Contribution of prostanoids to endothelium-dependent vasodilatation in the digital circulation of women with primary Raynaud’s disease

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ABSTRACT

In 15 women with PR (primary Raynaud’s) disease and in 15 matched control women, ACh (acetylcholine) was delivered by iontophoresis to the dorsum of the finger (seven 20 s pulses of 0.1 mA, followed by one 20 s pulse of 0.2 mA, applied at 60 s intervals). Cutaneous RCF (red cell flux) was recorded from the same site by the laser Doppler technique. ACh evoked progressive increases in RCF that were comparable in pre- and post-menopausal women with PR [maxima of $294 \pm 113$ and $259 \pm 59$ pu (perfusion units) respectively, $n = 7$ and 8 respectively], and in pre-menopausal controls (225 ± 92 pu, $n = 7$), but smaller in post-menopausal controls (140 ± 63 pu, $n = 8$; $P < 0.05$). Aspirin (600 mg, orally), a COX (cyclo-oxygenase) inhibitor, potentiated the ACh-evoked dilator responses in pre- and post-menopausal women with PR (343 ± 129 and 311 ± 48 pu respectively) and post-menopausal controls (277 ± 124 pu; $P < 0.05$), but had no effect in pre-menopausal controls (225 ± 92 pu). These results suggest that vasoconstrictor COX products limit ACh-evoked endothelium-dependent cutaneous dilatation in the digits in pre- and post-menopausal women with PR and in post-menopausal, but not pre-menopausal, control women. We propose that PR disease is associated with abnormality in the ability of oestrogen to modulate the synthesis of endothelium-dependent vasodilator and/or vasoconstrictor COX products.

INTRODUCTION

Raynaud’s phenomenon was first described by Maurice Raynaud more than 100 years ago as vasospasm that generally occurs in the fingers and is precipitated by cold or emotional stress. Since then, the diagnosis has been refined to more clearly differentiate PR (primary Raynaud’s) disease from secondary Raynaud’s phenomenon which manifests in a similar manner, but is associated with a range of connective tissue diseases [1]. The mechanisms underlying PR are still not clear. Raynaud originally suggested it was caused by hyper-reactivity of the sympathetic nervous system [1], whereas Lewis [2] argued that it was due to a ‘local fault’ at the level of the blood vessels. These two basic hypotheses persist, although ideas have gradually changed as to exactly how the sympathetic nervous system and local mechanisms might be involved.

In our own studies, an auditory stimulus and mild cooling of one hand both evoked the pattern of the

Key words: acetylcholine, endothelium, cyclo-oxygenase, prostanoid, Raynaud’s disease, vasodilatation.

Abbreviations: ABP, arterial blood pressure; ACh, acetylcholine; CGRP, calcitonin gene-related peptide; COX, cyclo-oxygenase; CV, coefficient of variation; EDHF, endothelium-derived hyperpolarizing factor; ET, endothelin; HR, heart rate; NO, nitric oxide; $O_2\cdot$, oxygen free radicals; PG, prostaglandin; PR, primary Raynaud’s; pu, perfusion units; RCF, red cell flux; DRCF, digital RCF; TXA$_2$, thromboxane A$_2$.

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alerting or defence response, which includes cutaneous vasoconstriction and forearm muscle vasodilatation, in subjects with PR and in age-matched controls. However, when the stimulus was repeated within an experimental session and over a period of 3 days, the controls showed habituation of the whole pattern of response, whereas, in those with PR, it persisted unchanged [3,4]. Furthermore, the mild cooling stimulus caused release of the vasoconstrictor peptide ET (endothelin) into the venous efflux of the cooled hand in those with PR but not controls. Thus we proposed that the tendency towards vasoconstriction is increased in PR by lack of habitation of cutaneous vasoconstrictor responses to everyday stimuli and accentuated release of ET-1 [4]. Consistent with these results, the component of sympathetic vasoconstriction that is mediated by α2 adrenoceptors is particularly pronounced in those with PR and α2-mediated vasoconstriction is facilitated by cooling [5,6]. Moreover, others reported graded ET release during graded cooling of one hand and high resting levels of ET in subjects with PR [7,8].

The latter findings are of particular interest in relation to endothelial function in the cutaneous vessels. Cleavage of ET from big ET, and therefore the release of ET, is normally inhibited by NO (nitric oxide). Moreover, the vasoconstrictor action of ET is limited by its action on endothelial ET receptors which stimulate NO synthesis and release [9]. Thus an obvious possibility is that cutaneous vasoconstrictor responses are exacerbated in PR disease because the mechanisms that produce endothelium-dependent dilatation are deficient.

In this connection, dilator responses evoked in the finger skin by iontophoresis of ACh (acetylcholine) were depressed in subjects with PR relative to controls [10]. This depression was not alleviated by oral supplementation, suggesting there is no deficit in the substrate available for NO synthesis in PR disease. However, digital dilatation evoked by iontophoresis of the NO donor, sodium nitroprusside, was also reduced in subjects with PR relative to controls. Furthermore, in our pilot study, dilator responses evoked in the finger by iontophoresis of ACh were of comparable magnitude in subjects with PR and their controls, but those evoked by sodium nitroprusside were again reduced in the subjects with PR. These results suggest that the actions of NO may be impaired in PR patients [11]. Given that ACh can evoke dilatation by releasing NO, PGs (prostaglandins) and/or EDHF (endothelium-derived hyperpolarizing factor) [12], the possibility raised is that the contribution of NO to endothelium-dependent dilatation is reduced in PR disease, but is compensated for by increased involvement of vasodilator PGs and/or EDHF.

Thus the major aim of the present study was to test cutaneous vascular responses evoked by iontophoresis of ACh in individuals with PR disease and their controls before and after a supramaximal dose of aspirin, which attenuates the COX (cyclo-oxygenase) pathway that produces PGs and thromboxane [13]. In control male subjects, COX inhibition had no effect on, or reduced, cutaneous dilator responses evoked by iontophoresis of ACh in the forearm [14,15]. By contrast, in young healthy male subjects, COX inhibition potentiated vasodilator responses evoked in the finger by iontophoresis of ACh, suggesting that, in young men, this digital cutaneous dilator response is limited by a vasoconstrictor product of the COX pathway [16]. The present study was performed on women with PR and age-matched control women. Although estimates of the prevalence of PR disease in the general population vary considerably, large epidemiological studies indicate the prevalence is at least twice as high in women as in men [17].

**METHODS**

Experiments were performed on 18 women with PR disease and 18 control women. The women with PR were recruited through the Rheumatology Department, University Hospital Trust, Birmingham, U.K. as volunteers from among University staff and students or from the Raynaud’s & Sclerodema Association. The controls were academic or support staff, or students of the University of Birmingham and were not related to those with PR. The patients had all been medically diagnosed using the criteria suggested by Allen and Brown [18] as in our previous studies [3,4]. In addition, they were also negative for anti-nuclear antibody and so considered unlikely to develop a connective tissue disorder [19]. All subjects had nail fold capillary patterns that demonstrated absence of connective tissue disease. The women with PR had no other known cardiovascular, neural or cutaneous disorders, and the control subjects were apparently fit and healthy. All women were non-smokers and drank less than 10 units of alcohol/week. None was taking any medication, including anti-inflammatory agents, the contraceptive pill or hormone replacement therapy. All subjects were asked about their menstrual cycle and meno-pausal status. Women were categorized as post-menopausal if > 2 years had elapsed since their last period. The others were experiencing regular menstrual cycles. They were asked to give details of the cycle and were not experimented upon within 2–3 days of the days we estimated represented ovulation and menstruation; at these times, high circulating oestrogen and/or progesterone levels have been shown to significantly affect vascular responsiveness [20]. On the day of the experiment, all subjects were asked to refrain from eating a heavy meal, drinking caffeinated drinks or undertaking physical exercise for at least 2 h prior to arriving at the laboratory.

All studies were approved by the Ethics Committee of the South Birmingham Health Authority and confirmed...
with the Declaration of Helsinki. Each subject was given written and verbal information about the protocol before attending, and familiarized with the equipment, before giving written consent.

Experimental methods

Experiments were performed in a small temperature-controlled room (21 ± 1°C; value is mean ± S.E.M.), all displays of recorded variables were out of sight of the subject and visual and auditory stimuli were kept to a minimum [3,4,7]. The subject reclined on a couch in a comfortable semi-supine position, with both arms supported at heart level. Cutaneous RCF (red cell flux) was recorded continuously from the dorsal surface of the finger (second or third digit, see below) of the left hand, and ACh or its vehicle was applied by iontophoresis to the same finger using methodology described in detail previously [16]. Briefly, a Perspex ring-shaped electrode chamber (Moor Instruments) was fitted to the dorsum of the finger with a double-sided adhesive ring, and an indifferent electrode mounted in a strap was fitted to the ipsilateral wrist. The chamber was fitted with either ACh or its vehicle, and a laser Doppler probe connected to a meter (DRT 4; Moor Instruments) was fitted into the chamber. The electrode chamber and indifferent electrode were connected to an iontophoresis controller (MICI; Moor Instruments). The outputs of the laser Doppler meter and the iontophoresis unit were connected to a computer (Power Mac 1600/60) via MacLab software (MacLab/8c) sampling at 4 Hz. The data were displayed in chart form using MacLab software enabling observation and analysis on- and off-line.

In addition, skin temperature was measured continuously from a thermostor connected to an electronic digital thermometer (3750 TH; Digitron), which was fixed to the first finger of the left hand. Furthermore, a semi-automatic sphygmomanometer was applied to the right forearm and used to record ABP (arterial blood pressure) and HR (heart rate) at intervals during the experiment (see below).

Protocols

Iontophoresis of ACh and its vehicle before and after aspirin treatment was performed on 15 women with PR (pre-menopausal, 34.4 ± 10 years; post-menopausal, 62.2 ± 7.9 years) and on 15 control women (pre-menopausal, 34.3 ± 10.3 years; post-menopausal, 60.9 ± 8.4 years; all values are means ± S.E.M.). All subjects rested for at least 30 min until the recorded variables had stabilized. ABP and HR were then measured three times, and the average values were used as a measure of resting ABP and HR. Skin temperature was also recorded, and a 60 s period of RCF was recorded. The iontophoresis protocol was then begun for either ACh or its vehicle. The order of applying ACh or vehicle and the finger to which each was applied (second or third finger) was randomized between experiments; a different iontophoresis chamber was used for ACh and for the vehicle to prevent contamination of the vehicle chamber with ACh. The iontophoresis protocol was comparable with that used by Morris and Shore [14] and Hendry and Marshall [16]. The electrode in the chamber was made positive as ACh carries a positive charge: seven 20 s pulses of 0.1 mA were delivered, followed by a final pulse of 0.2 mA for 20 s, with a 60 s rest period between each pair of pulses. At the end of the iontophoresis protocol when RCF had stabilized, further measurements of ABP, HR and skin temperature were made. A decision had been taken at the outset of these experiments that any patient or subject who showed a change in skin temperature, ABP or HR between the beginning and end of the iontophoresis protocol would be excluded from the analysis: in practice, this did not happen. The iontophoresis protocol was then repeated for either ACh or vehicle on the other finger.

After this, the subject took 600 mg of aspirin dissolved in 250 ml water and was allowed to rest in the semi-supine position for 30 min. The iontophoresis protocols for ACh and its vehicle were then repeated as described above at the same sites on the second and third fingers.

To investigate the effect of time on the responses evoked by ACh, the above protocol was performed on a group of three women with PR and three control women, but they simply experienced a 30 min rest period between successive iontophoresis protocols and did not take aspirin.

Drugs

ACh (Miochol) was obtained from CIBA Visions. The vehicle for ACh in this preparation was 3 % mannitol in sterile water. Thus, to test the effect of vehicle alone, mannitol (10% in water) was obtained from Baxter Healthcare and was diluted to a 3 % solution with water for injection (Braun Medical Ltd). Soluble aspirin was obtained in 300 mg tablets from The Boots Company plc, and two tablets were dissolved in 250 ml of tap water. All drugs were prepared immediately prior to use.

Analysis of results

All data are expressed as means ± S.E.M. Baseline values for each recorded variable before and after aspirin, or a 30 min rest period, were collected prior to the iontophoresis protocols, as described above. During the iontophoresis protocols, an RCF reading was taken at the end of the 60 s period between successive pulses and 60 s after the last pulse when RCF had stabilized. These absolute values were recorded (see Figures 1A and 1B). In addition, the values recorded during the iontophoresis of vehicle were subtracted from those taken during iontophoresis of ACh to indicate the effect of ACh alone (see Discussion, and [14]); these values had the appropriate baseline RCF subtracted from them to give
Table 1 Baseline values of recorded variables in women with PR and control women

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<thead>
<tr>
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<th>Control women</th>
<th>Women with PR disease</th>
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<tbody>
<tr>
<td>DRCF (pu)</td>
<td>53.3 ± 13.3</td>
<td>44.3 ± 11.1</td>
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<tr>
<td>Systolic pressure (mmHg)</td>
<td>130 ± 17.6</td>
<td>129 ± 10.7</td>
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<tr>
<td>Diastolic pressure (mmHg)</td>
<td>75.1 ± 10.3</td>
<td>66.8 ± 11.8</td>
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<tr>
<td>Mean ABP (mmHg)</td>
<td>90.1 ± 10.0</td>
<td>86.0 ± 9.6</td>
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<tr>
<td>HR (beats/min)</td>
<td>65.4 ± 12.6</td>
<td>66.0 ± 8.0</td>
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<tr>
<td>Skin temperature (°C)</td>
<td>28.1 ± 3.4</td>
<td>27.3 ± 3.9</td>
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Values are means ± S.E.M., n = 18 for each group. There were no significant differences between the groups.

The baseline values within groups were compared by paired Student’s t test, and between groups were compared by unpaired Student’s t tests. Comparisons within groups, for the effects of aspirin or time, and between groups, for responses evoked in the absence of aspirin by iontophoresis of vehicle or ACh, or for responses evoked by ACh minus vehicle, were made by ANOVA for repeated measures. These values were compared by paired or unpaired Student’s t test as appropriate. In all cases, P < 0.05 was considered to be significant. Sample size calculations indicated that seven subjects were required to provide a 90% probability of detecting a difference between RCF responses of one S.D. within or between groups at the 5% level. In addition, for the time control protocol, the CV (coefficient of variation) was calculated for the maximum response to ACh to assess the reproducibility of the response between the first and second application.

RESULTS

The baseline values for the complete group of women with PR and their controls are shown in Table 1. There were no significant differences between the groups. None of the women was hypertensive (systolic pressure > 140 mmHg and diastolic pressure > 90 mmHg).

In both groups, iontophoresis of ACh produced a progressive increase in RCF. Iontophoresis of vehicle had no significant effect on either group, although there was an apparent trend for an increase in the controls (Figure 1A and 1B). Not surprisingly, the change in RCF from baseline evoked by ACh minus vehicle showed a progressive increase in each group (Figure 1C). In the description below, we have concentrated on the responses evoked by ACh minus vehicle: these changes being described as responses evoked by ACh for the sake of simplicity. The statistically significant effects we have described were the same when the statistical analyses were performed on the absolute increase in RCF evoked by ACh alone (results not shown).

When the complete group of women with PR was compared with the complete group of controls, the responses evoked by ACh in the control subjects were smaller (Figure 1C). The explanation for this is clear when the groups are divided on the basis of age and menopausal status. There were no significant differences between the baseline values in the pre- and post-menopausal women with PR and controls (Table 2). However, RCF responses evoked by ACh in pre- and post-menopausal women with PR and pre-menopausal controls were comparable, but responses evoked in the
Table 2  Baseline values of recorded variables in subgroups of women with PR disease and control women

Values are means ± S.E.M., n = 7 for each younger pre-menopausal group, and n = 8 for each older post-menopausal group. There were no statistically significant differences between values recorded before and after aspirin in any group. *P < 0.05 between women with PR and their controls after aspirin.

<table>
<thead>
<tr>
<th></th>
<th>Pre-menopausal women</th>
<th>After aspirin</th>
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<tr>
<td></td>
<td>Control women</td>
<td>Women with PR disease</td>
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<tr>
<td>DRCF (µu)</td>
<td>52.2 ± 19.3</td>
<td>41.0 ± 12.2</td>
</tr>
<tr>
<td>Mean ABP (mmHg)</td>
<td>84.8 ± 6.7</td>
<td>86.9 ± 3.9</td>
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<tr>
<td>HR (beats/min)</td>
<td>61.2 ± 7.1</td>
<td>65.1 ± 2.5</td>
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<tr>
<td>Skin temperature (°C)</td>
<td>28.5 ± 1.9</td>
<td>26.6 ± 1.2</td>
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<th>Post-menopausal women</th>
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<tr>
<td></td>
<td>Control women</td>
<td>Women with PR disease</td>
</tr>
<tr>
<td>DRCF (µu)</td>
<td>46.4 ± 20.7</td>
<td>49.0 ± 11.9</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>92.0 ± 5.5</td>
<td>90.6 ± 5.4</td>
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<tr>
<td>HR (beats/min)</td>
<td>68.6 ± 5.9</td>
<td>68.5 ± 4.0</td>
</tr>
<tr>
<td>Skin temperature (°C)</td>
<td>29.3 ± 1.9</td>
<td>26.6 ± 1.9</td>
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post-menopausal control subjects were significantly smaller (P < 0.05) than those evoked in the other subgroups (see Figure 3).

Aspirin had no significant effect on the baseline of any of the recorded variables in either the complete groups of women with PR or controls (results not shown), or in the subgroups (Table 2). After aspirin, there were still no differences between pre-menopausal women with PR and their controls, but post-menopausal women with PR had lower baseline cutaneous RCF than their controls. In the complete groups of women with PR and controls, aspirin potentiated responses evoked by ACh (Figures 2A and 2B). Similarly, analysis of the subgroups revealed that, in both pre- and post-menopausal women with PR, aspirin potentiated the increases in RCF evoked by ACh (Figures 3B and D). By contrast, although a similar effect of aspirin occurred in the post-menopausal controls (Figure 3C), aspirin had no effect on RCF responses evoked by ACh in the pre-menopausal controls (Figure 3A).

In the ‘time-control’ group, the 30 min rest period between the iontophoresis protocols had no effect on the baseline values (results not shown). Furthermore, there was no difference between the RCF responses evoked by ACh before and after the 30 min rest period (Figure 2C). The CV of the maximum responses was 3.2 ± 0.6 %.

DISCUSSION

The main findings of the present study were that iontophoresis of ACh into the dorsal surface of the finger evoked substantial cutaneous vasodilatation both in women with PR and their controls; the dilatation was not depressed in the women with PR relative to the controls. Furthermore, aspirin, which inhibits COX, potentiated this ACh-evoked dilatation in pre- and post-menopausal women with PR and in post-menopausal, but not in pre-menopausal, control women. Before discussing these findings, it is important to consider the limitations of the study.

Limitations of the study

In the time-control protocol, the CV for the maximum response evoked by repeat iontophoretic application of ACh at the same site on the finger within the group of women with PR and controls was only 3.2 ± 0.6 %. Similar calculations of the CV from the time-control protocol for repeat iontophoretic application of ACh to the finger in our recent study on young men [16] yield a value of 2.8 ± 0.6 % (R. Hendry and J. M. Marshall, unpublished work). In on-going experiments on five young women controls we have obtained a value of 2.4 ± 0.7 %. These values are much lower than the CV of 42.0 ± 26.1 % reported for the maximum response to repeat application of ACh to the forearm in the study of Morris and Shore [14], which involved the same iontophoresis and laser Doppler equipment and essentially the same protocol as we have used. The pronounced disparity might be explained by the fact that we applied ACh to the finger or that our subjects were women, whereas Morris and Shore [14] used the forearm and a mixed group of men and women. However, it seems likely that the main reason is that we applied ACh to the same site on the finger each time, whereas Morris and Shore [14] compared responses evoked at two or three different
Effects of aspirin on responses evoked by ACh in women with PR and control women

Abscissae and ordinates as in Figure 1(C). (A and B) Responses (means ± S.E.M.; \( n = 15 \)) evoked in controls (A) and women with PR (B) before and 30 min after aspirin. In each case, dotted lines are used for data obtained after aspirin. (C) Responses evoked in a mixed group of women with PR (\( n = 6 \)) and control women (\( n = 8 \)) before and after a 30 min rest period in the absence of aspirin. †† \( P < 0.01 \) for the responses evoked before compared with after aspirin.

Figure 2

In view of all these factors, it seemed appropriate to present our results as the response to ACh at one site on the finger minus the response to vehicle at a site on a different finger, just as Morris and Shore [14] did, but by using different sites on the forearm. We acknowledge it would have been preferable to apply vehicle and ACh in vehicle to the same site on the same finger and that the response to the vehicle and ACh may not be simply additive (see [14]). Nevertheless, the approach we chose was the only practical way a priori of attempting to exclude any difference between the women with PR and controls in their response to the vehicle, and of differentiating any effect aspirin had on the response to the vehicle, from its effect on the response to ACh. In fact, the conclusions we can draw are the same whether the analysis is done on the response to ACh minus vehicle or
on the response to ACh alone (see the Results section). It is therefore very unlikely that the differences we describe in the digital dilator responses to ACh between the women with PR and control women reflect differences in sensory nerve-mediated dilatation.

Turning to the baseline values of the cardiovascular variables, lower DRCF (digital RCF) values and skin temperature might have been expected in the women with PR than the controls: they were not significantly different. This was also the case in our previous studies on relatively small groups of subjects with PR and their controls and in some, but not all, studies performed by other groups (see [3] for further discussion). Given the variance of the data, larger group sizes would probably have been required to detect any significant differences. This was not an objective of the present study. Rather, we aimed to test the effect of aspirin on the changes in DRCF evoked by ACh.

Responses evoked by ACh

Comparison between the complete groups of subjects might simply suggest that digital cutaneous dilator responses evoked by ACh in the women with PR are larger than in the controls. However, this difference can be attributed to the responses evoked in the post-menopausal control women as they were depressed relative to those evoked in the other subgroups; the responses evoked in pre-menopausal controls and both subgroups of women with PR were similar.

It is difficult to compare these results with previous findings as, even in studies in which the groups of subjects with PR and controls were age- and sex-matched, the results were not considered in relation to the age, sex or menopausal status of the individuals. Thus dilator responses evoked by iontophoresis of ACh in the cutaneous circulation of both the finger and the forearm were similar in mixed groups of male and female subjects with PR and controls [22,23]. By contrast, in mixed male/female (three/seven respectively) groups of subjects with PR and controls, ranging in age from 30–62 and 25–60 years respectively, dilator responses evoked in the finger by iontophoresis of ACh were depressed in those with PR relative to the controls [10]. On the other hand, in 12 women with PR disease (24–66 years of age), dilator responses evoked in forearm skin and in finger tip by intra-arterial infusion of ACh were enhanced compared with those evoked in a control group comprising eight women and six men (21–57 years of age) [24]. Finally, when we used the same iontophoresis protocol as in the present study, the dilator responses evoked by ACh in 14 women with PR (mean age 42.0 ± 3.3 years) and in 14 age-matched control women (mean age 41.9 ± 3.6) were apparently comparable [11]. All of these apparently disparate findings might have been compatible with the present findings had age, sex and menopausal status been taken into consideration.

The present results allow two important proposals. First, endothelium-dependent dilatation induced in the finger is not depressed in women with PR compared with their own controls, even though PR disease has been associated with endothelial dysfunction [1,25]. Secondly, women with PR do not show the depression of ACh-evoked digital dilatation that occurs in control women in association with aging and/or the menopause.

That the digital cutaneous response to ACh was depressed in post-menopausal, relative to pre-menopausal, control women is consistent with previous reports that forearm cutaneous dilator responses to ACh decrease with age in both women and men [26] and that vascular responses in whole forearm to intra-arterial infusion of ACh are decreased in old relative to young control men [27]. More specifically, dilator responses evoked in whole forearm by intra-arterial infusion of ACh were progressively depressed with aging in male control subjects from approx. 30 years old, but in women they were progressively depressed only after the menopause [28–30]. Furthermore, in pre-menopausal women, the ACh-evoked dilator responses were depressed 1 month after ovariectomy, but restored 3 months after oestrogen replacement therapy [31]. Thus it seems reasonable to propose that in normal women the deleterious effect of aging on endothelium-dependent dilator responses in forearm and in the finger is abrogated until the menopause by the facilitatory effects of female hormones, particularly oestrogen.

Seen in this light, our finding that digital cutaneous dilator responses to ACh were similar in pre- and post-menopausal women with PR suggests that the effects of aging on these responses are weaker in PR disease and/or that women with PR do not show the normal facilitatory effect of oestrogen on endothelium-dependent dilatation (for further discussion, see below).

Effects of aspirin

Any changes in the baseline levels of cutaneous RCF after aspirin did not achieve statistical significance in the complete groups of control women or women with PR, or in the subgroups. Given the variance of the data, larger group sizes would have been required to detect any differences. In previous studies on control subjects who were predominantly male, aspirin similarly had no effect on baseline cutaneous RCF in the forearm [14,15]. There have been no comparable studies on the finger or in subjects with PR. After aspirin, baseline cutaneous RCF was significantly lower in post-menopausal women with PR than their controls, but any difference between pre-menopausal women with PR and their controls did not reach significance. There is no reason to suppose that differences in baseline RCF values between subgroups were responsible for the different effects of aspirin on responses evoked by ACh in the subgroups, as responses in post-menopausal women with PR and their controls...
were similarly affected, whereas those in pre-menopausal women with PR and their controls were not.

In the control women, aspirin had no effect on digital cutaneous responses evoked by ACh in the pre-menopausal controls, but potentiated those evoked in the post-menopausal controls. Thus it seems that, in the pre-menopausal women, either ACh did not stimulate the synthesis of vasoactive COX products or the effects of vasodilator and vasoconstrictor COX products counteracted one another. By contrast, vasoconstrictor COX products whose synthesis was stimulated by ACh were apparently largely responsible for the depressed dilator responses seen in the post-menopausal control women (see Figure 3).

In previous studies performed predominantly on forearm cutaneous circulation of male subjects who were less than 50 years old, aspirin either had no effect [14,32] or reduced dilator responses evoked by iontophoresis of ACh, implicating vasodilator prostanoids in the response [15,33]. By contrast, in young male control subjects, we [16] recently showed that aspirin potentiated cutaneous vasodilation evoked in the finger by ACh, suggesting vasoconstrictor COX products limited this dilatation. Taken together, these results suggest that the balance is more easily tipped in favour of the production of vasoconstrictor COX products limited this dilatation.

Considering the age-related depression of dilator responses evoked by intra-arterially infused ACh in the whole forearm of men, this was reversed by L-arginine supplementation in the early stages (30–60 years old), implying a deficit in the substrate available for NO-induced dilatation, but was ameliorated in men of >60 years old by inhibition of COX [30,34]. Moreover, in pre-menopausal women, COX blockade had no effect on dilator responses evoked in the whole forearm by intra-arterial ACh, whereas, after ovariectomy, COX blockade potentiated these responses, again implicating vasoconstrictor COX products [35]. Thus placing the present findings in the context of these results allows the proposal that, in pre-menopausal control women, the ability of vasoconstrictor COX products to limit ACh-evoked dilatation in the digital cutaneous circulation is opposed by the actions of oestrogen, whereas, in post-menopausal control women, the influence of oestrogen on cutaneous circulation as in whole forearm circulation [35].

Turning to the women with PR, the fact that aspirin potentiated digital cutaneous dilator responses to ACh in both the pre- and post-menopausal women allows the novel proposal that women with PR lack these same opposing effects of oestrogen on the synthesis or action of vasoconstrictor COX products.

The COX pathway in endothelial cells can produce several different vasoconstrictor products, including vasoconstrictor PGs (prostaglandins), TXA₂ (thrombox-
endothelium-dependent dilatation evoked by ACh in the finger is not depressed in women with PR. Indeed, these ACh-evoked dilator responses were potentiated in post-menopausal women with PR relative to their post-menopausal controls and were depressed in these controls relative to all other subgroups. Moreover, aspirin potentiated the ACh-evoked dilator responses in pre- and post-menopausal women with PR, but had this effect only in the post-menopausal control women. We propose that, in women with PR, the facilitatory effect of oestrogen on endothelium-dependent cutaneous dilatation is impaired and/or the ability of oestrogen to promote the synthesis of vasoconstrictr COX products is accentuated.

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