Local nitric oxide release does not affect tachyphylaxis to angiotensin II in dorsal hand veins in man in the presence of prostaglandin synthesis inhibition

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Aims Local prostaglandin (PG) production contributes to tachyphylaxis to angiotensin II (ANGII) in veins. Our aim was to assess the hypothesis that local nitric oxide (NO) generation is also, in part, responsible for tachyphylaxis to ANGIO in veins, using the Aellig dorsal hand vein technique.

Methods Eight healthy male volunteers received 600 mg of aspirin (orally) to inhibit PG production. The venoconstrictor effects of ANGIO and noradrenaline (NA) were then compared in dorsal hand veins during co-infusion of the NO synthase inhibitor L-NMMA or saline, on separate occasions.

Results ANGIO and NA produced a similar degree of initial venoconstriction. However, the response to ANGIO was significantly attenuated by 12 min compared with NA (AUC 147 ± 38 vs 196 ± 40 , respectively; [95% confidence interval for difference: 7, 92], $P=0.02$). Infusion of L-NMMA did not influence the response to ANGIO or NA ($P=0.2$ and $P=0.3$, respectively).

Conclusions Tachyphylaxis to ANGIO in dorsal hand veins is not dependent on local NO release.

Keywords: angiotensin II, endothelium, human, L-NMMA, prostaglandins, tachyphylaxis, veins

Introduction

The renin-angiotensin system plays an important role in cardiovascular homeostasis. Indeed, angiotensin II (ANGII) is one of the most powerful endogenous vasoconstrictors in man. However, responses to ANGIO develop rapid desensitization (tachyphylaxis) in veins [1] but not in human resistance arteries [2, 3].

Recent evidence suggests that vasodilatory prostaglandins (PG), which are released by ANGIO [4], are involved in tachyphylaxis to ANGIO in humans, as pre-treatment with indomethacin reduces, but does not abolish, tachyphylaxis [5]. However, ANGIO also induces NO release in both arteries and veins [4]. Therefore, we

hypothesized that NO release may, in part, be responsible for tachyphylaxis of ANGIO in veins.

The aim of the present study was to test this hypothesis in dorsal hand veins *in vivo*, using L-NMMA as an inhibitor of local NO generation and noradrenaline (NA) as a comparative venoconstrictor, after inhibition of PG production with oral aspirin.

Methods

Subjects

Eight healthy, non-smoking, male volunteers, of mean age 30 years (range: 22–53) were recruited. The study was approved by the Local Research Ethics Committee, and written informed consent was obtained from each subject.

Drugs

All drugs, except for aspirin, were prepared aseptically from fresh opened vials, using 0.9% saline (Baxter, Norfolk, UK). Angiotensin II (Clinalfa, Laufelfingen,

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Switzerland) was infused intravenously at a dose of 50 pmol min^{-1} , as previous studies have reported tachyphylaxis with this dose [5]. Noradrenaline (Levophed, Abbott, Maidenhead, UK) was infused at a dose of 50 pmol min^{-1} [1]. N^G -monomethyl-L-arginine (L-NMMA, Clinalfa, Laufelfingen, Switzerland), a specific substrate analogue inhibitor of NO synthase (NOS), was administered at a dose of $100 \text{ nmol min}^{-1}$ [6]. The total rate of intravenous infusion was kept constant throughout all studies at 0.25 ml min^{-1} . Acetylsalicylic acid, an irreversible inhibitor of cyclo-oxygenase (Aspirin; 600 mg soluble tablets; AH Cox & Co Ltd, Barnstaple, UK) was suspended in 200 ml water and administered orally 30 min before other drugs were infused, to inhibit PG generation. When given at this dose, aspirin inhibits bradykinin-stimulated endothelial prostacyclin but has no direct effects on blood pressure or basal venous tone [7].

Protocol

The study was conducted in a single-blind, randomized manner, with two visits separated by 1 week. Subjects abstained from alcohol and caffeine for 24 h before each visit, and all studies were conducted after an overnight fast, following 20 min rest in a semirecumbent position, in a quiet room, maintained at a constant temperature ($25\text{--}27^\circ \text{C}$). The left hand was supported above the level of the heart. A non-branching dorsal hand vein was cannulated with a 22-gauge 'butterfly' needle (Abbott, Queensborough, UK) in the direction of flow and the same vein was used at both visits. The internal diameter (ID) of the vein, distended by inflation of an upper arm cuff to 40 mmHg, was measured by a light-weight magnetized rod resting on the summit of the vein using the technique of Aellig [8], as described previously [9].

On each occasion, aspirin was administered orally, and saline was then infused for a minimum of 20 min, until a stable baseline (two readings within 10% of each other) was obtained [9]. On one occasion saline, and on the other L-NMMA ($100 \text{ nmol min}^{-1}$), was infused, in a randomized order, together with ANGII (50 pmol min^{-1}) and NA (50 pmol min^{-1}), which were each infused for 12 min. Hand vein ID was recorded every 3 min, and a minimum washout period of 15 min was observed between drugs, to allow ID to return to baseline. The order of the ANGII and NA infusions was randomized, but kept the same for each individual subject.

Statistical analysis

Data are expressed as means $\pm$ s.e. mean (mV) or [95% confidence interval for differences]. The area under the curve (AUC) was calculated, using the trapezoid method, and statistical analysis was performed using paired Student's

t-tests to compare differences between noradrenaline and ANGII. Analysis of variance (ANOVA) for repeated measurements was used to compare differences between drug infusions with and without L-NMMA. $P < 0.05$ was considered significant.

Results

The hand vein diameter did not differ significantly between baseline and the last washout measurement (ID: 2.3 ± 0.3 vs $2.4 \pm 0.4 \text{ mV}$; $P = 0.7$), or at baseline between study days (ID: 2.6 ± 0.2 vs $2.3 \pm 0.3 \text{ mV}$; $P = 0.5$).

When co-infused with saline, NA caused a significant venoconstriction ($56 \pm 12\%$ from baseline to 3 min; $P = 0.002$), which was stable throughout the 12 min infusion period ($P = 0.9$; ANOVA). In contrast, although ANGII and NA caused similar degrees of initial venoconstriction (mean difference 6 [−15, 27]%; $P = 0.5$), thereafter, the response to ANGII was significantly attenuated (50 ± 13 at 3 min vs $32 \pm 9\%$ at 12 min; [−1, −35] $P = 0.043$). The mean AUC for the NA infusion was significantly greater than for ANGII (196 ± 40 vs 147 ± 38 , respectively [7, 92]; $P = 0.023$), in keeping with the reduced venoconstrictor effect of ANGII with time. The responses to NA and ANGII (Figures 1 and 2) were similar during co-infusion of L-NMMA and saline (mean difference 45 [−42, 134] $P = 0.3$; and 24 [−17, 64] $P = 0.2$, respectively).

Discussion

The main findings of the present study are the confirmation that the venoconstrictor response to ANGII but not NA undergoes tachyphylaxis in dorsal hand veins, but that local NO generation is not involved in this phenomenon.

We have confirmed earlier reports of rapid tachyphylaxis to ANGII but not to NA in the dorsal hand vein, during a constant infusion [1, 5]. The mechanism responsible for tachyphylaxis to ANGII has not been fully elucidated, but may involve local PG and NO generation by the venous endothelium in response to ANGII-induced venoconstriction. Indeed, although PG and NO do not contribute to resting tone in dorsal hand veins *in vivo*, ANGII induces release of PG and NO from venous and arterial endothelial cells *ex vivo* [4]. Moreover, pre-treatment with indomethacin, which inhibits PG generation, has been recently shown to attenuate, but not to abolish, the tachyphylaxis to ANGII in human dorsal hand veins *in vivo* [5]. However, the role of NO production in the development of tachyphylaxis has not been previously investigated *in vivo*.

In the present study, any potential confounding effects of PG release were removed by oral administration of 600 mg aspirin, a dose that has been previously shown to inhibit bradykinin-stimulated endothelial prostacyclin

Figure 1 Effect of noradrenaline on dorsal hand vein diameter. Venoconstriction (mean $\pm$ s.e. mean) in dorsal hand veins during infusion of 50 pmol min^{-1} noradrenaline and either saline (□) or L-NMMA (■) ($n=8$).

Figure 2 Effect of angiotensin II on dorsal hand vein diameter. Venoconstriction (mean $\pm$ s.e. mean) in dorsal hand veins during infusion of 50 pmol min^{-1} ANGII and either saline (□) or L-NMMA (■), ($n=8$).

generation without influencing blood pressure or basal venous tone [7]. Despite giving aspirin, there was still marked tachyphylaxis to ANGII in the present study. However, co-infusion of L-NMMA, a specific inhibitor of NOS, at a dose previously demonstrated to block acetylcholine- and bradykinin-induced NO generation [6], did not alter this response. This indicates that PG release does not completely account for tachyphylaxis to ANGII *in vivo*, as has been previously demonstrated [5], and that local NO generation is not involved in the development of tachyphylaxis. Nevertheless, NO may modulate the degree of venoconstriction produced by ANGII in different circumstances, especially in injured vessels [12], or other parts of the venous system [4].

In summary, tachyphylaxis to ANGII in dorsal hand veins is not accounted for by local NO generation.

Although PG production may be responsible, in part, for the observed tachyphylaxis, alternative mechanisms such as changes in receptor density [10] or responsiveness [11], or the release of other modulatory factors may also be important.

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