

Adrenomedullin (ADM) in the human forearm vascular bed: effect of neutral endopeptidase inhibition and comparison with proadrenomedullin NH₂-terminal 20 peptide (PAMP)

Ian B. Wilkinson,^{1,†} Carmel M. McEniery,^{1,†} Katherine H. Bongaerts,¹ Helen MacCallum,¹ David J. Webb¹ & John R. Cockcroft²

¹Clinical Pharmacology Unit, Department of Medical Sciences, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU and ²Department of Cardiology, Wales Heart Research Institute, University Hospital, Cardiff CF14 4XN

Aims To compare the haemodynamic responses of proadrenomedullin N-terminal 20 peptide (PAMP) and adrenomedullin (ADM) in the forearm vascular bed of healthy male volunteers, and to investigate the role of neutral endopeptidase (NEP) in the metabolism of ADM.

Methods On two separate occasions, ADM (1–30 pmol min⁻¹) and PAMP (100–3000 pmol min⁻¹) were infused into the brachial artery of eight male subjects, and forearm blood flow (FBF) assessed using venous occlusion plethysmography. In a second study, eight male subjects received the same doses of ADM, co-infused with either the NEP inhibitor thiorphan (30 nmol min⁻¹) or the control vasoconstrictor noradrenaline (120 pmol min⁻¹), on separate occasions. Both studies were conducted in a double-blind, randomized manner.

Results ADM and PAMP produced a dose-dependent increase in FBF ($P \leq 0.002$). Based on the dose producing a 50% increase in FBF, ADM was ~60 times more potent than PAMP. Thiorphan and noradrenaline produced similar reductions in FBF of $14 \pm 4\%$ (mean \pm s.e. mean) and $22 \pm 6\%$, respectively ($P = 0.4$). However, the area under the dose–response curve was significantly greater during co-infusion of ADM with thiorphan than with noradrenaline ($P = 0.028$), as was the maximum increase in FBF ratio (2.1 ± 1.0 vs 1.2 ± 0.2 ; $P = 0.030$).

Conclusions ADM and PAMP both produce a local dose-related vasodilatation in the human forearm, but PAMP is ~60 times less potent than ADM. In addition, NEP inhibition potentiates the haemodynamic effects of ADM. These findings suggest that PAMP may not play a role in the physiological regulation of blood flow. However, in pathophysiological conditions such as hypertension and heart failure, NEP inhibition may exert a beneficial effect by increasing the biological activity of ADM.

Keywords: adrenomedullin, arteries, forearm blood flow, neutral endopeptidase, PAMP

Introduction

Adrenomedullin (ADM) and proadrenomedullin NH₂-terminal 20 peptide (PAMP) are recently discovered hypotensive peptides encoded by a single gene [1, 2].

Correspondence: Dr John Cockcroft, Department of Cardiology, Wales Heart Research Institute, University Hospital, Cardiff CF14 4XN. Tel.: 02920 743489; Fax: 02920 743500.

[†]Current address: Clinical Pharmacology Unit, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 2QQ.

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Transcription yields a precursor peptide, proadrenomedullin, from which ADM and PAMP are enzymatically cleaved. Both peptides have a similar widespread tissue distribution throughout the body [3], and are present in plasma in healthy subjects in concentrations of ~5–10 pmol l⁻¹ [4, 5]. In addition, ADM is actively synthesized and secreted by human vascular endothelial and smooth muscle cells, which also possess ADM receptors [3, 6, 7]. In animal models, ADM and PAMP are both potent vasodilators and result in sustained hypotension, although PAMP appears to be 30–100 times less potent than ADM [8–11]. Adrenomedullin

also has a number of other diverse biological actions including natriuresis, diuresis, positive inotropism and antiproliferative effects [3].

We have previously shown that ADM produces a dose-dependent vasodilatation in the human forearm vascular bed [12]. Moreover, systemic administration of ADM, in healthy subjects, results in a sustained decrease in arterial pressure [13]. In contrast, data concerning the effects of PAMP in humans are limited. Our preliminary data [14] and data from a Japanese population [15] indicate that PAMP is less potent than ADM, suggesting that PAMP may not play an important physiological role in the regulation of vascular tone or blood pressure [15].

In patients with congestive cardiac failure, plasma ADM concentrations are raised, suggesting that ADM may play a role in the regulation of pressure and volume homeostasis in this condition [4, 16]. Interestingly, infusion of ADM in patients with congestive cardiac failure reduces pulmonary capillary wedge pressure, increases cardiac output, causes a natriuresis, but has little effect on peripheral arterial pressure, suggesting that potentiation of ADM may be beneficial in heart failure [17]. The pathways responsible for ADM clearance remain to be fully elucidated but recent evidence suggests that neutral endopeptidase (NEP) may be involved in the breakdown of ADM [18]. Therefore, potentiation of ADM by NEP inhibition may be clinically relevant, especially in patients with heart failure.

Based on animal studies, and data from a Japanese population, we hypothesized that PAMP would induce vasodilatation in humans *in vivo* but be less potent than ADM, and that inhibition of NEP would potentiate the vasodilator effect of ADM. The aim of this study was to test these hypotheses in the human forearm vascular bed using intra-arterial drug administration and venous occlusion plethysmography to assess forearm blood flow (FBF).

Methods

Subjects

Sixteen healthy, normotensive, male volunteers between 26 and 47 years of age participated in the two studies. Both investigations were approved of the Local Research Ethics Committee, and written informed consent was obtained from each subject. All subjects were non-smokers and free from medication, and abstained from alcohol and caffeine-containing products for 24 h before the study. Following an overnight fast, subjects rested supine in a quiet temperature-controlled laboratory (21–24°C) for 30 min before the start of the study.

Drugs

All drugs were prepared aseptically from freshly opened vials, using 0.9% saline (Baxter; Norfolk; UK) as a diluent. Adrenomedullin (The Peptide Institute; Osaka, Japan) was infused intra-arterially at doses of 1, 3, 10, and 30 pmol min⁻¹, and PAMP (The Peptide Institute) at doses of 100, 300, 1000, and 3000 pmol min⁻¹. The doses of PAMP were based on comparative animal data and pilot studies conducted in two healthy volunteers (data not shown). Thiorphan (Sigma-Aldrich; Poole, UK) was infused at 30 nmol min⁻¹, based on previous studies [19, 20], and noradrenaline (Levophed; Abbott, Maidenhead, UK) at 120 pmol min⁻¹, a dose selected from the published literature to produce a similar reduction in FBF to thiorphan [21]. The total infusion rate was kept constant at 1 ml min⁻¹ throughout each study.

Intra-arterial administration

A 27 gauge steel cannula (Cooper's Needle Works; Birmingham, UK), attached to a 16 gauge epidural catheter (Portex; Kent, UK), was inserted into the left brachial artery under local anaesthesia, using ~1 ml of 1% lignocaine hydrochloride (Antigen Pharmaceuticals; Southport, UK). Patency was maintained by continuous infusion of 0.9% saline or drugs at a rate of 1.0 ml min⁻¹, via a P1000 IVAC infusion pump (Alaris; Hampshire, UK).

Forearm blood flow

Blood flow was measured simultaneously in both arms using venous occlusion plethysmography, with temperature-compensated, indium/gallium-in-silastic strain gauges applied to the widest part of the forearm, as previously described [22]. The hands were excluded from the circulation during each measurement period by inflation of a wrist cuff to 220 mmHg. Upper arm cuffs were inflated intermittently to 40 mmHg for 10 s in every 15 s to prevent, temporarily, venous outflow from the forearm and, thus, obtain plethysmographic recordings. Blood flows were recorded during the final 3 min of each infusion period, and the mean of the final five measurements used for analysis. Data were collected using a dual channel strain-gauge plethysmograph (Hokanson) and a computer-based, R wave triggered, system for on-line, semicontinuous monitoring of FBF, as described previously [23].

Brachial artery blood pressure

Peripheral blood pressure was measured in the brachial artery of the dominant arm using the validated Omron HEM-705CP oscillometric sphygmomanometer [24].

Mean arterial pressure (MAP) was calculated as the diastolic pressure plus one-third of the pulse pressure.

Study 1 Comparison of adrenomedullin and PAMP

Eight male volunteers, aged 26–47 years, attended on two separate occasions at least 1 week apart. Basal values of FBF were established during a 30 min period of intra-arterial infusion of saline. Adrenomedullin was then infused in a stepwise manner at doses of 1, 3, 10 and 30 pmol min⁻¹, each for 6 min and, on another occasion, PAMP at doses of 100, 300, 1000 and 3000 pmol min⁻¹, each for 6 min. The order of the infusions was randomized and administration was double-blind. Blood pressure and heart rate were recorded at the end of each infusion period.

Study 2 Adrenomedullin with co-infusion of thiorphan or noradrenaline

Eight healthy male volunteers, aged 26–44 years, were studied on two occasions, separated by at least 1 week. At each visit, saline was infused intra-arterially for 30 min, followed by 48 min of either thiorphan 30 nmol min⁻¹ or noradrenaline 120 pmol min⁻¹. The order of the infusions was randomized, and administration was double-blind. Adrenomedullin was then co-infused at doses of 1, 3, 10 and 30 pmol min⁻¹, each dose for 6 min. Blood pressure and heart rate were recorded at the end of each infusion period.

Data analysis and statistics

The ratio of FBF in the infused to non-infused arm was calculated for each time point, as previously described [25], and expressed as the percentage change from baseline or, in the case of Study 2, as the percentage change from the last recording during infusion of thiorphan or noradrenaline alone [19]. Data are expressed as means \pm s.e. mean and [95% confidence limits] for differences, and were examined using analysis of variance (ANOVA), or paired Student's *t*-tests, as appropriate. The dose of peptide

producing a 50% increase in the FBF ratio (Study 1), or area under the dose–response curve (AUC) and FBF ratio at the 30 pmol min⁻¹ dose of ADM (Study 2) were used as predefined summary measure statistics. *P* < 0.05 was considered as significant.

Results

Study 1

Basal MAP, heart rate and the FBF ratio were similar on the two study days, and there was no difference in blood flow between the two arms. There was no significant change in blood flow in the noninfused (control) arm, heart rate or MAP, throughout either study period (Table 1), confirming that the doses of ADM and PAMP infused did not produce systemic haemodynamic effects. Adrenomedullin and PAMP caused a dose-dependent increase in the FBF ratio (*P* = 0.001 and *P* = 0.002, respectively), as shown in Figure 1. Blood flow in the infused arm is presented in Table 1. The estimated dose required to produce a 50% increase in FBF ratio was significantly lower for ADM compared with PAMP

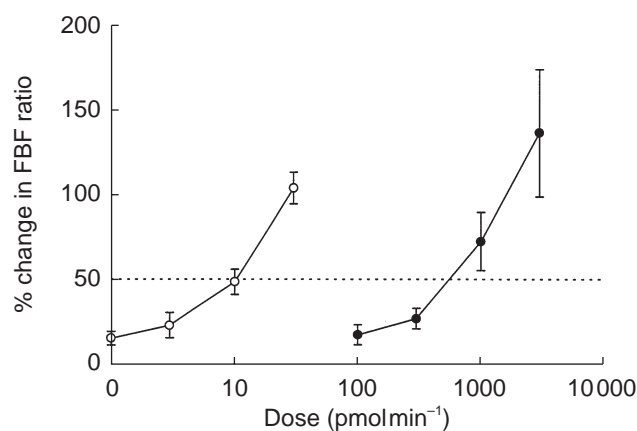


Figure 1 Comparison of the effect of intra-arterial infusion of adrenomedullin (○) and proadrenomedullin NH₂-terminal 20 peptide (●) on forearm blood flow. The dotted line represents a 50% increase in the FBF ratio, which was used as a summary measure statistic to compare the potency of the two drugs. *n* = 8.

Table 1 Mean arterial pressure, heart rate and blood flow in the infused arm during Study 1.

	ADM (pmol min ⁻¹)					PAMP (pmol min ⁻¹)				
	Saline	1	3	10	30	Saline	100	300	1000	3000
FBF (ml 100 ml ⁻¹ min ⁻¹)	5.2 ± 0.9	5.8 ± 1.0	5.8 ± 0.9	6.6 ± 1.0	8.7 ± 1.2	4.2 ± 0.7	3.9 ± 0.6	5.4 ± 0.8	6.8 ± 0.9	9.2 ± 1.2
MAP (mmHg)	86 ± 2	89 ± 4	87 ± 3	87 ± 3	92 ± 3	92 ± 3	93 ± 3	91 ± 3	90 ± 3	90 ± 3
Heart rate (beats min ⁻¹)	65 ± 1	69 ± 3	68 ± 2	64 ± 3	66 ± 3	66 ± 1	65 ± 3	65 ± 4	65 ± 3	65 ± 2

ADM = adrenomedullin; PAMP = proadrenomedullin NH₂-terminal 20 peptide; FBF = absolute blood flow in the infused forearm; MAP = mean arterial pressure. Values are means \pm s.e. mean, *n* = 8.

(11 ± 2 vs 656 ± 106 pmol min⁻¹; mean difference 645 [349, 941]; $P=0.015$).

Study 2

Basal MAP, heart rate and the FBF ratio were similar on the two study days, and there was no difference in blood flow between the two arms. There was no significant change in blood flow in the noninfused (control) arm, heart rate or MAP, throughout either study period (Table 2). There was a $14 \pm 4\%$ reduction in the FBF ratio following infusion of thiorphan ($P=0.030$), and a $22 \pm 6\%$ reduction after noradrenaline ($P=0.018$). Neither, the percentage change in the FBF ratio (mean difference 8% [-14, 35]; $P=0.4$), nor the FBF ratio immediately prior to ADM infusion (1.1 ± 0.1 vs 0.9 ± 0.1 ; mean difference 0.2 [-0.2, 0.6]; $P=0.4$), differed significantly between the two drugs. Co-infusion of 1, 3, 10 and 30 pmol min⁻¹ ADM with noradrenaline resulted in a dose-dependent increase in the FBF ratio ($P=0.007$), as did coinfusion with thiorphan ($P=0.003$) (Figure 2). However, the area under the dose-response curve was significantly greater during co-infusion of ADM with thiorphan compared with noradrenaline (51 ± 8 vs 31 ± 3 AU; mean difference 20 [3, 37]; $P=0.028$), as was the maximum FBF ratio (2.1 ± 1.0 vs 1.2 ± 0.2 ; mean difference 0.9 [0.1–1.8]; $P=0.030$). Compared with infusion of ADM alone in Study 1, the response to ADM was reduced during co-infusion of noradrenaline ($P \leq 0.001$), but not thiorphan ($P=0.7$).

Discussion

The aims of the present study were to compare the response to ADM and PAMP in forearm resistance vessels *in vivo*, and to examine the effect of the NEP inhibitor thiorphan and the control vasoconstrictor noradrenaline on the response to ADM. The main findings were that, although ADM and PAMP both produced vasodilatation in the forearm vascular bed in healthy male subjects, PAMP was ~ 60 times less potent than ADM, and that the response to ADM was substantially potentiated by thiorphan.

Adrenomedullin is a potent vasodilator in a number of vascular beds in several species including the rat [8] and cat [10, 26], and we have previously demonstrated vasodilatation to ADM in the human forearm vascular bed [12]. Proadrenomedullin N-terminal 20 peptide causes hypotension when injected intravenously into anaesthetized rats [2], and is a vasodilator in cat mesenteric and hind limb resistance vessels [10, 11], although in these models it is between 30 and 100 times less potent than ADM. Only one previous study has investigated the relative potencies of the two peptides in the human forearm vascular bed, concluding that PAMP is ~ 100 times less potent than ADM, in Japanese subjects [15]. The results of the present study are in broad agreement with these observations as we found PAMP to be ~ 60 times less potent a vasodilator than ADM, in Caucasian subjects. The lack of change in the control arm provides evidence that the dose-dependent vasodilatation occurs in response to a local effect of ADM or PAMP in the forearm, rather than via a centrally mediated mechanism. However, given that the circulating concentrations of ADM and PAMP are similar (in the range 5–10 pmol l⁻¹), and that the local concentration of PAMP in the forearm during the highest

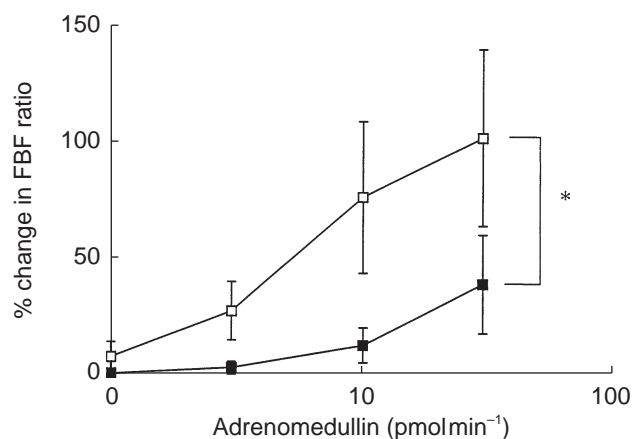


Figure 2 Comparison of the effect of intra-arterial adrenomedullin on forearm blood flow when co-infused with either thiorphan (□) or noradrenaline (■). $n=8$; $*P=0.03$ (AUC).

Table 2 Mean arterial pressure, heart rate and blood flow in the infused arm during Study 2.

Adrenomedullin (pmol min ⁻¹)	Baseline	Thiorphan				Noradrenaline				
		1	3	10	30	Baseline	1	3	10	30
FBF (ml 100 ml ⁻¹ min ⁻¹)	3.9 ± 0.7	4.1 ± 0.7	4.5 ± 0.6	5.5 ± 0.7	7.2 ± 0.8	4.1 ± 0.8	3.9 ± 0.6	4.0 ± 0.7	4.2 ± 0.8	4.7 ± 0.8
MAP (mmHg)	91 ± 3	88 ± 2	88 ± 1	87 ± 2	90 ± 2	91 ± 4	93 ± 4	92 ± 3	92 ± 3	94 ± 3
Heart rate (beats min ⁻¹)	59 ± 1	54 ± 2	59 ± 1	60 ± 1	58 ± 2	63 ± 2	63 ± 2	64 ± 2	63 ± 2	63 ± 1

FBF = absolute blood flow in the infused forearm; MAP = mean arterial pressure. Values are means \pm s.e. mean, $n=8$.

infusion rate would have been $\sim 60 \text{ nmol l}^{-1}$ (assuming a FBF of 50 ml min^{-1}), it seems unlikely that PAMP is an important circulating physiological regulator of arterial tone. Although PAMP may be more important in regulating blood flow in other vascular beds, it should be recognized that the forearm has proved a reliable predictor of the systemic response to number of peptides; including angiotensin II [27, 28], endothelin-1 [29, 30], bradykinin [31, 32], and ADM [12, 13]. However, the issue of the physiological relevance of PAMP can only really be resolved with the use of receptor antagonists, which are not currently available. It has also been suggested that PAMP may produce vasodilatation through inhibition of peripheral sympathetic neurotransmission [9], although others have been unable to confirm this [10]. If, indeed, PAMP does inhibit sympathetic activity, then it may produce a more profound vasodilatation in situations of increased sympathetic nervous system activity, such as congestive heart failure.

Plasma ADM levels are increased in subjects with heart failure [4, 16, 33, 34], possibly as a compensatory response protecting against further elevation of peripheral vascular resistance [16, 33]. This view is supported by the observation that, as with other neurohormones, plasma concentrations of ADM decrease following effective treatment for heart failure [34]. *In vivo* studies indicate that NEP may be involved in the breakdown of ADM [18]. Therefore, in the present study we investigated the effect of the selective NEP inhibitor, thiorphan, on haemodynamic responses to ADM.

We have previously demonstrated that thiorphan reduces FBF by $\sim 15\%$ in healthy subjects [20]. Therefore, we elected to use noradrenaline as a control vasoconstrictor, to reduce FBF to a similar degree, to avoid any potential confounding influence that precontraction might have on the response to ADM [35, 36]. As expected, thiorphan and noradrenaline produced similar reductions in resting FBF and there was little difference in the FBF ratio immediately before infusion of ADM. Despite this, the increase in FBF produced by the highest dose of ADM (30 pmol min^{-1}) was substantially greater when it was co-infused with thiorphan rather than noradrenaline, as was the area under the dose-response curve, indicating a potentiation of the response to ADM by NEP inhibition. Although, *in vitro* evidence supporting a role for NEP in ADM degradation is, at present, conflicting [37, 38], our observations are consistent with the work of Lisy *et al.* [18], who have previously reported a rise in plasma ADM, and potentiation of the natriuretic effect of ADM, in dogs following administration of the selective NEP inhibitor candoxatrilat [18]. However, since the response to ADM was reduced during co-infusion of noradrenaline, compared with infusion of ADM alone, we cannot exclude the possibility that there was physiological antagonism of the

dilator effect of ADM by noradrenaline. Nevertheless, the degree of vasoconstriction induced by noradrenaline and thiorphan was similar.

In summary, PAMP is a less potent vasodilator in human resistance vessels than ADM, and the haemodynamic response to ADM is potentiated by NEP inhibition. This may be clinically relevant since infusion of ADM has a beneficial haemodynamic effect in patients with congestive cardiac failure [17]. Indeed, potentiation of haemodynamic and natriuretic properties of endogenous ADM may be a useful strategy for patients with heart failure and may, in part, explain the favourable haemodynamic response to NEP inhibitors [39] and combined NEP/angiotensin converting enzyme inhibitors in patients with heart failure [40].

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