR, cardiac output and MAP in postmenopausal women.

Methods

Participants

After the protocol was approved by the Institutional Review Board of the Mayo Clinic, 17 young men, 17 young women and 15 postmenopausal women gave written informed consent to participate in this study (Table 1 for demographics). The subjects were non-smokers with no history of cardiovascular or other chronic diseases. Participants were excluded if their body mass index was ≥ 28 kg m^{-2} or if they were taking any medications other than oral contraceptives. In the young women, 11 out of 17 subjects were taking oral contraceptives. Young women using an inter-uterine device were excluded. Postmenopausal women were excluded if they were taking hormone replacement therapy. In the older women, we accepted participants who were taking medication for hypothyroidism. We did this since a large percentage of the postmenopausal population is prescribed drugs for hypothyroidism. Thirteen out of fifteen were taking this type of medication. Women treated for hypothyroidism have thyroid hormones (e.g. thyroid stimulating hormone) that remain within a normal range. Postmenopause was defined as at least 1 year since last menstruation (Gracia *et al.* 2005). To minimize the effects of the reproductive hormones on autonomic control or cardiovascular function, all women were studied in the early follicular phase of the menstrual cycle or in the low hormone phase of oral contraceptive use (Minson *et al.* 2000). All women of child bearing age were asked to complete a pregnancy test at least 48 h before the study day. Subjects were asked not to consume anything within 2 h before the experiment, and not to consume caffeine or alcohol for 24 h before the experiment.

Table 1. Demographic characteristics in young men, young women and postmenopausal (PM) women

Demographics	Young men ($n = 17$)	Young women ($n = 17$)	PM women ($n = 15$)
Height (m)	1.78 ± 0.01	1.68 ± 0.03†	1.65 ± 0.01†
Weight (kg)	79.1 ± 2.8	65.0 ± 3.1†	66.5 ± 1.5†
BMI (kg m^{-2})	24.9 ± 0.6	23.1 ± 0.7	24.4 ± 0.6
Age (years)	25 ± 1	25 ± 2	59 ± 2

†$P \leq 0.05$ *vs.* young men. Mean $\pm$ SEM.

Procedures

All of the studies were performed in a CTSA Clinical Research Unit laboratory at the Mayo Clinic, where ambient temperature was controlled between 22°C and 24°C. On arrival at the laboratory, subjects rested in the supine position during instrumentation. After local anaesthesia with 2% lidocaine, a 5 cm, 20-gauge arterial catheter was placed in the brachial artery of the non-dominant arm, using aseptic technique. The catheter was connected to a pressure transducer, which was positioned at the level of the heart and interfaced with a personal computer to monitor arterial pressure. A 3-lead ECG was used for continuous recordings of heart rate.

Multiunit MSNA was measured from the right peroneal nerve at the fibular head using insulated tungsten microelectrodes. A muscle sympathetic fascicle was identified when taps on the muscle belly or passive muscle stretch evoked mechanoreceptive impulses, and no afferent neural response was evoked by skin stimuli (Sundlof & Wallin, 1977, 1978*b*) The recorded signal was amplified 80,000-fold, band pass filtered (700 to 2000 Hz), rectified, and integrated (resistance-capacitance integrator circuit time constant 0.1 s) by a nerve traffic analyser.

Stroke volume was measured from the brachial artery using Modelflow analysis. Briefly, the brachial pressure wave was downloaded onto a personal computer and analysed offline using data acquisition software (WinDaq194; DATAQ instruments, Ohio, USA). Beat-to-beat stroke volume was then calculated using Modelflow which computes an aortic waveform based on non-linear pressure–volume, pressure–compliance and pressure–characteristic impedance equations, incorporating age, sex, height and body mass (Wesseling *et al.* 1993). Cardiac output was calculated as stroke volume × heart rate and TPR was calculated as MAP/cardiac output.

Forearm blood flow (FBF) was measured using mercury-in-silastic strain gauge plethysmography (Joyner *et al.* 2001). Briefly, a paediatric blood pressure cuff was placed around the wrist and inflated to suprasystolic levels (220 mmHg) to arrest the circulation of the hand, and a venous occlusion cuff was placed on the upper arm and rapidly inflated to 50 mmHg every 7.5 s, yielding one blood flow every 15.0 s. FBF was expressed as milliliters per 100 ml of tissue per minute.

Brachial artery infusion of noradrenaline

Noradrenaline infusion was adjusted for forearm volume and administered via the brachial artery catheter at total rates of 2 to 4 ml min^{-1}. Noradrenaline (noradrenaline bitartrate, Bedford Laboratories) was administered at 2, 4 and 8 ng (100 ml)$^{-1}$ min^{-1}.

Systemic β-adrenergic blockade

Non-selective β-blockade was achieved using intravenous infusion of propranolol. A 0.25 mg kg^{-1} bolus of propranolol was followed by a continuous infusion of 0.004 mg kg^{-1} min^{-1} propranolol to maintain β-blockade. This dose of propranolol has been previously proven to cause total β-blockade in adult humans (Bell *et al.* 2001)

Protocol

After the placement of arterial catheters, ECG leads and instrumentation for plethysmography, subjects rested supine during instrumentation for microneurography. Once a good electrode site for measurement of MSNA was found, 5 min of baseline data (cardiac output, arterial pressures and MSNA) were recorded with the subject resting quietly. After this baseline period, forearm dose–response trials were performed for noradrenaline at the doses noted above. Each dose–response trial included 2 to 4 min of resting FBF measurement, followed by 2 min infusions of each dose. β-Blockade was then achieved via infusion of propranolol. Once a stable heart rate had been reached ($\sim$5 min) MSNA, arterial pressures, heart rate and cardiac output were again recorded over 5 min. FBF responses to noradrenaline were then repeated.

Data analysis

Data were sampled at 250 Hz and stored on a personal computer for offline analysis. MSNA, heart rate, mean arterial pressure, systolic blood pressure (SBP) and

Table 2. Average haemodynamic and neural variables at rest and during β-blockade (BB) in young men, young women and post-menopausal (PM) women

Haemodynamic and neural variables	Young men (n = 14)		Young women (n = 15)		PM women (n = 14)	
	Pre BB	During BB	Pre BB	During BB	Pre BB	During BB
SBP (mmHg)	125 ± 2	126 ± 3	130 ± 3	130 ± 4	143 ± 5†$	145 ± 5†$
DBP (mmHg)	72 ± 2	74 ± 2*	73 ± 2	75 ± 2*	73 ± 2	74 ± 2
MAP (mmHg)	90 ± 2	93 ± 2	93 ± 2	96 ± 2*	99 ± 3†	98 ± 2
HR (beats min^{-1})	59 ± 2	51 ± 2*	61 ± 3	54 ± 3*	62 ± 2	51 ± 2*
SV (ml)	99 ± 3	87 ± 6	85 ± 4†	83 ± 4	66 ± 2†$	68 ± 3†$
CO (l min^{-1})	5.9 ± 0.2	4.9 ± 0.2*	5.1 ± 0.2†	4.4 ± 0.2*	4.1 ± 0.2†$	3.5 ± 0.2*†$
TPR (mmHg l^{-1} min^{-1})	15.5 ± 0.2	19.2 ± 0.8*	18.8 ± 0.9†	22.2 ± 1.1*	25.1 ± 1.3†$	29.3 ± 1.8*†$
FBF (ml/100 ml min^{-1})	2.31 ± 0.16	2.05 ± 0.15*	2.51 ± 0.23	2.00 ± 0.13	1.87 ± 0.18	1.70 ± 0.14†$
FVC (ml min^{-1} mmHg^{-1})	2.47 ± 0.16	2.14 ± 0.17*	2.63 ± 0.21	2.14 ± 0.14	1.96 ± 0.19	1.69 ± 0.15†$
MSNA (bursts (100 heart beats)$^{-1}$)	38 ± 4	46 ± 3	31 ± 4	35 ± 5	62 ± 3†$	74 ± 5*†$
MSNA (bursts min^{-1})	24 ± 3	23 ± 2	18 ± 2	19 ± 2	38 ± 2†$	37 ± 2†$

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance, MSNA, muscle sympathetic nerve activity. *$P \leq 0.05$ *vs.* pre β-blockade (i.e. within group), †$P \leq 0.05$ *vs.* young men, $$P \leq 0.05$ *vs.* young women. For example, PM women had a significantly lower CO during β-blockade *vs.* pre condition. They also had a lower CO *vs.* young men and women during β-blockade. Mean ± SEM.

diastolic blood pressure (DBP) were assessed as 5 min averages at the end of the initial baseline period. Sympathetic bursts in the integrated neurogram were identified by a custom-manufactured semiautomated analysis program (Kienbaum *et al.* 2001; Charkoudian *et al.* 2005); burst identification was controlled visually by a single investigator. The program then compensated for baroreflex latency and associated each sympathetic burst with the appropriate cardiac cycle.

FBF was determined from the slope of the plethysmographic recording during venous occlusion (Joyner *et al.* 2001). FBF data are presented as an average of four measurements during the last minute of each dose of drug infusion, when steady-state vasoconstriction was observed. Vasoconstrictor responses were analysed both as absolute change and as the percentages of change in FBF and forearm vascular conductance from baseline. Forearm vascular conductance was calculated as FBF/MAP × 100.

Statistics

Data were analysed statistically using commercially available software (Sigma Stat 2.03, SPSS Inc., Chicago, IL, USA). Group data are expressed as means ± SEM. A multifactorial ANOVA was used to examine whether neural and cardiovascular variables were different in response to β-blockade within and between each group, with a Bonferroni adjustment for pairwise comparisons. To measure whether there was a relationship of baseline MSNA to cardiovascular variables, linear regression analysis was performed and Pearson's correlation coefficients calculated. The α-level was set at 0.05.

Results

Average group differences in neural–haemodynamic variables

Average group haemodynamic and neural variables before and during β-blockade are outlined in Table 2. We did not gain a recording site for MSNA in one postmenopausal woman and we also lost the recording site for MSNA in three men and two young women during β-blockade. Therefore average haemodynamic and neural data reflect that from 14 young men, 15 young women and 14 postmenopausal women. Before β-blockade there was no difference in arterial blood pressures, heart rate and MSNA between young men and women. However, cardiac output and stroke volume were lower in young women *vs.* young men (P < 0.05). Conversely, TPR was higher in the young women compared to the men (P < 0.05). In the postmenopausal women, SBP, TPR and MSNA were higher compared to young men and women (P < 0.05), whereas stroke volume and cardiac output were lower (P < 0.05). Differences between young men and women were abolished after β-blockade, whereas differences between older women and the young groups persisted after β-blockade.

During β-blockade, heart rate and cardiac output where reduced, whereas TPR was increased in all groups (P < 0.05). The changes in TPR, HR and cardiac output were not different among groups. Arterial pressures were unchanged by β-blockade in all groups, with the exception of DBP, which increased in young men and women (P < 0.05). MSNA (burst incidence and burst frequency) was unchanged during propranolol infusion in young men and women, but increased

in the postmenopausal women (bursts incidence only, $P < 0.05$).

Forearm blood flow and conductance responses to noradrenaline before and during propranolol

Baseline forearm blood flow and conductance before and during β-blockade. We recorded blood flow response to noradrenaline in all subjects (young men, $n = 17$; young women, $n = 17$; postmenopausal women, $n = 15$). There was no difference in baseline FBF before β-blockade between young men and women (Table 2, $P < 0.05$). There was also no difference in FVC between young men and

women before β-blockade. Resting FBF and FVC were lower in postmenopausal women ($P < 0.05$) compared to the two younger groups. During β-blockade, baseline FBF was similar among groups ($P > 0.05$). However, there was a trend towards a lower FVC in older women during β-blockade compared to the two younger groups ($P = 0.06$).

Changes in forearm blood flow and conductance to noradrenaline before and during β-blockade. All young men had a dose-dependent vasoconstrictor response to noradrenaline before and during β-blockade. In 2 of the 17 young women, no dose-dependent vasoconstriction was
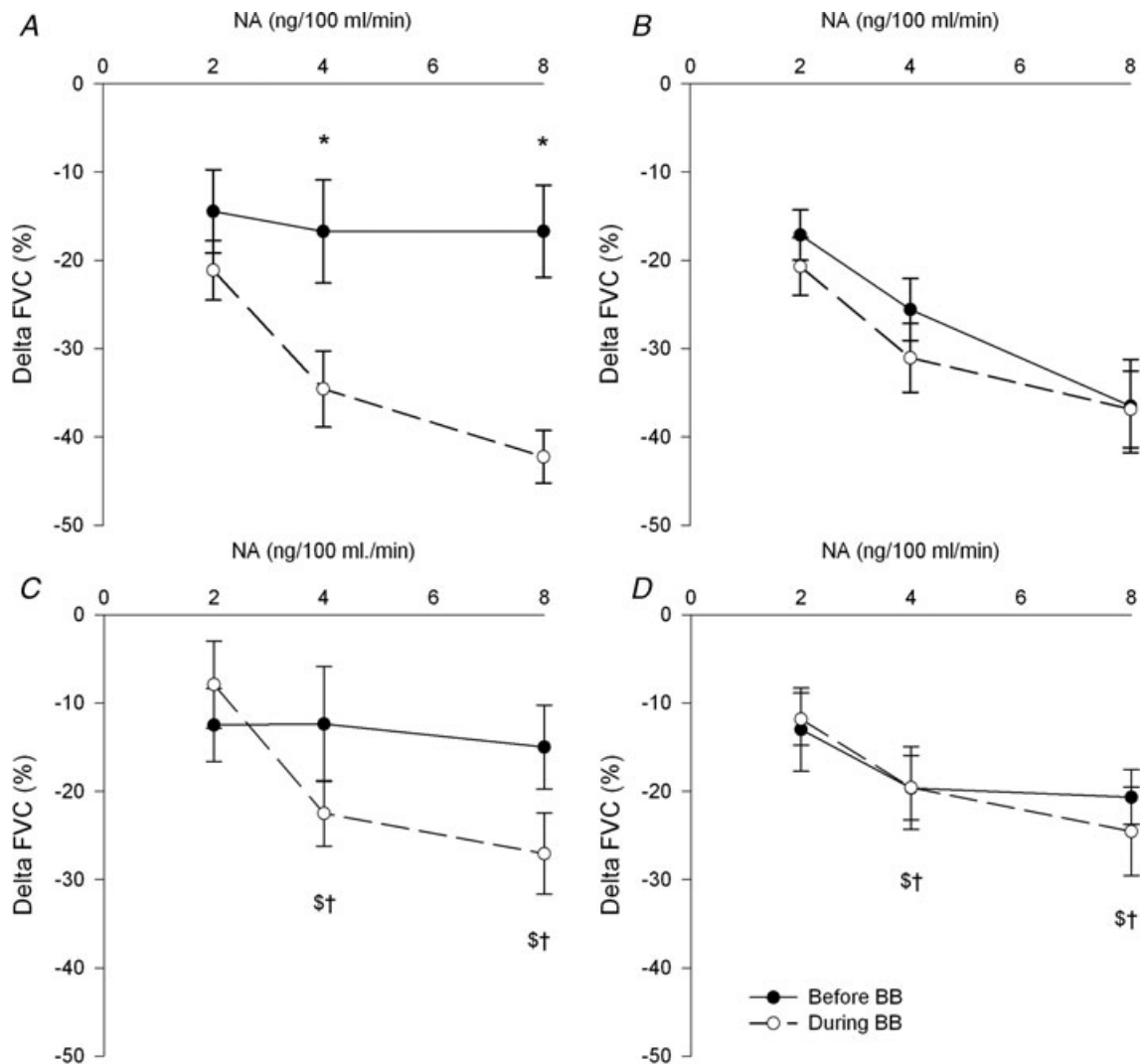

Figure 1. Percentage change in forearm vascular conductance (FVC) to increasing doses of noradrenaline (NA) in: A, young women ($n = 17$); B, young men ($n = 17$), and postmenopausal (PM) women with (C) outliers included ($n = 15$) and (D) excluded ($n = 13$)
*$P < 0.05$ vs. before β-blockade, $^{\$}P < 0.05$ vs. young women during β-blockade, $^{†}P < 0.05$ vs. young men during β-blockade, $^{‡}P < 0.05$ vs. young women before β-blockade and $^{λ}P < 0.05$ vs. before β-blockade young men. All comparisons between groups are vs. the same condition (for example during β-blockade in postmenopausal women vs. during β-blockade in young men). Removal of the PM outliers does not make any difference to this group's response to noradrenaline before and during β-blockade.

observed before β-blockade, but became apparent during β-blockade. In addition, 2 of the 15 postmenopausal women demonstrated an increase in blood flow in response to noradrenaline at all doses of noradrenaline indicating vasodilatation. We analysed FBF and FVC responses in the postmenopausal group both with and without these two outliers, and we present both sets of data.

Figure 1 demonstrates percentage changes in FVC to noradrenaline before and during β-blockade in all groups. In the young men, the percentage reduction in FVC was similar before and during β-blockade at

all doses of noradrenaline. As expected, in the young women the percentage reduction in FVC was greater during β-blockade at the last two doses of noradrenaline. Interestingly, the percentage reduction in FVC in post-menopausal women was similar before and during β-blockade at all doses of noradrenaline. This was true whether or not the two outliers were included in the postmenopausal averages. Results are similar when expressed as absolute changes in FVC (Fig. 2). Furthermore, percentage and absolute changes in FBF reflected the differences observed in FVC before and during β-blockade within groups (Table 3).
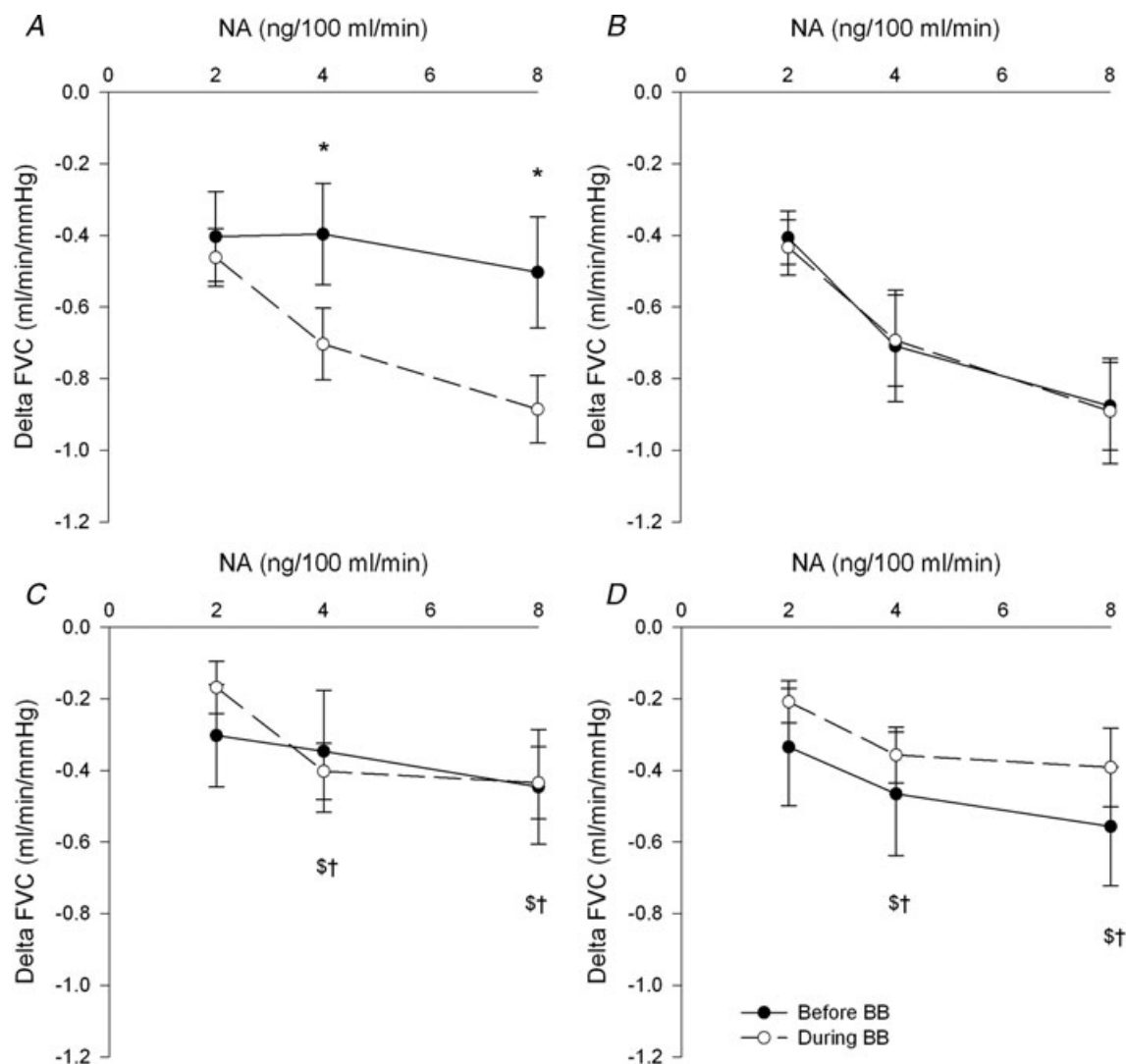

Figure 2. Delta forearm vascular conductance (FVC) responses to increasing doses of noradrenaline (NA) before and during β-blockade in: *A*, young women; *B*, young men; and postmenopausal (PM) women with outliers included (*C*, *n* = 15) and *D*, outliers excluded (*n* = 13)
*$P < 0.05$ *vs.* before β-blockade, $^\$P < 0.05$ *vs.* young women during β-blockade, $^\dagger P < 0.05$ *vs.* young men during β-blockade, $^\ddagger P < 0.05$ *vs.* young women before β-blockade. All comparisons between groups are *vs.* the same condition (for example during β-blockade in postmenopausal (PM) women *vs.* during β-blockade in young men). Removal of the PM outliers does not make any difference to this group's response to noradrenaline before and during β-blockade.

Table 3. Absolute and percentage changes in forearm blood flow (FBF) in response to noradrenaline (NA) before and during β-blockade (BB) in young men, young women and postmenopausal (PM) women

FBF response to NA	Young men ($n = 17$)		Young women ($n = 17$)		PM women ($n = 15$)	
	Before BB	During BB	Before BB	During BB	Before BB	During BB
Absolute ΔFBF (ml min^{-1} (100 ml)$^{-1}$)						
2	-0.42 ± 0.06	-0.38 ± 0.13	-0.50 ± 0.12	-0.43 ± 0.07	-0.19 ± 0.06	$-0.20 \pm 0.06^{\dagger\$}$
4	-0.63 ± 0.12	-0.62 ± 0.11	-0.57 ± 0.14	-0.67 ± 0.08	-0.33 ± 0.04	$-0.35 \pm 0.07^{\dagger\$}$
8	$-0.76 \pm 0.16^{\$}$	-0.77 ± 0.16	-0.63 ± 0.16	$-0.85 \pm 0.08^{*}$	$-0.41 \pm 0.06^{\dagger}$	$-0.44 \pm 0.1^{\dagger\$}$
% ΔFBF						
2	-17 ± 3	-21 ± 3	-20 ± 5	-21 ± 3	-9 ± 2	-12 ± 3
4	-25 ± 7	-30 ± 4	-21 ± 3	-33 ± 3	-20 ± 2	$-20 \pm 5^{\dagger\$}$
8	$-37 \pm 5^{\$}$	-36 ± 5	-21 ± 3	$-43 \pm 3^{*}$	$-24 \pm 3^{\dagger}$	$-22 \pm 5^{\dagger\$}$

$^{*}P \leq 0.05$ *vs.* pre β-blockade (i.e. within group), $^{\dagger}P \leq 0.05$ *vs.* young men, $^{\$}P \leq 0.05$ *vs.* young women. For example, the absolute change in FBF was greater in young men *vs.* young women at 8 ng (100 ml)$^{-1}$ min^{-1} before BB. Mean $\pm$ SEM. For changes in forearm vascular conductance see Figs 1 and 2.

Group differences in the vasoconstrictor response to noradrenaline. The percentage reduction in FVC before β-blockade was similar between men and women at 2 and 4 ng (100 ml)$^{-1}$ min^{-1} of noradrenaline, but was greater in young men at the final dose of noradrenaline. This difference was abolished during β-blockade. The percentage reduction in FVC to all doses of noradrenaline was lower in postmenopausal women *vs.* young men before and during β-blockade. The percentage reduction in FVC was greater in young women *vs.* postmenopausal women during β-blockade at the final two doses of noradrenaline. Again these differences were similar when expressed as absolute FVC (Fig. 2) or absolute and percentage change in FBF (Table 3).

Relationships between neural and haemodynamic variables before and during β-blockade

As previously demonstrated (Narkiewicz *et al.* 2005; Hart *et al.* 2009*a*) there was no relationship of MSNA to MAP before β-blockade among young men and women (Fig. 3), whereas there was a positive relationship between MSNA and MAP in the postmenopausal women ($r = 0.55$, $P < 0.05$). Notably, the relationship between MSNA and MAP among young women became positive during β-blockade ($r = 0.58$, $P < 0.05$, Fig. 3). Among young men the lack of relationship between MSNA and MAP persisted during β-blockade. During β-blockade, the relationship between MSNA and MAP was no longer significant in postmenopausal women.

As expected, before propranolol, the relationship between MSNA and TPR was positive in young men ($r = 0.64$, $P < 0.05$) but was not evident in young women ($P > 0.05$, Fig. 4). Interestingly, the relationship between MSNA and TPR among postmenopausal women was positive ($r = 0.61$, $P < 0.05$). During β-blockade, the relationship between MSNA and TPR became positive

in young women ($r = 0.59$, $P < 0.05$). The relationship between MSNA and TPR in young men and postmenopausal women remained positive during β-blockade.
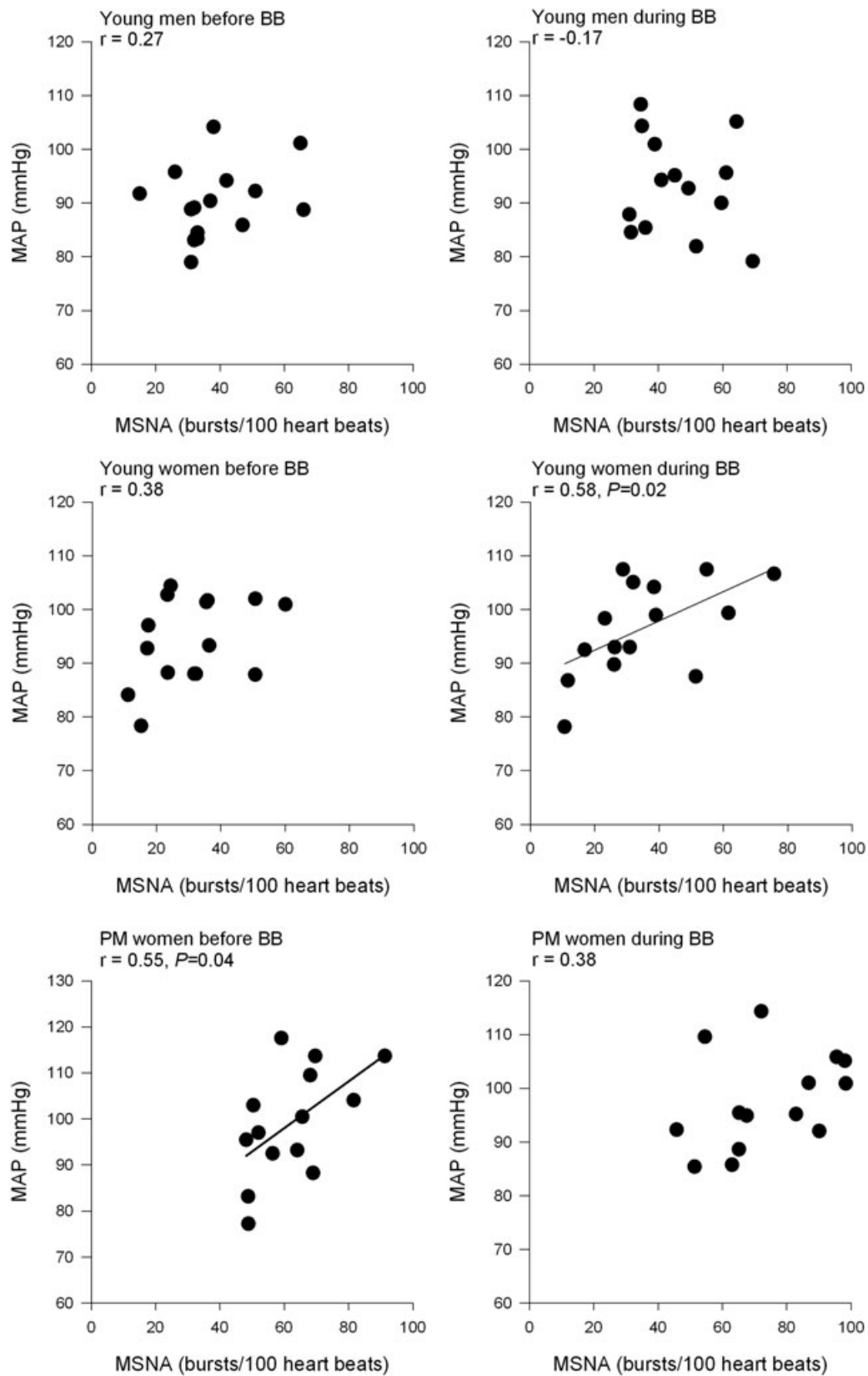
Before β-blockade, there was an inverse relationship between MSNA and cardiac output among young men ($r = -0.55$, $P = 0.04$) whereas no such relationship observed in the young women ($r = -0.14$, $P = 0.2$). Furthermore, there was no relationship between MSNA and cardiac output among the postmenopausal women ($r = -0.39$, $P = 0.15$). Propranolol infusion had no effect on relationships (or lack thereof) between MSNA and cardiac output observed in any group (young men; $r = -0.77$; $P = 0.001$, young women; $r = -0.23$; $P = 0.15$ and postmenopausal women; -0.43; $P = 0.13$).

Discussion

The major new findings of this study are: (a) there is a positive relationship of MSNA to TPR and MAP among postmenopausal women, (b) β-adrenergic blockade causes the relationship of MSNA to MAP and TPR to become positive in young women, which is similar to the relationship in postmenopausal women, and (c) the forearm vasoconstrictor responsiveness to noradrenaline is augmented in young women during β-blockade, but not in young men or postmenopausal women. These data suggest that enhanced β-adrenergic vasodilator responsiveness in young women is important in balancing the vasoconstrictor effects of MSNA on arterial pressure. This mechanism appears to be absent in postmenopausal women and young men.

Resting arterial pressure regulation and MSNA in postmenopausal women

In the present and previous studies we have found that MSNA is positively related to TPR but inversely related to

Young men before BB
r = 0.27

Young men during BB
r = -0.17

Young women before BB
r = 0.38

Young women during BB
r = 0.58, *P*=0.02

PM women before BB
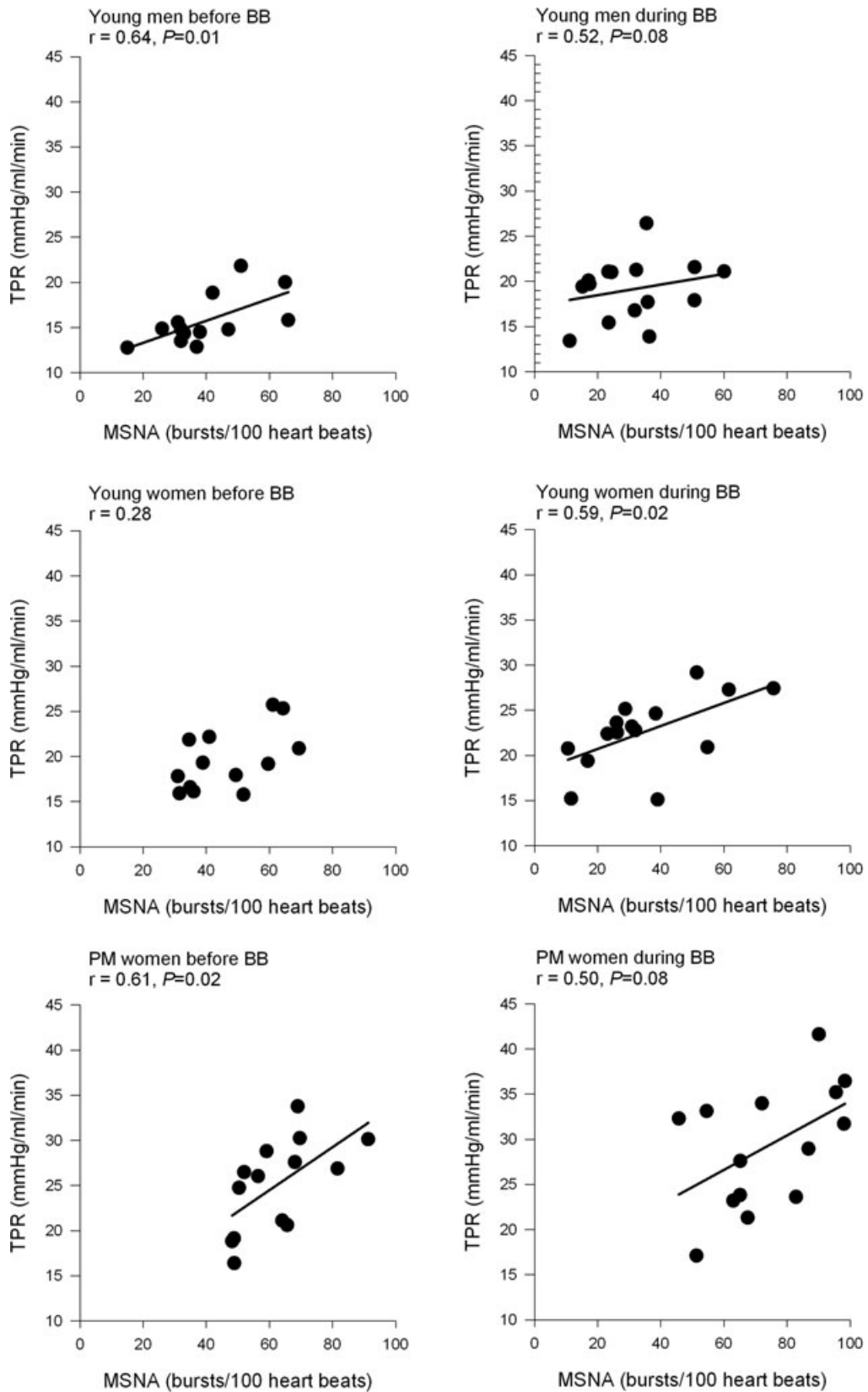r = 0.55, *P*=0.04

PM women during BB
r = 0.38

Figure 3.
Linear regression analysis of the relationship between muscle sympathetic nerve activity (MSNA) and mean arterial pressure (MAP) in young men (*n* = 14), young women (*n* = 15) and postmenopausal (PM) women (*n* = 15) before and during β-blockade (BB).

Figure 4.
Linear regression analysis of the relationship between muscle sympathetic nerve activity (MSNA) and total peripheral resistance (TPR) in young men, young women and postmenopausal (PM) women before and during β-blockade (BB).

cardiac output in young men. Thus, men with high MSNA and high TPR have a lower cardiac output (Charkoudian *et al.* 2005; Hart *et al.* 2009*a*). Consequently, the net effect of resting MSNA on the level of resting arterial pressure is minimal in young men. This is demonstrated by the lack of relationship between MSNA and arterial pressure in young men. Among young women, there is no relationship between MSNA and TPR, thus high (or low) levels of MSNA do not need to be balanced by cardiac output in young women (Hart *et al.* 2009*a*). This led us to hypothesize that other key factors are also important in determining vasoconstrictor tone in young women, including important influences of female sex hormones.

In the present study, we report that in postmenopausal women the relationship of MSNA to TPR is positive, but the lack of relationship between MSNA and cardiac output persists. Therefore, there is a positive relationship between MSNA and MAP in postmenopausal women, which has also been reported previously (Matsukawa *et al.* 1998; Narkiewicz *et al.* 2005). Consequently, postmenopausal women with high MSNA have a high TPR. In addition, because the vasoconstrictor effects of MSNA are not balanced by cardiac output in postmenopausal women, arterial pressure becomes directly related to MSNA. This is different to what has been observed in older men, where the relationship of MSNA to TPR and cardiac output does not exist (Hart *et al.* 2009*b*). Therefore, the effect of ageing on the interaction between the sympathetic nerves and the vasculature appears to differ between men and women. It is possible that other non-sympathetic factors may become more important in maintaining vasoconstrictor tone in older men, such as endothelin 1 and vasopressin. However, there is no scientific evidence to support this as yet. In contrast, in postmenopausal women, the sympathetic nerves may play a more dominant role in maintaining peripheral vasoconstrictor tone. Along these lines, we hypothesised that the female sex hormones (directly or indirectly) modify the transduction of MSNA into vaso-constrictor tone.

Sex differences in forearm vasoconstrictor responses: effect of the *β*-adrenergic receptors

Earlier studies investigating sex differences in adrenergic vasoconstrictor responsiveness demonstrated that young women demonstrated less forearm vasoconstriction to noradrenaline when compared to young men (Freedman *et al.* 1987). Kneale *et al.* (2000) demonstrated that this was due to greater *β*-adrenergic vasodilatation in young women, which balanced the *α*-adrenergic vaso-constrictor effect of noradrenaline. Thus, forearm vaso-constrictor responses to noradrenaline were enhanced in young women following *β*-blockade so their responses became similar to young men.

Our present findings are consistent with those of Kneale *et al.*, and we extend those findings to include implications for changes in *β*-adrenergic responsiveness with ageing and menopause. In our group of young women, the reduction in FBF during noradrenaline infusion was greater after *β*-blockade with propranolol. In young men there was no difference in FBF responses to noradrenaline before and after *β*-blockade. In addition, we report a key new finding: in post-menopausal women, the vasoconstrictor response to noradrenaline was similar before and during *β*-blockade. This suggests that in postmenopausal women, the ability of the *β*-adrenergic receptors to offset *α*-mediated vaso-constriction caused by noradrenaline is minimized or abolished.

Resting blood pressure regulation and the *β*-adrenergic receptors

Since the reduction in FBF and FVC to noradrenaline was greater after *β*-blockade in young women, we hypothesized that the *β*-adrenergic receptors are a key mechanism by which the effect of MSNA on the vasculature is modified. In the present study, we demonstrate for the first time that following *β*-adrenergic blockade, the relationship between MSNA and TPR became positive in the young women, so that it looked similar to the MSNA–TPR relationship in postmenopausal women (Fig. 4). In addition, MAP became positively related to MSNA during *β*-blockade in the young women (Fig. 3). Therefore, following *β*-adrenergic blockade, young women with higher MSNA had higher TPR and MAP. In the young men and postmenopausal women, the relationship between MSNA and TPR was positive, and did not change with *β*-blockade.

Our data therefore suggest that vascular *β*-adrenergic receptors (most probably vasodilating *β₂*-receptors) are fundamental to resting arterial pressure regulation in young women. Moreover, as women age the ability of the *β*-adrenergic receptors to offset the vasoconstrictor effects of MSNA disappears. Thus, TPR becomes related to the level of MSNA (Fig. 4). The loss of ability of the *β*-receptors to offset the vasoconstrictor effects of resting sympathetic traffic directed to the vasculature, combined with an increase in MSNA in postmenopausal women (Narkiewicz *et al.* 2005), probably contributes to the marked increase in risk of hypertension that occurs in women after menopause.

Sex hormones and the *β*-adrenergic receptors

Sherwood *et al.* (2010) recently demonstrated that cardiac *β*-adrenergic receptor responsiveness to noradrenaline was reduced in postmenopausal compared

to pre-menopausal women. Our data in the peripheral vasculature suggest that β-adrenergic receptor sensitivity to noradrenaline is reduced in postmenopausal women, although we did not assess this directly. There are several possible mechanisms which may lead to modulations in β-adrenergic receptor sensitivity in postmenopausal women. First, the increase in sympathetic nerve activity which occurs with age in women (Narkiewicz *et al.* 2005) may lead to decreased β-adrenergic sensitivity via agonist-mediated receptor downregulation (or desensitization). However, it is not possible to evaluate from our present data whether increases in sympathetic nerve activity are a result rather than the cause of receptor desensitization.

Second, it is possible that the female sex hormones have a direct effect on the β-adrenergic receptors. In this context, Ferrer *et al.* (1996) demonstrated that oestrogen supplementation in ovariectomised rats increased iso-proterenol (isoprenaline)-mediated relaxation in the rat mesenteric arteries. This response was abolished when propranolol was infused. Furthermore, increased vasodilatation to isoproterenol in the treated rats was not altered when the endothelium was removed, suggesting that oestrogen may enhance β-adrenergic receptor sensitivity through an endothelium-independent mechanism. Whether oestrogen (or other female sex hormones) has a similar direct effect on the vascular β-adrenergic receptors in humans is unknown. In addition to studies focusing on oestrogen, other animal-based experiments indicate that progesterone may increase β_2-adrenergic receptor expression in the myometrium (Vivat *et al.* 1992). Whether progesterone has a similar effect on the expression of β-adrenergic receptors in the vasculature is unknown. Finally, the female sex hormones may enhance β-adrenergic receptor sensitivity in women via an indirect mechanism. It is well known that the female sex hormones increase nitric oxide availability (Sudhir *et al.* 1996; Miller & Duckles, 2008). Since the β-adrenergic receptors cause vasodilatation partially through a nitric oxide mechanism (Cardillo *et al.* 1997; Ferro *et al.* 1999; Jordan *et al.* 2001; Eisenach *et al.* 2002), increased nitric oxide availability may increase β-adrenergic-mediated vasodilatation in the peripheral vasculature.

Other observations

We also noted that in postmenopausal women, there was reduced α-adrenergic receptor responsiveness to noradrenaline during β-blockade compared to young men and young women. This suggests that α-adrenergic receptor sensitivity in the forearm is reduced in post-menopausal women. This is similar to that previously reported in older men (Dinenno *et al.* 2002) where α-adrenergic receptor sensitivity in the forearm was

reduced compared to young men. However, this appeared to be specific to the forearm, as Dinenno *et al.* (2001) indicated that α-adrenergic receptor sensitivity in leg vasculature was preserved in healthy older men. Whether this occurs in postmenopausal women is unknown. It is important to determine whether there is a global decrease in α-adrenergic receptor sensitivity in post-menopausal women since these receptors are fundamental in blood pressure regulation. A decrease in sensitivity to noradrenaline in older women may explain why this group suffers from increased rates of orthostatic intolerance.

Possible mechanisms which may underlie the reduction in α-adrenergic receptor sensitivity include chronic over-stimulation of these receptors. Along these lines MSNA increases as women age, thus the receptors are chronically exposed to high levels of noradrenaline, which typically leads to desensitization, downregulation or changes in receptor subtype expression. However, it should be noted that from these data we cannot make inferences according to whether all or one α-subtype is desensitized (or downregulated) in our group of post-menopausal women.

Limitations

We used a systemic infusion of the non-selective β-antagonist, propranolol, to block the β-adrenergic receptors. Consequently, all β-adrenergic receptors were blocked, including those on the myocardium. Thus, cardiac output was reduced due to the effect of propranolol on heart rate. However, we do not feel this change in cardiac output limited our ability to interpret our data relative to the relationships among MSNA, TPR and arterial pressure in our groups of subjects.

Additionally, we focused on adrenergic vasoconstrictor responsiveness in the forearm before and during β-blockade. Consequently, these findings are only specific to this vascular bed. Whether α-adrenergic receptor responsiveness to noradrenaline is altered during β-blockade in other vascular beds in young and older women needs to be elucidated.

In addition, we used Modelflow analysis to determine stroke volume (and thus cardiac output) from the brachial arterial wave form. It is possible that this analysis may underestimate cardiac output compared to the more accepted thermodilution method in certain experimental conditions (Shibasaki *et al.* 2011). In our investigation individuals were resting in a normothermic environment. Under these conditions Modelflow-derived cardiac output has been shown to be similar to cardiac output derived from thermodilution. In our previous experiments focusing on resting neural–haemodynamic balance in men and women, we used the open-circuit acetylene approach to measure cardiac output. In the current study we have found similar relationships between

MSNA and cardiac output derived from the Modelflow in men and women compared to our previous study where we used the open circuit acetylene method. We did not use the open circuit method to measure cardiac output because the Modelflow technique simplified the logistical elements of this complex experiment.

Perspectives

According to the data presented in this study, the β-adrenergic receptors offset resting sympathetic nerve activity directed towards the peripheral vasculature in young women, and therefore may prevent increases in resistance when *resting* MSNA increases. However, in older women this phenomenom does not occur. In older women, MSNA is very high *vs.* young men and women. In addition MSNA becomes related to the resting level of arterial pressure. Thus, the lack of ability for the β-adrenergic receptors to offset vasoconstrictor tone may explain, in part, why the incidence of hypertension increases in this population (Burt *et al.* 1995). Furthermore, it is possible that since the β-adrenergic receptors do not balance high levels of MSNA, further increases in MSNA due to mental stress, for example, could explain why the risk of cerebrovascular and other cardiovascular-related events increase in ageing women in the UK and USA (Rothwell *et al.* 2005; Roger *et al.* 2011).

Conclusions

In summary, we report that the β-adrenergic receptors appear to offset the vasoconstrictor effects of sympathetic nerve activity in young women at rest. This mechanism appears to be minimal or non-existent in young men and in postmenopausal women. Our data therefore suggest that the β-adrenergic receptors play a fundamental role in resting arterial pressure regulation in young women. Furthermore, these changes in the contributions of β-adrenergic receptors with ageing, combined with an increase in MSNA, probably contribute to the marked increase in risk of hypertension that occurs in women after menopause.

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