Haemodynamic effects of adrenomedullin in human resistance and capacitance vessels

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Aims The haemodynamic effects of adrenomedullin and calcitonin gene-related peptide (CGRP) were studied in resistance and capacitance vessels of healthy volunteers.

Methods Adrenomedullin and CGRP were infused into the brachial artery of eight healthy subjects on two separate occasions at doses between 0.3–30 pmol min⁻¹. Forearm blood flow was measured using venous occlusion plethysmography. Venodilatation to adrenomedullin and CGRP was assessed in a further eight subjects by infusing the peptides at doses between 0.3–10 pmol min⁻¹ into a dorsal hand vein preconstricted with noradrenaline. Venodilator responses were measured as percentage reduction in noradrenaline preconstriction.

Results Adrenomedullin and CGRP at a dose of 30 pmol min⁻¹ produced an increase in forearm blood flow of 288 ± 42% and 252 ± 30% respectively (mean ± s.e. mean, P < 0.001). At doses between 3 and 10 pmol min⁻¹ adrenomedullin was significantly more potent than CGRP. The vasodilatation to both peptides was of similar duration with a biological half-life of approximately 18 min. Adrenomedullin reversed constriction in dorsal hand veins by 84 ± 2% (P < 0.001) at a dose of 10 pmol min⁻¹. CGRP produced a similar effect reversing constriction by 72 ± 12% at the same dose (P < 0.01). In veins, adrenomedullin was also more potent than CGRP at doses between 0.3 and 3 pmol min⁻¹.

Conclusions The lowest dose of adrenomedullin producing significant arteriolar dilatation was calculated to produce plasma levels similar to those found in heart failure. These findings suggest that in pathophysiological conditions such as heart failure circulating levels of adrenomedullin may be within a range capable of influencing vascular resistance directly.

Keywords: adrenomedullin, CGRP, blood flow, arteries, veins

Introduction

Adrenomedullin is a novel, 52 amino acid vasodilator peptide, originally discovered in human phaeochromocytoma [1], but which is also present in the plasma of healthy subjects and patients with hypertension [2]. The presence of adrenomedullin in normal tissues and plasma [3], and its synthesis and release by endothelial cells in vitro [4], suggests that the peptide has a physiological role in cardiovascular control. In animal studies, adrenomedullin exerts a potent hypotensive effect [1, 5] which is due to vasodilatation [6]. Bolus injections of adrenomedullin increase blood flow in the renal, mesenteric and hindquarter vascular beds of conscious rats [7]. Recently increased plasma levels of adrenomedullin have been reported in patients with hypertension [2, 8] suggesting that the peptide may exert a beneficial arteriolar vasodilator effect in this condition. However, there are no data on regional haemodynamic changes in human vascular beds, in response to adrenomedullin.

The amino acid sequence of adrenomedullin has a degree of homology with another naturally occurring vasodilator peptide, calcitonin gene-related peptide (CGRP) [1], and it has been suggested that adrenomedullin may act via binding to receptors for CGRP [9]. In the isolated mesenteric vascular bed of the rat, adrenomedullin causes vasodilation which can be blocked by the CGRP receptor antagonist, CGRP [8–37] [10]. Since endogenous CGRP is localized to perivascular nerve fibres in the mesenteric vascular bed, this finding could be explained either by adrenomedullin interacting with CGRP receptors and/or by releasing endogenous CGRP. In this context it is notable that, in conscious rats, the regional haemodynamic effects of exogenous adrenomedullin are not influenced by CGRP [8–37] [7]. Our objectives in this study were to determine whether adrenomedullin was a vasodilator in the resistance vessels of the human forearm and dorsal hand veins, and to compare its haemodynamic effects with those of CGRP.

Methods

Experiments were performed in 16, healthy, normotensive volunteers (aged 19–41 year) who gave written, informed consent. The protocols had the approval of the University of Nottingham Medical School Ethical Committee. Studies
were performed after subjects had rested supine in a quiet clinical laboratory for a minimum of 30 min, at a room temperature of 21–24°C for arterial studies, or semi-recumbent at 24–26°C for vein studies.

Forearm blood flow was measured in both arms simultaneously using venous occlusion plethysmography, with temperature-compensated, mercury-in-elastic strain gauges [11, 12]. This method gives values for flow in ml min\(^{-1}\) 100 ml \(^{-1}\) forearm tissue. Flows were recorded for 10 s in every 15 s during the final 3 min of each infusion period, with the mean of the final five measurements used for analysis. A 27 gauge steel cannula (Cooper Needle Works, Birmingham, UK) was inserted into the left brachial artery under local anaesthesia, using less than 1 ml of 1% lignocaine (Antigen Limited, Ireland). Saline (NaCl 140 mM, Travenol, Thetford, UK), and all drugs and peptides (Peptide Institute, Osaka) were infused at a constant rate of 1.0 ml min\(^{-1}\).

For vein studies, subjects rested semi-recumbent with the left hand supported by an arm rest above the level of the heart. A dorsal hand vein was then selected and cannulated with a 23 G butterfly needle (Abbott, Sligo, Republic of Ireland) without local anaesthesia. The same vein was used in each study. The internal diameter of the dorsal hand vein, distended by inflation of an upper arm cuff to 30 mm Hg, was measured using the technique previously described by Aellig [13]. Briefly, 30 min after cannulation, a linear variable differential transformer (LVDT) (Lucas, UK) was mounted onto the dorsum of the hand by means of a tripod. The LVDT’s lightweight core was placed over the summit of the vein being studied, approximately 10 mm downstream from the tip of the infusion cannula. Under the laboratory conditions used, veins have no intrinsic tone [14], therefore, in measuring responses to vasodilators, veins were first preconstricted by 60–70% of maximum with noradrenaline and CGRP did not produce systemic effects.

Adrenomedullin increased forearm blood flow from 3.4 ± 0.5 ml 100 ml \(^{-1}\) min\(^{-1}\) to 13.0 ± 1.1 ml 100 ml \(^{-1}\) min\(^{-1}\) at the highest dose, 30 pmol min\(^{-1}\) (P < 0.001) (Figure 1). CGRP, also at a dose of 30 pmol min\(^{-1}\), increased forearm blood flow from 3.0 ± 0.2 ml 100 ml \(^{-1}\) min\(^{-1}\) to 11.4 ± 1.1 ml 100 ml \(^{-1}\) min\(^{-1}\) (P < 0.001).

Data analysis and statistics

Percentage changes in blood flow were calculated from the change in the ratio of flow between arms. Data are expressed as mean ± s.e. mean and analysis was by repeated measures analysis of variance (ANOVA). P values of < 0.01 were considered significant.

Results

Effect of brachial artery infusion of adrenomedullin or CGRP on blood flow

Blood flow in the control (non-infused) arm did not change significantly throughout the experimental period on any study day, confirming that at the doses infused adrenomedullin and CGRP did not produce systemic effects.

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Figure 1 Percentage increase in forearm blood flow (mean ± s.e. mean) produced by brachial artery infusion of adrenomedullin (closed squares) and CGRP (open circles) in eight healthy volunteers, each studied on two occasions. *P < 0.001.
Dorsal hand vein studies

Adrenomedullin reversed constriction in dorsal hand veins by 62 ± 9% at 0.3 pmol min\(^{-1}\) (\(P < 0.03\)), and by 84 ± 2% at the highest dose (10 pmol min\(^{-1}\), \(P < 0.001\)) (Figure 2). Despite a decrease in venodilatation at the second dose, CGRP reversed constriction by 72 ± 12% at the highest dose (\(P < 0.01\)) (Figure 2). In agreement with its effect in the forearm resistance vessels, adrenomedullin produced significantly greater venodilatation than CGRP at doses between 0.3 and 3 pmol min\(^{-1}\).

Discussion

Studies in animals have demonstrated vasodilatation to adrenomedullin in rat isolated mesenteric vessels [10], and the pulmonary vessels of the cat [15]. However, there have been conflicting reports as to the relative potencies of adrenomedullin and CGRP. A recent study demonstrated that adrenomedullin was 10-fold less potent than CGRP in relaxing isolated rat mesenteric arteries, although the two peptides were equipotent in their vasodilator effects on hindquarters blood flow [10]. In our own studies in conscious rats, we have demonstrated that the hypotensive and tachycardic actions of adrenomedullin are two-fold greater than those of CGRP [7]. Although bolus injections of adrenomedullin produced significant and long lasting increases in renal and mesenteric blood flow, equiparal doses of CGRP caused reductions in renal and mesenteric flows, and only transient increases in renal and mesenteric vascular conductances [7]. The haemodynamic profiles of adrenomedullin and CGRP also differ in conscious sheep. Equinolar doses of adrenomedullin and CGRP produced a similar reduction in blood pressure, but adrenomedullin produced a three-fold greater vasodilatation, as assessed by total peripheral conductance [8]. Systemic administration of adrenomedullin to small numbers of human volunteers, at doses of up to 1.3 pmol kg\(^{-1}\) min\(^{-1}\), for 1.5 h produced a significant reduction in blood pressure, without an increase in heart rate [16]. The present study demonstrates, for the first time, that adrenomedullin is a potent vasodilator in human resistance vessels, and that its haemodynamic effects are greater in magnitude than those of CGRP at doses between 10 and 30 pmol min\(^{-1}\), although the duration of effect was similar for the two peptides. The lack of change in the control arm provides evidence that the dose-dependent vasodilatation occurs in response to a local action of adrenomedullin or CGRP on the brachial arterial vasculature, rather than via a centrally-mediated mechanism. The dilator response to adrenomedullin (34 ± 6%) was apparent at a dose of 1 pmol min\(^{-1}\). The local concentration of adrenomedullin in the forearm circulation at this dose would be in the order of 20 pmol l\(^{-1}\), which is similar to that reported in heart failure (5–15 pmol l\(^{-1}\)) [17], but higher than that reported in normal humans (2–4 pmol l\(^{-1}\)) and subjects with hypertension (4–8 pmol l\(^{-1}\)) [8]. However, since adrenomedullin can be synthesised by vascular endothelial cells [4], local tissue levels may be higher than those measured in the circulation. Levels of adrenomedullin in pathophysiological conditions such as hypertension are raised in proportion to disease severity [8], and thus these studies would support the hypothesis that circulating levels of adrenomedullin in heart failure may be in a range capable of influencing peripheral vascular resistance, and therefore, adrenomedullin may be of functional importance in such pathophysiological conditions.

Adrenomedullin caused significant venodilatation in pre-constricted human dorsal hand veins. Because superficial hand veins are involved in thermoregulation, venous tone is highly dependent upon ambient temperature. Using the temperature-controlled laboratory conditions of the present study, we were confident of an absence of venous tone by repeated measurements of basal diameter before infusions commenced. Investigating inhibition by dilator agents or the effects of constrictors in resistance vessels can be complicated by the fact that these vessels will always have some degree of tone. Thus, the absence of intrinsic tone in capacitance vessels provides an advantage over arterial studies in this respect [14, 18], providing a more comprehensive understanding of the pharmacology of agents being studied.

CGRP also caused venodilatation which, in agreement with the arterial studies, was less potent than adrenomedullin. This contrasts with a previous study in which CGRP produced no increase in venous diameter at doses of 5 pmol ml\(^{-1}\) min\(^{-1}\) or less [19]. Although, in the present study, we used up to 10 pmol ml\(^{-1}\) min\(^{-1}\), venodilatation was also produced at lower doses. Interestingly, one individual in our sample did not respond to CGRP administered to the dorsal hand vein. The study was repeated in this subject on a separate day with the same result. As this subject did respond to adrenomedullin, it is likely that these two peptides act at different receptors [7]. However,
ongoing work using specific receptor antagonists may provide further evidence in this respect.

In conclusion, the present study shows that adrenomedullin is a potent and long-lasting vasodilator in resistance and capacitance vessels of the human forearm. The haemodynamic effects of adrenomedullin were similar but greater in magnitude than those of CGRP. The lowest dose of adrenomedullin associated with vasodilatation suggests that circulating levels in pathophysiological conditions, such as heart failure, are within a range which may directly affect vascular tone. Whether or not adrenomedullin plays a physiological role in the maintenance of normal vascular tone remains to be established.

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References

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