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Venous dilator effect of apelin, an endogenous peptide ligand for the orphan APJ receptor, in conscious rats

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Abstract

Apelin is an endogenous depressor peptide for the G protein-coupled APJ receptor. Our hypothesis is that apelin is a venodilator, and it reduces mean circulatory filling pressure (MCFP; index of venous tone). Dose–response curves of apelin (10, 20 and 40 nmol/kg) or vehicle (0.9% NaCl) were constructed in two groups each of conscious, unrestrained rats: unblocked rats and rats pretreated with mecamylamine (Mec; ganglionic blocker) and noradrenaline (NA; to restore vascular tone). The vehicle had no effects in the unblocked or ganglionic-blocked rats. Apelin decreased mean arterial pressure (MAP) and increased heart rate (HR), but it did not alter mean circulatory filling pressure in the unblocked rats. In the ganglionic-blocked rats, apelin did not alter heart rate but decreased mean arterial pressure and mean circulatory filling pressure. These results show that apelin is an arterial and venous dilator in vivo. The depressor effect of apelin is accompanied by tachycardia which is abolished by ganglion blockade.

Keywords: Apelin; Arterial pressure, mean; Circulatory filling pressure, mean; Venous tone; Capacitance vessel; Ganglionic blockade

1. Introduction

The biological action of peptide hormones are principally mediated through the activation of G protein-coupled receptors. Some G protein-coupled receptors are called ‘‘orphan G protein-coupled receptors’’ because the identity of their ligands is not established. O’Dowd et al. (1993) reported a novel orphan G protein-coupled receptor, the APJ receptor, which most closely resembled the angiotensin AT_1 receptor. The APJ receptor did not, however, bind to angiotensin II (Tatemoto et al., 1998). Tatemoto et al. (1998) isolated from the bovine stomach a novel endogenous 36-amino-acid ligand for the APJ receptor, and they named the peptide apelin-36 (Tatemoto et al., 1998). Further studies showed that two C-terminal fragments of apelin-36, namely, apelin-13 and apelin-17, were 54- and 8-fold, respectively, more potent than apelin-36 in cells expressing the APJ receptor (Tatemoto et al., 1998). APJ binding sites and/or mRNA encoding the APJ receptors have been detected in various human and rat tissues, which include the lung, heart, artery, vein, skeletal muscle, kidney, brain, liver and ovary (De Mota et al., 2000; Hosoya et al., 2000; Lee et al., 2000; O’Carroll et al., 2000; Katugampola et al., 2001; Reaux et al., 2002). Moreover, apelin mRNA or immunoreactive apelin were detected in various tissues and organs such as the stomach, brain, heart, blood vessels, lung, testis, uterus and mammary gland (Tatemoto et al., 1998; Habata et al., 1999; Lee et al., 2000; O’Carroll et al., 2000; Kawamata et al., 2001; Brailoiu et al., 2002). The presence of apelin receptors and apelin in the heart and blood vessels suggest that this peptide may have a cardiovascular role. Indeed, intravenous injection of apelin in anaesthetized rats decreased blood pressure (Lee et al., 2000); the depressor response was abolished after pretreatment of the rats with an inhibitor of nitric oxide synthase (Tatemoto et al., 2001). Apelin was also reported to have positive inotropic action in the isolated rat heart (Szokodi et al., 2002).

The venous system plays a crucial role in the regulation of cardiac output. Drugs that interfere with sympathetic venomotor tone (e.g., α-adrenoceptor antagonists and ganglionic blockers) or have direct venodilator action (e.g., nitrovasodilators) are known to cause considerable reduction of cardiac

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output and orthostatic hypotension (Pang, 2001). In vitro studies show that apelin-13 causes concentration-dependent contraction of the endothelium-denuded human saphenous vein with a high potency (low EC_{50}) but low efficacy (20% maximum response as 100 mM KCl) of response (Katugampola et al., 2001). To our knowledge, there is no published report on the in vivo venous action of apelin. Our hypothesis is that apelin has venodilator action in vivo.

The primary aim of the present study was to determine the effect of apelin-12 on mean circulatory filling pressure (MCFP) of conscious rats. Apelin-12 has been shown to be a more potent depressor agent than apelin-13 and apelin-36 (Tatemoto et al., 2001). Mean circulatory filling pressure is the pressure that would occur throughout the circulation if all pressures were brought to an equilibrium (Guyton, 1955). In the absence of a change in blood volume, a decrease in mean circulatory filling pressure denotes a reduction in body venous tone (Tabrizchi and Pang, 1992; Pang, 2000). Because the venodilator action of a drug is best revealed in animal with suppressed sympathetic nerve activity and/or elevated venomotor tone (Tabrizchi and Pang, 1992; Pang, 2000), the rats in the current study were pretreated with mecamylamine (Mec) to obliterate autonomic reflex and continuously infused with noradrenaline (NA) to elevate venomotor tone.

2. Materials and methods

2.1. Animal preparation

Male Sprague–Dawley rats (380–450 g) were obtained from Charles River Canada. The rats were maintained under a 12:12-h light–dark cycle (lights on from 7 a.m. to 7 p.m.) and supplied with a standard laboratory chow diet (PMI Feeds) and water ad libitum. The rats were anaesthetized with halothane (4% in air for induction and 1.5% for maintenance). A polyethylene (PE_{50}) catheter was introduced into the left iliac artery to record mean arterial pressure (MAP) by a pressure transducer (PD23DB, Gould, Statham, CA, USA). Heart rate (HR) was derived electronically from the upstroke of the arterial pulse pressure by a Grass 7P4G tachograph. The vehicle or drugs were administered through two catheters inserted into the right iliac vein and the left external jugular vein. The left iliac vein was also cannulated to allow the insertion of a catheter into the inferior vena cava for the measurement of central venous pressure by another pressure transducer (P23DB, Gould). A saline-filled, balloon-tipped catheter was advanced into the right atrium through the right external jugular vein. The proper positioning of the balloon was tested by transiently inflating the balloon, which when correctly placed, caused a simultaneous decrease in mean arterial pressure to 20–25 mm Hg and an increase in central venous pressure within 5 s of circulatory arrest. All cannulae were filled with heparinized normal saline (25 IU/ml) and tunneled subcutaneously to the back of the neck, exteriorized and secured. The rats were allowed 6 h to recover from the effects of surgery and anaesthesia before further use.

The experiment has been carried out in accordance with the Declaration of Helsinki and/or with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institute of Health.

2.2. Measurements of mean circulatory filling pressure

The method for determining mean circulatory filling pressure has been described in detail elsewhere (Pang, 2000). Briefly, steady-state readings of mean arterial pressure and central venous pressure were noted at 4–5 s after temporarily stopping the circulation by inflation of the atrial balloon. To correct for the incomplete equilibration of arterial and venous pressures during circulatory arrest, mean circulatory filling pressure was calculated by the following equation: mean circulatory filling pressure = venous plateau pressure + 1/60(final arterial pressure − venous plateau pressure), where 1/60 represents the ratio of arterial to venous compliance.

2.3. Experimental protocol

The rats were divided into four groups ($n = 6–7$ each). Mean arterial pressure, heart rate and central venous pressure were continuously displayed on a Grass Polygraph (Model RPS 7C8). The rats were given 45 min to stabilize before baseline values of mean arterial pressure, heart rate and mean circulatory filling pressure were obtained. Afterwards, two groups of rats were given intravenous injections of apelin (10, 20 and 40 nmol/kg) or an equivalent volume of the vehicle (0.9% NaCl) at dose intervals of 10 min. All the measurements were taken at the plateau phase of depressor response to apelin (between 1–1.5 and 2–3 min for the low and high dose, respectively), and at the same time points in the vehicle-treated rats. Two other groups of rats were pretreated with mecamylamine (50 μm/kg i.v. bolus), and measurements were taken 10 min later. The dose of mecamylamine used was found to abolish ganglionic transmission for more than 2 h (Wang and Pang, 1991). Afterwards, noradrenaline was continuously infused (6 nmol/kg/min), and measurements were again taken at 10 min after the initiation of infusion. This was followed by injections of single doses of apelin or the vehicle as described for the intact (non-ganglionic blocked) rats.

2.4. Statistical analysis

All data are presented as mean ± S.E.M. Baseline data were analyzed by one-way (among groups of rats) analysis of variance. Haemodynamics and responses to drugs or vehicle among the four groups were analyzed by two-way

repeated-measures analysis of variance followed by multiple comparison of group data using Tukey test (SigmaStat statistical software), with $P < 0.05$ selected as the criterion for statistical significance.

2.5. Drugs

Apelin-12 (Human, Bovine, Phoenix Pharmaceuticals, CA, USA) was dissolved in distilled water and kept in aliquots at −70 °C until the day of the experiment when it was diluted with normal saline (0.9% NaCl). Mecamylamine and noradrenaline (Sigma, St. Louis, MO, USA) were also dissolved in normal saline.

3. Results

3.1. Effects of mecamylamine and noradrenaline

There are no significant differences in baseline readings of mean arterial pressure, heart rate and mean circulatory filling pressure among the four groups of conscious rats (Table 1).

To facilitate elucidation of the venodilator action of drugs, two groups of rats were pretreated with mecamylamine followed by noradrenaline prior to construction of the dose–response curve of apelin. Intravenous injection of mecamylamine significantly decreased mean arterial pressure and heart rate, whereas subsequent intravenous infusion of noradrenaline increased mean arterial pressure, heart rate and mean circulatory filling pressure in both groups of rats (Table 1).

3.2. Effects of apelin

Saline did not significantly alter mean arterial pressure, heart rate or mean circulatory filling pressure in either the intact or ganglionic-blocked groups of time-control rats (Fig. 1A,B). Curve analyses show that apelin significantly and dose-dependently reduced mean arterial pressure and increased heart rate in the intact rats, relative to the corresponding changes in the time-control rats. Apelin did not significantly alter mean circulatory filling pressure (Fig. 1A).

In the ganglionic-blocked rats, apelin significantly and dose-dependently decreased mean arterial pressure and mean circulatory filling pressure but did not significantly affect heart rate, relative to the corresponding changes in the time-control rats (Fig. 1B). Curve analyses show that the changes in mean arterial pressure and mean circulatory filling pressure in the ganglionic-blocked rats were greater than those of the intact rats.

Table 1
Baseline readings of mean arterial pressure (MAP), heart rates (HR) and mean circulatory filling pressure (MCFP) in four groups ($n = 6–7$) of conscious, unrestrained rats prior to, and following, pretreatment with mecamylamine (Mec, 50 μm/kg i.v.) and noradrenaline (NA, 6 nmol/kg/min)

		Intact rats		Ganglionic-blocked rats	
		Saline	Apelin	Saline	Apelin
MAP	Baseline	107 ± 3	110 ± 6	108 ± 7	106 ± 5
(mm Hg)	After Mec			67 ± 2[a]	71 ± 3[a]
	After NA			149 ± 6[a,b]	151 ± 2[a,b]
HR	Baseline	400 ± 17	410 ± 15	406 ± 9	409 ± 6
(beats/min)	After Mec			355 ± 15[a]	364 ± 7[a]
	After NA			438 ± 28[a,b]	425 ± 10[a,b]
MCFP	Baseline	7.4 ± 0.3	7.3 ± 0.3	7.3 ± 0.1	7.5 ± 0.2
(mm Hg)	After NA			9.6 ± 0.5[a]	10.0 ± 0.4[a]

[a] Significantly different from baseline reading.
[b] Significantly different from the reading after mecamylamine pretreatment.

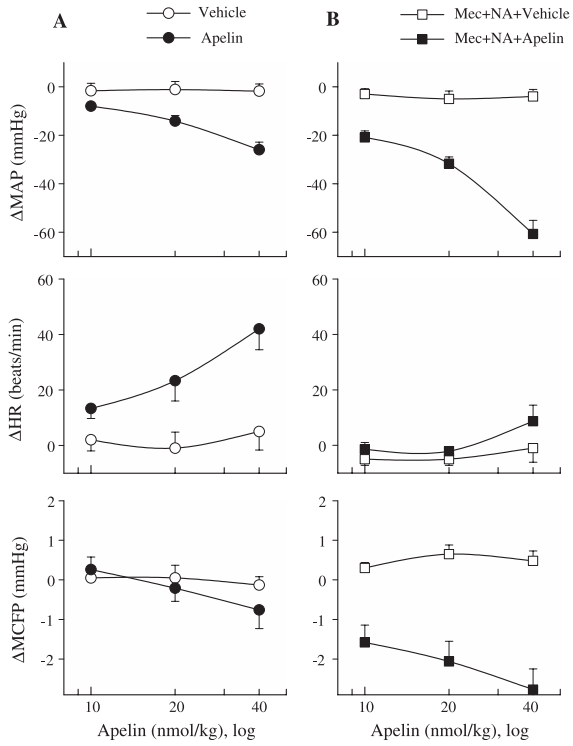

Fig. 1. Dose–response effects (means ± S.E.M, $n = 6–7$ per group) of intravenous apelin or the same volume of the vehicle (0.9% NaCl) on mean arterial pressure (ΔMAP), heart rate (ΔHR) and mean circulatory filling pressure (ΔMCFP) in: (A) conscious, intact rats and (B) rats pre-treated with mecamylamine (Mec, 50 μm/kg) and noradrenaline (NA, 6 nmol/kg/min).

4. Discussion

Intravenous injection of apelin caused a dose-dependent decrease in mean arterial pressure and an increase in heart rate in conscious, intact (unblocked) rats. Apelin caused greater reductions in mean arterial pressure but no change in heart rate in the ganglionic-blocked rats. These results suggest that the increase of heart rate in the intact rats was likely an indirect action due to hypotension, as it was

abolished by ganglionic blockade. The mechanism by which apelin caused tachycardia in the intact rats is unclear and could be through sympathetic activation and/or reduced vagal influence. The absence of significant reflex tachycardia in the ganglionic-blocked rats suggests that apelin neither has a direct positive or negative chronotropic action nor modulates cholinergic or adrenergic activity through action at the postganglionic prejunctional nerve terminal. Depressor responses to intravenous injections of apelin in anaesthetized rats have been reported (Lee et al., 2000; Tatemoto et al., 2001); however, unlike our findings, there was no significant tachycardia accompanying the depressor responses. The discrepancy in the heart rate response is likely due to our use of conscious rats that have a more active baroreflex system.

There is evidence that apelin causes vasodilatation via the activation of the nitric oxide/L-arginine system. The depressor response of apelin in anaesthetized rats was accompanied by an increase of the plasma concentrations of nitrite/nitrate (NO_x) and was inhibited by pretreatment with the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (Tatemoto et al., 2001). Apelin has also been shown to increase contractility in the isolated perfused rat heart, and the response was shown to involve activation of G protein-coupled phospholipase C, protein kinase C, and sarcolemmal Na^+-Ca^{2+} and Na^+-H^+ exchanges (Szokodi et al., 2002). Taken together, these reports indicate that apelin may activate differential second messenger systems in different tissues or organs.

Apelin insignificantly reduced mean circulatory filling pressure in intact rats. In rats pretreated with mecamylamine to cause ganglionic blockade followed by noradrenaline to restore venomotor tone, apelin caused a dose-dependent decrease in mean circulatory filling pressure despite the greater reduction in mean arterial pressure. These results demonstrate that apelin has a prominent venodilator action. In our experience, a depressor response to a drug (e.g., nitrovasodilators and Ca^{2+} channel blockers) is often associated with an increase in mean circulatory filling pressure, and the increase is the result of hypotension-induced reflex activation of the sympathetic nervous system (Pang, 2000). Apelin is therefore a more efficacious venodilator than verapamil, flunarizine and nifedipine (Ca^{2+} channel blockers, Waite et al., 1988), hydralazine (D'Oyley and Pang, 1990), isoprenaline (β-adrenoceptor agonist, Abdelrahman and Pang, 1990), zaprinast (type V phosphodiesterase inhibitor, Ng and Pang, 1998), nitroglycerin (Poon and Pang, 2002) and pinacidil (potassium channel opener, Waite et al., 1995), which all decrease mean arterial pressure but increase mean circulatory filling pressure in intact rats.

To summarize, apelin caused a depressor response and tachycardia in intact (unblocked) rats. In rats pretreated with mecamylamine and noradrenaline, it did not alter heart rate but decreased mean arterial pressure as well as mean circulatory filling pressure. These results show that apelin is an arterial as well as a venous dilator in vivo. The depressor effect of apelin is accompanied by tachycardia, which is abolished by ganglion blockade.

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