

Vascular Effects of Apelin In Vivo in Man

Alan G. Japp, MRCP,* Nicholas L. Cruden, PhD,* David A. B. Amer,* Vivienne K. Y. Li, BSc,* Ewan B. Goudie, BSc,* Neil R. Johnston, MSc,* Sushma Sharma, PhD,† Ilene Neilson, BSc,† David J. Webb, MD, FRCP,* Ian L. Megson, PhD,† Andrew D. Flapan, MD, MRCP,‡ David E. Newby, PhD, FRCP*

Edinburgh and Inverness, United Kingdom

Objectives

This study was designed to establish the direct vascular effects of apelin in vivo in man.

Background

Apelin is the endogenous ligand for the previously orphaned G-protein-coupled receptor, APJ. This novel pathway is widely expressed in the cardiovascular system and is emerging as an important mediator of cardiovascular homeostasis. In pre-clinical models, apelin causes venous and arterial vasodilation.

Methods

Vascular effects of apelin were assessed in 24 healthy volunteers. Dorsal hand vein diameter was measured by the Aellig technique during local intravenous infusions (0.1 to 3 nmol/min) of apelin-36, (Pyr¹)apelin-13, and sodium nitroprusside (0.6 nmol/min). Forearm blood flow was measured by venous occlusion plethysmography during intrabrachial infusions of apelin-36 and (Pyr¹)apelin-13 (0.1 to 30 nmol/min) and subsequently in the presence or absence of a “nitric oxide clamp” (nitric oxide synthase inhibitor, L-N^G-monomethylarginine [8 μmol/min], coinfused with nitric oxide donor, sodium nitroprusside [90 to 900 ng/min]), or a single oral dose of aspirin (600 mg) or matched placebo.

Results

Although sodium nitroprusside caused venodilation ($p < 0.0001$), apelin-36 and (Pyr¹)apelin-13 had no effect on dorsal hand vein diameter ($p = 0.2$). Both apelin isoforms caused reproducible vasodilation in forearm resistance vessels ($p < 0.0001$). (Pyr¹)apelin-13-mediated vasodilation was attenuated by the nitric oxide clamp ($p = 0.004$) but unaffected by aspirin ($p = 0.7$).

Conclusions

Although having no apparent effect on venous tone, apelin causes nitric oxide-dependent arterial vasodilation in vivo in man. The apelin-APJ system merits further clinical investigation to determine its role in cardiovascular homeostasis. (J Am Coll Cardiol 2008;52:908–13) © 2008 by the American College of Cardiology Foundation

Apelin is the endogenous ligand for the orphan G-protein-coupled receptor, APJ (1,2). The full-length mature peptide, apelin-36, comprises 36 amino acids but the most potent isoform is the pyroglutamated form of apelin-13, (Pyr¹)apelin-13 (2–4). The APJ receptors are present on endothelial cells, vascular smooth muscle cells, and cardiomyocytes (5).

In rodent models, exogenous apelin administration causes a rapid nitric oxide (NO)-dependent fall in blood pressure and mean capillary filling pressure, indicating powerful vasodilator and venodilator effects (3). In ex vivo myography studies, apelin

causes NO-dependent vasorelaxation in human mesenteric arteries (6) and venoconstriction in endothelium-denuded human saphenous veins (7). As a first step toward characterizing the in vivo cardiovascular profile of apelin in man, we sought to determine its direct vasomotor effects in the peripheral venous and arterial circulation.

Methods

Subjects. Twenty-four healthy male volunteers (age 19 to 24 years) participated following written informed consent, with the approval of the local research ethics committee, and in accordance with the Declaration of Helsinki. Participants were not taking regular medication and abstained from alcohol for 24 h and from food and caffeine-containing drinks for at least 4 h before studies.

Vascular studies. Studies were conducted in a quiet temperature-controlled room (22°C to 25°C). Heart rate and blood pressure were monitored at regular intervals with a semiautomated, oscillometric sphygmomanometer (HEM 705CP, Omron, Tokyo, Japan).

From the *Centre for Cardiovascular Science, University of Edinburgh, Chancellor's Building, Edinburgh, United Kingdom; †Department of Diabetes, UHI Millennium Institute, Inverness, United Kingdom; and the ‡Department of Cardiology, Royal Infirmary of Edinburgh, Edinburgh, United Kingdom. Dr. Japp currently receives funding through a British Heart Foundation Clinical Research and Training Fellowship (FS/06/064). This study was also supported by a project grant from Chest, Heart, and Stroke, Scotland (Res06.A98). The authors had full access to the data and take responsibility for their integrity. All authors have read and agree to the manuscript as written.

Manuscript received November 12, 2007; revised manuscript received March 26, 2008, accepted June 3, 2008.

VENOUS STUDIES. Intravenous infusions (0.25 ml/min) were administered through a 23-gauge needle in a non-branching dorsal hand vein (DHV). Venous tone was measured by the Aellig technique during norepinephrine (Hospira, Illinois; 1 to 32 ng/min) precontraction (50% to 70% reduction in diameter) as described previously (8). Six subjects (Fig. 1, protocol 1) received incremental infusions of (Pyr¹)apelin-13 or apelin-36 (Clinalfa AG, Läufelfingen, Switzerland; 0.1 to 3 nmol/min) followed, after saline washout, by a single dose of sodium nitroprusside (SNP) (Mayne Pharma Plc, Warwickshire, United Kingdom) at 0.6 nmol/min. A further 3 subjects received (Pyr¹)apelin-13 and apelin-36 in the absence of norepinephrine precontraction.

ARTERIAL STUDIES. Intra-arterial infusions (1 ml/min) were administered through a 27-standard-wire-gauge steel brachial artery needle. Forearm blood flow (FBF) was measured in both forearms by venous occlusion plethysmography as described previously (9,10). In protocol 2A, blood samples (10 ml) were drawn from each arm into ethylenediaminetetraacetic acid and centrifuged to obtain plasma that was stored at –80°C. Plasma apelin concentrations were measured using a commercially available enzyme-linked immunosorbant assay (Phoenix Pharmaceuticals Inc., Burlingame, California.). In protocol 3, a “NO clamp” (L-N^G-

monomethylarginine [8 μmol/min] coinfused with SNP [90 to 900 ng/min]) was used to allow simulation of normal basal NO activity during complete inhibition of endogenous NO synthesis, as described previously (11). Dispersible aspirin (600 mg; Aspar Pharmaceuticals Ltd., London, United Kingdom) was administered orally 30 min before study commencement to inhibit production of prostaglandins (12).

Eight subjects attended on 4 occasions at least 1 week apart to receive incremental infusions of apelin-36 or (Pyr¹)apelin-13 (0.1 to 30 nmol/min) in the presence and absence of saline washouts (protocol 2). A further 8 subjects attended on 4 occasions to receive, in a randomized order: 1) the NO clamp and oral aspirin; 2) the NO clamp and oral placebo; 3) saline placebo and oral aspirin; and 4) saline placebo and oral placebo, followed on all occasions by sequential infusions of (Pyr¹)apelin-13 (0.3 to 3 nmol/min) and acetylcholine (Novartis AG, Basel, Switzerland; 5 to 20 μg/min) in a double-blind manner (protocol 3).

Abbreviations and Acronyms
ANOVA = analysis of variance
APJ = apelin receptor
AUC = area under the curve
DHV = dorsal hand vein
FBF = forearm blood flow
NO = nitric oxide
SNP = sodium nitroprusside

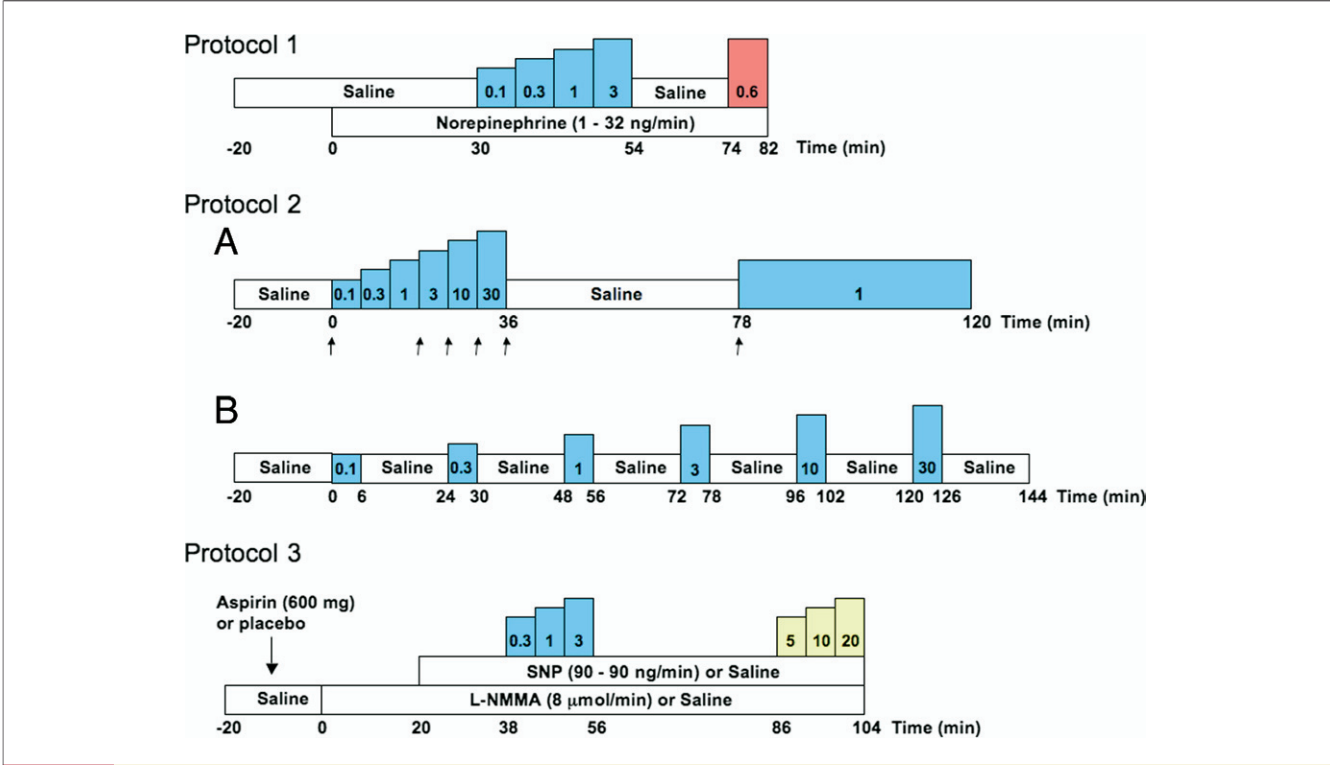


Figure 1 Study Design

Protocol 1: Dorsal hand vein infusions of (Pyr¹)apelin-13 or apelin-36 (blue; nmol/min), sodium nitroprusside (SNP) (red; nmol/min) and saline, with coinfusion of norepinephrine. **Protocol 2 (A and B):** Intrabrachial infusions of (Pyr¹)apelin-13 or apelin-36 (blue; nmol/min) and saline with venous blood sampling (↑). **Protocol 3:** Intrabrachial infusions of (Pyr¹)apelin-13 (blue; nmol/min) and acetylcholine (yellow; μg/min) with coadministration of aspirin or placebo, and “nitric oxide clamp” or saline placebo. L-NMMA = L-N^G-monomethylarginine.

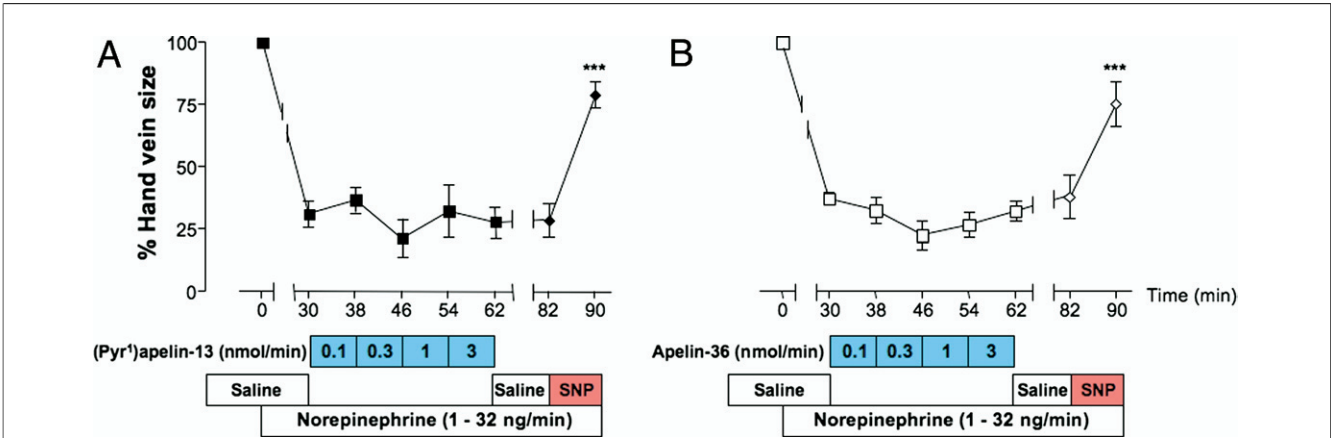


Figure 2 DHV Diameter During Coinfusion of Norepinephrine

DHV diameter during coinfusion of norepinephrine and (A) incremental doses of (Pyr¹)apelin-13 (solid squares) and single dose of SNP (solid diamonds; 0.6 nmol/min) and (B) incremental doses of apelin-36 (open squares) and single dose of SNP (open diamonds; 0.6 nmol/min). ***p < 0.001, paired Student *t* test. DHV = dorsal hand vein; other abbreviations as in Figure 1.

Data and statistical analyses. The DHV (13) and FBF (9) data were analyzed as described previously. The effects of the NO clamp and aspirin on FBF responses to (Pyr¹)apelin-13 and acetylcholine were assessed by calculating area under the curve (AUC) using the trapezoid method. Apelin concentrations were logarithmically transformed prior to analysis. Variables are

reported as mean ± standard error of mean and analyzed using repeated-measures analysis of variance (ANOVA) with post-hoc Bonferroni corrections, regression analysis, and 2-tailed Student *t* test as appropriate (Graph-Pad Prism, GraphPad Software, Inc., San Diego, California). Statistical significance was taken at the 5% level.

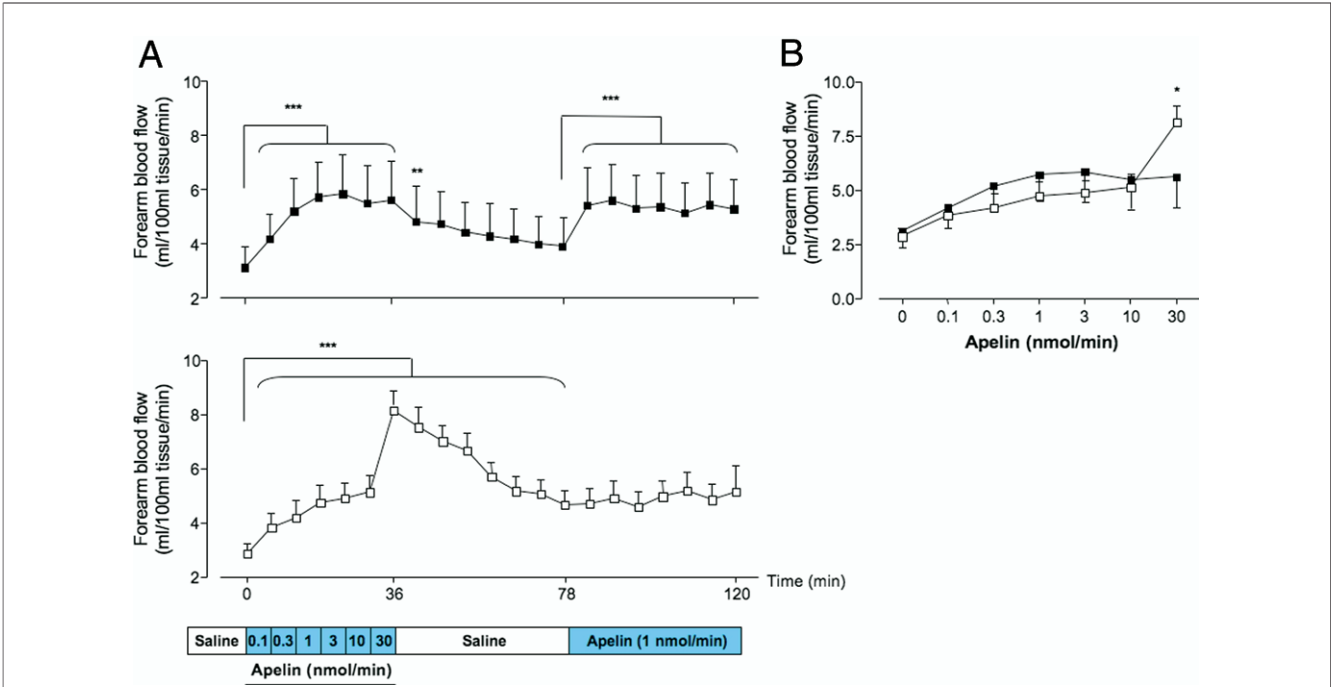


Figure 3 Infused FBF

Infused FBF (A) in protocol 2A during infusion of (Pyr¹)apelin-13 (top, solid squares; 1-way ANOVA) and apelin-36 (bottom, open squares; 1-way ANOVA) and (B) during incremental infusion of (Pyr¹)apelin-13 (solid squares) versus apelin-36 (open squares); 2-way ANOVA with repeated measures. *p < 0.05, **p < 0.01, ***p < 0.001, post-hoc Bonferroni tests. ANOVA = analysis of variance; FBF = forearm blood flow.

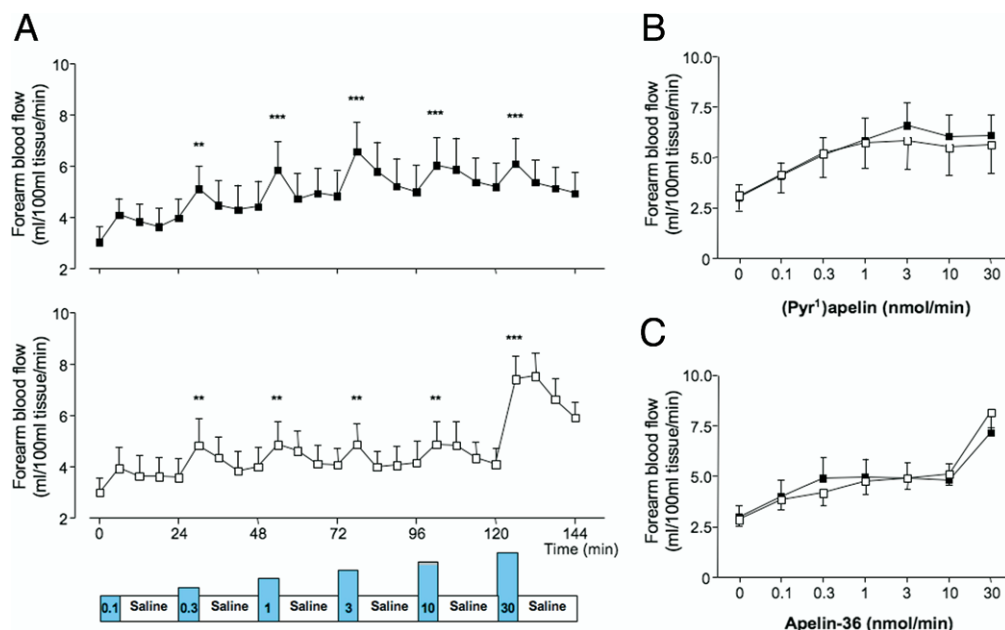


Figure 4 Infused FBF

Infused FBF (**A**) in protocol 2B during infusion of (Pyr¹)apelin-13 (**top**, solid squares; 1-way ANOVA) and apelin-36 (**bottom**, open squares; 1-way ANOVA) and during incremental infusion of (Pyr¹)apelin-13 (**B**) and apelin-36 (**C**) in the presence (solid squares) and absence (open squares) of saline washouts (for both: $p > 0.05$, 2-way ANOVA with repeated measures). ** $p < 0.01$, *** $p < 0.001$, post-hoc Bonferroni tests. Abbreviations as in Figure 3.

Results

In all studies, apelin infusions were well tolerated with no serious adverse events. There were no changes in heart rate or blood pressure.

Neither (Pyr¹)apelin-13 nor apelin-36 caused venoconstriction in relaxed DHVs (data not shown). Following precontraction, DHV diameter was unaffected by (Pyr¹)apelin-13 ($p = 0.18$, 1-way ANOVA) or apelin-36 ($p = 0.24$, 1-way ANOVA) but increased by SNP ($p < 0.0001$) (Fig. 2).

(Pyr¹)apelin-13 and apelin-36 increased FBF in the infused arm ($p < 0.001$) (Figs. 3 and 4). Vasodilation to apelin-36 was dose-dependent ($r^2 = 0.41$, $p < 0.0001$) but plateaued rapidly with (Pyr¹)apelin-13 ($r^2 = 0.003$, $p = 0.73$). After continuous dose escalation of each apelin isoform, FBF declined gradually over 42 min (for both: $p < 0.0001$, ANOVA). With apelin-36, FBF remained elevated during saline washout ($p < 0.0001$) and failed to rise further during subsequent reinfusion (Fig. 3). With (Pyr¹)apelin-13, FBF remained elevated at 6 min during saline washout ($p < 0.05$) but not thereafter, and further vasodilation was demonstrable during subsequent reinfusion ($p < 0.0001$) (Fig. 3).

At the highest dose of 30 nmol/min, infused FBF was greater with apelin-36 compared with (Pyr¹)apelin-13 ($p < 0.05$) (Fig. 3B). The inclusion of saline washouts between doses did not affect vasodilation to either (Pyr¹)apelin-13 ($p = 0.68$) (Fig. 4B) or apelin-36 ($p = 0.93$) (Fig. 4C). With both apelin isoforms, FBF increased

in the noninfused arm ($p < 0.0001$, ANOVA) at 30 nmol/min ($p < 0.05$, post-hoc Bonferroni tests; data not shown).

Plasma apelin concentrations in the infused arm rose with increasing doses of apelin-36 ($p < 0.0001$) (Fig. 5) and increased in the noninfused arm from 10 nmol/min ($p < 0.01$).

L-N^G-monomethylarginine reduced infused FBF (3.48 ± 0.41 ml/100 ml/min to 2.10 ± 0.17 ml/100 ml/min; $p <$

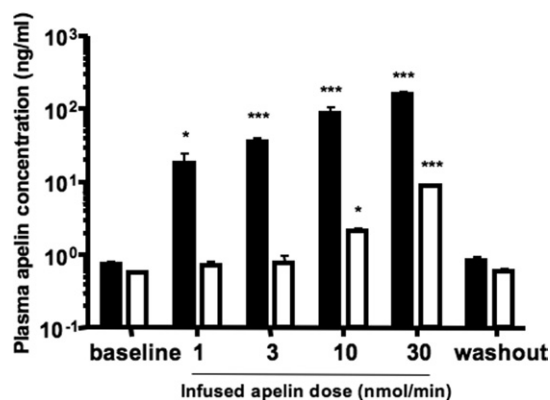


Figure 5 Plasma Apelin Concentration

Plasma apelin concentration in venous blood samples from infused (solid bars) and noninfused arm (open bars) during intrabrachial apelin-36 infusion. ** $p < 0.01$, *** $p < 0.001$, 1-way ANOVA with post-hoc Bonferroni tests. Abbreviation as in Figure 3.

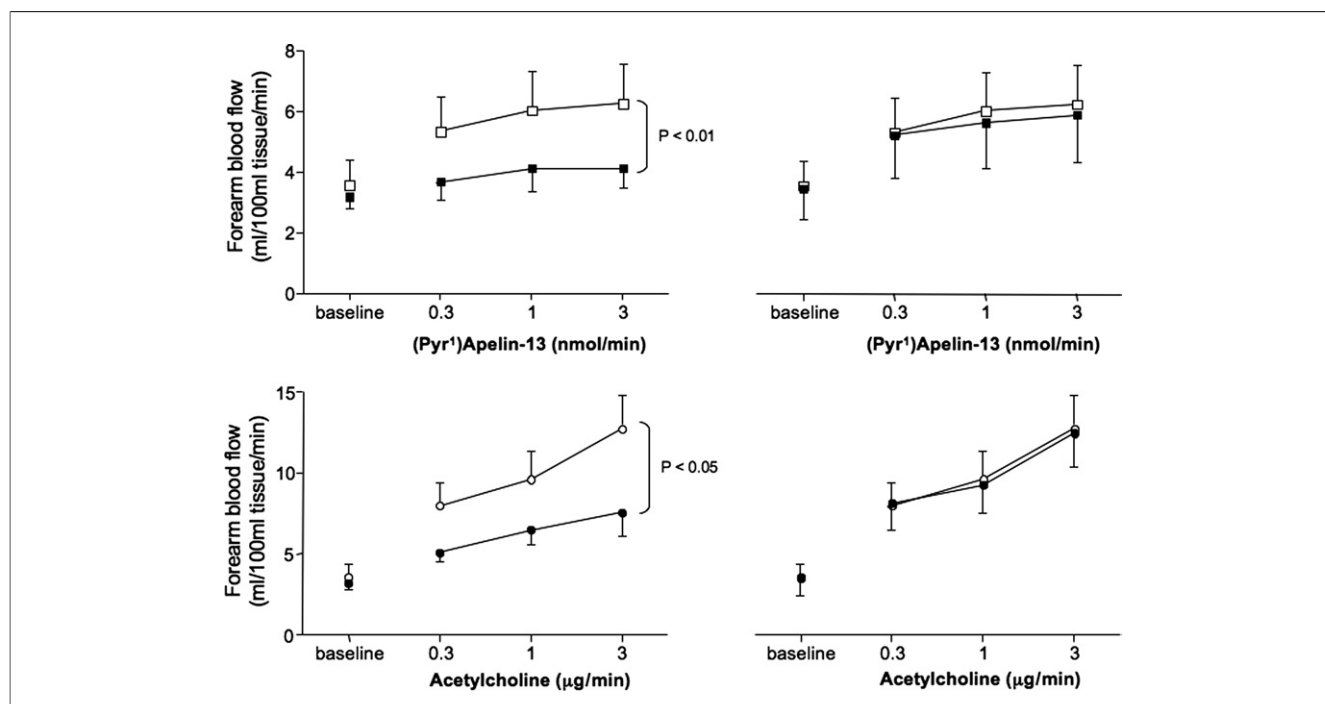


Figure 6 Infused FBF in Subjects During Infusion

Infused FBF in subjects during infusion of (top row) (Pyr¹)apelin-13 in the presence (solid squares) or absence (open squares) of nitric oxide (NO) clamp (left) or aspirin (right) and (bottom row) acetylcholine in the presence (solid circles) or absence (open circles) of NO clamp (left) or aspirin (right). $p < 0.0001$; 1-way ANOVA for dose responses to (Pyr¹)apelin-13 and acetylcholine alone. For placebo versus NO clamp (AUC): (Pyr¹)apelin-13, $p < 0.01$; acetylcholine, $p < 0.05$. For placebo versus aspirin (AUC): (Pyr¹)apelin-13, $p = 0.7$; acetylcholine, $p = 0.9$. AUC = area under the curve; other abbreviations as in Figure 3.

0.001) but this was restored with SNP coinfusion (3.20 ± 0.33 ml/100 ml/min; $p > 0.05$ vs. baseline). Both (Pyr¹)apelin-13 ($p < 0.001$) and acetylcholine ($p < 0.001$) increased infused FBF (Fig. 6). Coinfusion of the NO clamp inhibited the response to (Pyr¹)apelin-13 (AUC: 4.7 ± 0.9 AU vs. 1.7 ± 0.7 AU; $p = 0.004$) and acetylcholine (AUC: 12.8 ± 2.3 AU vs. 6.4 ± 1.5 AU; $p = 0.02$) but aspirin had no effect ($p > 0.05$ for both) (Fig. 6).

Discussion

This is the first study to examine the in vivo vascular actions of apelin in man. Using robust well-validated techniques, we have assessed the direct effects of 2 endogenous apelin isoforms on vascular tone. Although not affecting peripheral venous tone, apelin peptides cause vasodilation in peripheral resistance vessels through a NO-dependent mechanism.

Effects of apelin on peripheral arteriolar tone. In keeping with pre-clinical models and studies of human mesenteric vessels in vitro, we have demonstrated that apelin causes reproducible vasodilation in human forearm resistance vessels in vivo. We studied 2 different apelin isoforms, apelin-36 and (Pyr¹)apelin-13, and both caused a rapid onset of vasodilation. Apelin-36 exhibited a slower offset, with vasodilation persisting for at least 42 min after cessation of infusion. This prolonged offset of action is unusual but has been described with other agonists, such as the V2 agonist, desmopressin (14).

It is consistent with in vitro data showing that apelin-36 exhibits a much slower dissociation from the APJ receptor than (Pyr¹)apelin-13 and causes prolonged biological activity in microphysiometric assays (4). In addition, apelin appears to be rapidly cleared from the circulation with a short plasma half-life of no longer than 8 min. Although shorter apelin peptides have more potent depressor activity in rats (15), we saw no difference in potency between the 2 isoforms and a greater response to apelin-36 at the highest dose. This was due, at least in part, to a plateauing of the response to (Pyr¹)apelin-13 at higher doses that may have resulted from APJ receptor internalization (16) combined with a shorter duration of receptor activation.

Vasodilation to (Pyr¹)apelin-13 was attenuated by two-thirds during the NO clamp, indicating that vasodilation to apelin is mediated predominantly by endothelial NO generation. As expected, the NO clamp also inhibited acetylcholine-mediated vasodilation, which is known to be partly mediated by NO (17). In contrast, systemic inhibition of prostanoid generation with oral aspirin did not alter vasodilation to either (Pyr¹)apelin-13 or acetylcholine, alone or in combination with the NO clamp. The dose of aspirin used in this study inhibits bradykinin-induced endothelial production of prostacyclin by at least 85% (12), suggesting that prostanoids do not provide a major contribution to apelin-mediated vasodilation. Our in vivo findings are in close agreement with Salcedo et al. (6) who

recently demonstrated that NO synthase, but not cyclooxygenase, inhibition attenuated relaxation to apelin in human mesenteric arteries in vitro.

Effects of apelin on peripheral venous tone. We demonstrated constrictor and dilator responses to norepinephrine and SNP, respectively, and employed doses of apelin 10-fold higher than those required to cause vasodilation in the forearm arterial circulation. It is unlikely therefore that the lack of venous response to apelin reflected either inadequate dosing or sensitivity of the technique. However, we examined the effects of apelin in a single peripheral venous bed and cannot exclude the possibility of a direct effect on central capacitance veins. Further clinical studies are required to determine the in vivo effects of apelin in specific venous beds and on overall systemic venous tone.

Study limitations. We measured changes in FBF and plasma apelin concentrations during local intrabrachial apelin infusions. At infusion rates >3 nmol/min, we detected a rise in plasma apelin concentrations accompanied by an increase in FBF in the noninfused arm, indicating spillover of infused apelin into the systemic circulation. This spillover limits interpretation of blood flow changes at the higher doses of apelin because of potential concomitant effects on myocardial contractility or activation of neurohumoral reflexes. However, at apelin doses ≤ 3 nmol/min, changes in blood flow can be solely ascribed to direct local vascular effects.

We have described the effects of exogenous apelin on resistance vessels in the forearm vascular bed. Although concordance between vasomotor responses in the forearm and other vascular beds is generally good (18), differences do exist and further studies will be needed to confirm our findings in other vascular beds such as the coronary circulation.

Conclusions

We have shown that acute apelin administration in vivo in man causes NO-mediated arterial vasodilation but does not appear to affect peripheral venous tone. Increasing evidence from pre-clinical models has suggested that apelin-APJ signaling mediates important effects on cardiovascular homeostasis. Our findings provide the strongest evidence to date of a role for the apelin-APJ system in human cardiovascular regulation.

Acknowledgments

The authors thank the staff in the Wellcome Trust Clinical Research Facility for their help with these studies.

Reprint requests and correspondence: Dr. Alan G. Japp, Centre for Cardiovascular Science, The University of Edinburgh, Chancellor's Building, Edinburgh, EH16 4SB, United Kingdom. E-mail: alan.japp@ed.ac.uk.

REFERENCES

1. O'Dowd BF, Heiber M, Chan A, et al. A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11. *Gene* 1993;136:355–60.
2. Tatemoto K, Hosoya M, Habata Y, et al. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochem Biophys Res Commun* 1998;251:471–6.
3. Japp AG, Newby DE. The apelin-APJ system in heart failure: pathophysiologic relevance and therapeutic potential. *Biochem Pharmacol* 2008;75:1882–92.
4. Hosoya M, Kawamata Y, Fukusumi S, et al. Molecular and functional characteristics of APJ. Tissue distribution of mRNA and interaction with the endogenous ligand apelin. *J Biol Chem* 2000;275:21061–7.
5. Kleinz MJ, Skepper JN, Davenport AP. Immunocytochemical localisation of the apelin receptor, APJ, to human cardiomyocytes, vascular smooth muscle and endothelial cells. *Regul Pept* 2005;126:233–40.
6. Salcedo A, Garijo J, Monge L, et al. Apelin effects in human splanchnic arteries. Role of nitric oxide and prostanooids. *Regul Pept* 2007;144:50–5.
7. Katugampola SD, Maguire JJ, Matthewson SR, Davenport AP. [(125)I]-(Pyr(1))Apelin-13 is a novel radioligand for localizing the APJ orphan receptor in human and rat tissues with evidence for a vasoconstrictor role in man. *Br J Pharmacol* 2001;132:1255–60.
8. Aellig WH. A new technique for recording compliance of human hand veins. *Br J Clin Pharmacol* 1981;11:237–43.
9. Newby DE, Wright RA, Ludlam CA, Fox KA, Boon NA, Webb DJ. An in vivo model for the assessment of acute fibrinolytic capacity of the endothelium. *Thromb Haemostasis* 1997;78:1242–8.
10. Newby DE, Wright RA, Labinjoh C, et al. Endothelial dysfunction, impaired endogenous fibrinolysis, and cigarette smoking: a mechanism for arterial thrombosis and myocardial infarction. *Circulation* 1999;99:1411–5.
11. Stroes ES, Luscher TF, de Groot FG, Koomans HA, Rabelink TJ. Cyclosporin A increases nitric oxide activity in vivo. *Hypertension* 1997;29:570–5.
12. Heavey DJ, Barrow SE, Hickling NE, Ritter JM. Aspirin causes short-lived inhibition of bradykinin-stimulated prostacyclin production in man. *Nature* 1985;318:186–8.
13. Haynes WG, Strachan FE, Webb DJ. Endothelin ETA and ETB receptors cause vasoconstriction of human resistance and capacitance vessels in vivo. *Circulation* 1995;92:357–63.
14. Affolter JT, McKee SP, Helmy A, Jones CR, Newby DE, Webb DJ. Intra-arterial vasopressin in the human forearm: pharmacodynamics and the role of nitric oxide. *Clin Pharmacol Ther* 2003;74:9–16.
15. Tatemoto K, Takayama K, Zou MX, et al. The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. *Regul Pept* 2001;99:87–92.
16. Zhou N, Fan X, Mukhtar M, et al. Cell-cell fusion and internalization of the CNS-based, HIV-1 co-receptor, APJ. *Virology* 2003;307:22–36.
17. Calver A, Collier J, Vallance P. Inhibition and stimulation of nitric oxide synthesis in the human forearm arterial bed of patients with insulin-dependent diabetes. *J Clin Invest* 1992;90:2548–54.
18. Wilkinson IB, Webb DJ. Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. *Br J Clin Pharmacol* 2001;52:631–46.

Key Words: apelin ■ APJ receptor ■ endothelium ■ vasodilator.