Apelin-13 induces a biphasic haemodynamic response and hormonal activation in normal conscious sheep

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Abstract

Whilst the tissue distribution and range of biological actions reported for apelin suggest a role for the peptide in pressure/volume homeostasis, conflicting reports make the precise role unclear. Furthermore, few integrated studies have been performed and there are no reports of bioactivity of apelin in a large animal model. Accordingly, we have examined the haemodynamic, hormonal and renal effects of apelin in ten normal conscious sheep. Apelin (1 mg i.v. bolus) induced a biphasic haemodynamic response characterised by an acute fall in arterial pressure and a rise in heart rate followed immediately by a rise in arterial pressure and a fall in heart rate. The secondary hypertensive phase was associated with a fall in cardiac output ($P=0.015$) and significant rises in calculated total peripheral resistance (CTPR) ($P<0.001$) and right atrial pressure (RAP) ($P=0.031$). Electrocardiogram

changes were also observed in four of ten sheep, most notably varying degrees of atrioventricular block. Apelin also induced significant rises in plasma arginine vasopressin ($P=0.009$), adrenocorticotrophin ($P=0.012$), aldosterone ($P=0.001$), cortisol ($P=0.014$), atrial ($P=0.036$) and brain ($P<0.001$) natriuretic peptide, cyclic GMP ($P=0.003$) and cyclic AMP ($P=0.002$) levels with no effect on renal indices. In conclusion, high dose administration of apelin to normal conscious sheep induces a significant biphasic response in arterial pressure and heart rate associated with rises in RAP and CTPR and a fall in cardiac output. Apelin also increases circulating levels of a number of vasoactive hormones. Taken together, these results suggest a potential role for apelin in pressure/volume homeostasis.

Journal of Endocrinology (2006) **189**, 701–710

Introduction

Apelin, a peptide recently isolated from bovine stomach extracts, appears to act as an endogenous ligand for the previously orphaned G-protein-coupled APJ receptor (Tatemoto *et al.* 1998). The pre-propeptide consists of 77 amino acids with mature apelin likely derived from the C-terminal region as either a 36 or a 13 amino acid peptide. Apelin mRNA and peptide expression have subsequently been described in various tissues including gastrointestinal tract, adipose tissue, brain, lung, kidney, liver and the cardiovascular system (Lee *et al.* 2000, Kawamata *et al.* 2001). Within the cardiovascular system apelin is strongly expressed in the heart (Lee *et al.* 2000) with expression also present in the large conduit vessels, coronary vessels and endothelial cells (Kleinz *et al.* 2005). Expression of mRNA for the APJ receptor is similarly distributed throughout the brain and periphery including cardiovascular tissue (Lee *et al.* 2000, Katugampola *et al.* 2001, Kleinz *et al.* 2005).

Haemodynamic actions have been attributed to apelin. Several studies show that the peptide lowers arterial pressure in anaesthetised (Lee *et al.* 2000, Tatemoto *et al.* 2001,

El Messari *et al.* 2004) and conscious (Cheng *et al.* 2003) rats, possibly via a nitric oxide dependent mechanism (Tatemoto *et al.* 2001). However, there are also contradictory reports of apelin increasing arterial pressure in conscious rats (Kagiyama *et al.* 2005). In ganglion-blocked rats, mean circulatory filling pressure is reduced by apelin, suggesting that it is a venodilator (Cheng *et al.* 2003). In contrast, apelin potently constricts human saphenous veins (Katugampola *et al.* 2001). Other haemodynamic actions of apelin include positive inotropic effects reported in isolated rat hearts (Szokodi *et al.* 2002) and an increase in the first derivative of left ventricular pressure or the rate of change in pressure with time (dP/dt) in normal and heart failure rats (Berry *et al.* 2004). Whilst apelin reduces preload and afterload in mice, it appears to exhibit differential acute (reduction) and chronic (increase) effects on cardiac output (Ashley *et al.* 2005).

The APJ receptor has also been localised in the hypothalamus and its expression responds to both osmotic and stress stimuli (O'Carroll & Lolait 2003, O'Carroll *et al.* 2003). Whilst both i.p. (Lee *et al.* 2000) and i.c.v. (Taheri *et al.* 2002) administration of apelin has been shown to increase drinking, other authors have demonstrated a

reduced drinking response to i.c.v. apelin in dehydrated rats (Reaux *et al.* 2001). There are conflicting reports on the effects of apelin on arginine vasopressin (AVP) secretion including increased AVP release from hypothalamic explants (Taheri *et al.* 2002) but reduced AVP secretion reported *in vivo* in response to i.c.v. apelin (Reaux *et al.* 2001, De Mota *et al.* 2004). Apelin also increases corticotrophin releasing hormone release from hypothalamic explants (Taheri *et al.* 2002) and increases adrenocorticotrophin (ACTH) (Taheri *et al.* 2002) and corticosterone (Taheri *et al.* 2002, Jaszberenyi *et al.* 2004) release *in vivo*.

Whilst this range of biological actions reported for apelin suggest a role for the peptide in pressure/volume homeostasis, contrasting reports make its precise role unclear. Furthermore, few integrated studies have been performed and there are no reports of bioactivity of apelin in a large animal model. Accordingly, we have examined the haemodynamic, hormonal and renal effects of apelin in normal conscious sheep.

Materials and Methods

The Animal Ethics Committee of the Christchurch School of Medicine and Health Sciences approved the study protocol. Ten Coopworth ewes weighing 44–67 kg (Lincoln University Farm, Christchurch, New Zealand) were housed in an air-conditioned light-controlled room and received a diet of lucerne chaff and food pellets providing 75 mmol sodium and 150 mmol potassium per day. Under general anaesthesia (induced by 17 mg/kg thiopentone sodium and maintained by a mixture of halothane, nitrous oxide and oxygen) a carotid artery was cannulated (16 G Angiocath; Becton Dickinson, Sandy, UT, USA) for direct measurement of arterial pressure and heart rate. Polyethylene catheters were placed in the jugular veins for blood sampling and measurement of right atrial pressure (RAP) and a Swan-Ganz thermodilution catheter (Edwards Life Sciences, Irvine, CA, USA) was placed in the pulmonary artery via the jugular vein for measurements of cardiac output. A foley catheter was placed per urethra in the bladder to allow continuous collection of urine. Stainless steel safety pins were placed through the skin of the four limbs for subsequent attachment of electrocardiogram (ECG) leads. The animals recovered for at least 7 days before experiments.

Each animal was studied on two occasions two days apart receiving vehicle control (0.9% saline) and human apelin-13 (American Peptides, Sunnyvale, CA, USA). Apelin was administered in incremental doses as i.v. boli injections (10 ml given over 15 s) of 10 µg, 100 µg and 1 mg at 1-h intervals. Control solution and apelin were administered in a balanced, random order design.

Arterial pressure, RAP and ECG were recorded using an in-house online data acquisition system, commencing 30 min before bolus administration and continuing for

60 min beyond the last bolus. Heart rate and pressures were digitally integrated in 1-min recording periods and data were recorded at preset intervals throughout the study. Cardiac output (thermodilution) was measured in triplicate (three values within 10%) at preset intervals for the duration of infusions. Calculated total peripheral resistance (CTPR) was calculated as mean arterial pressure (MAP) divided by cardiac output.

Venous blood was drawn at preset intervals during the study protocol. Blood was taken into chilled EDTA tubes, centrifuged and the plasma was stored at -80°C before assay for AVP (Sadler *et al.* 1983), ACTH (Donald 1977), aldosterone (Lun *et al.* 1983), atrial natriuretic peptide (ANP) (Charles *et al.* 1990), brain natriuretic peptide (BNP) (Pemberton *et al.* 1997), catecholamines (Goldstein *et al.* 1981), cortisol (Lewis *et al.* 1992), cyclic GMP (Charles *et al.* 1990), endothelin-1 (Rademaker *et al.* 2000), plasma renin activity (PRA) (Dunn & Espiner 1976), urocortin (Rademaker *et al.* 2002) and cyclic AMP by a commercially available kit (Biotrak; Amersham). Haematocrit was measured with each blood sample.

Statistical analysis

Results are expressed as means $\pm$ S.E.M. Two-way ANOVA with time as a repeated measure was used to determine time and treatment differences between the apelin and control arms of the study. Statistical significance was assumed at $P < 0.05$. Where significant differences were identified by ANOVA, *a priori* Fisher's protected least square difference (LSD) tests were used to identify time-points significantly different from time-matched control.

Results

Experiments were completed without mishap and data collection was complete. There was no significant difference in baseline levels of any variables between study days. Furthermore, with the exception of plasma BNP, all variables were similar to time-matched controls immediately prior to each bolus.

The lower doses of apelin (10 and 100 µg) had no significant effect on haemodynamic parameters (Fig. 1). However, the 1 mg bolus of apelin induced a significant ($P < 0.001$) biphasic response in MAP, with an initial fall in pressure (approximately 12 mmHg below the time-matched control value at 2 min post-bolus) followed by a rise in MAP peaking approximately 12 mmHg above the control value at 4 min post-bolus. These effects were short-lived with pressures returning to control levels by 15 min post-bolus. Both systolic ($P = 0.015$) and diastolic ($P < 0.001$) arterial pressures were affected similarly by the 1 mg dose of apelin with no significant effect on pulse pressure (data not shown). Heart rate response to 1 mg

Figure 1 Haemodynamic response to i.v. bolus injections of apelin (●) or vehicle control (○) in ten sheep. Values are means $\pm$ S.E.M. Significant differences were observed for mean arterial pressure ($P<0.001$), heart rate ($P=0.017$), cardiac output ($P=0.015$), calculated total peripheral resistance (CTPR) ($P<0.001$) and right atrial pressure ($P=0.031$). Individual time points significantly different from time-matched control (Fisher's protected LSD) are indicated by * $P<0.05$ and † $P<0.01$.

Table 1 Haematocrit and plasma hormone responses in ten sheep to 1 mg bolus dose of apelin or vehicle control administered at 120 min

	Baseline	120 min	135 min	150 min	180 min
Haematocrit (%)					
Control	26.0 ± 1.5	24.7 ± 1.3	24.4 ± 1.3	24.2 ± 1.3	24.6 ± 1.2
Apelin	26.2 ± 1.4	25.0 ± 1.5	25.4 ± 1.4	24.2 ± 1.6	24.9 ± 1.5
Plasma endothelin (pmol/l)					
Control	1.8 ± 0.2	2.1 ± 0.3	2.2 ± 0.3	2.1 ± 0.3	2.1 ± 0.3
Apelin	2.3 ± 0.3	2.7 ± 0.3	2.7 ± 0.2	2.9 ± 0.3	3.0 ± 0.3
Plasma renin activity (nmol/l/h)					
Control	0.25 ± 0.05	0.18 ± 0.05	0.18 ± 0.05	0.18 ± 0.04	0.18 ± 0.04
Apelin	0.22 ± 0.04	0.15 ± 0.03	0.16 ± 0.03	0.15 ± 0.03	0.18 ± 0.04
Plasma norepinephrine (pmol/l)					
Control	6400 ± 1380	4790 ± 930	5600 ± 1390	5660 ± 1550	4870 ± 1160
Apelin	6460 ± 1390	5700 ± 1730	5600 ± 1200	5220 ± 1010	4960 ± 1140
Plasma epinephrine (pmol/l)					
Control	598 ± 99	450 ± 51	467 ± 55	431 ± 55	514 ± 69
Apelin	504 ± 44	454 ± 89	476 ± 76	441 ± 81	482 ± 64
Plasma urocortin (pmol/l)					
Control	16.4 ± 0.9	15.1 ± 0.8	15.5 ± 1.1	14.7 ± 0.9	14.6 ± 0.3
Apelin	15.7 ± 0.6	15.1 ± 1.1	15.3 ± 0.8	14.1 ± 0.6	14.5 ± 0.7

Values are means ± S.E.M. In addition, 10 and 100 µg doses were administered at 0 and 60 min respectively and had no significant effect (data not shown).

apelin was likewise biphasic ($P=0.017$), but was a mirror image of the response of MAP with an immediate (2 min) rise in heart rate followed by a fall to below control levels between 4 and 20 min post-bolus (Fig. 1). The high dose of apelin also induced a significant but short lived (peak effects 5 min post-bolus) fall in cardiac output ($P=0.015$) and rise in RAP ($P=0.031$). CTPR was also raised in response to 1 mg apelin bolus at 5–10 min post-bolus ($P<0.001$). ECG changes, including sinus tachycardia and sudden onset of sinus bradycardia (with or without changes in arterial pressure), were observed in four out of ten sheep following the 1 mg dose. However, the most consistent ECG change observed in these four sheep was varying degrees of atrioventricular (AV) block lasting up to 15 min post-bolus. Prolonged periods of 2:1 AV block were observed with 3:1 and 4:1 block also observed in one sheep. AV block was also observed in one of the sheep following the 100 µg dose. Apelin had no significant effect on haematocrit (Table 1).

The response of the hypothalamic–pituitary–adrenal (HPA) axis is shown in Fig. 2. Whilst the lower two doses of apelin had no effect, the 1 mg bolus of apelin induced a significant rise in plasma AVP ($P=0.009$), ACTH ($P=0.012$), aldosterone ($P=0.001$) and cortisol ($P=0.014$) levels. Levels were increased 15–30 min post-bolus but had returned to time-matched control levels by 60 min. Plasma ANP ($P=0.036$) and BNP ($P<0.001$) were also elevated in response to apelin (Fig. 3). Plasma ANP levels peaked 15 min following the 1 mg bolus but were still significantly raised 60 min post-bolus.

Plasma BNP concentrations were increased above time-matched control levels at 30 min following the 100 µg dose of apelin and were increased further in response to the 1 mg dose, again remaining significantly above control levels 60 min post-bolus. Plasma cyclic GMP levels were also significantly increased ($P=0.003$) in response to apelin, again with levels significantly higher 30 min following the 100 µg dose and further augmented following the 1 mg dose (Fig. 3). Plasma cyclic AMP levels were not affected by the lower two doses of apelin but were significantly raised ($P=0.002$) in response to the 1 mg bolus (Fig. 3). Apelin administration had no significant effects at any dose on plasma endothelin-1, PRA, norepinephrine, epinephrine or urocortin (Table 1).

Urinary volume and sodium excretion tended to be higher during the apelin arm of the study but this increased output was apparent before the first dose of apelin (Fig. 4). As such, administration of apelin at all three doses had no significant effect on urinary volume, sodium, potassium or creatinine excretion.

Discussion

Whilst the tissue distribution and range of biological actions reported for apelin suggest a role for the peptide in pressure/volume homeostasis, conflicting reports make the precise role unclear. Furthermore, few integrated studies have been performed and there are no reports of bioactivity of apelin in a large conscious animal model.

Figure 2 Hypothalamo–pituitary–adrenal axis response to i.v. bolus injections of apelin (●) or vehicle control (○) in ten sheep. Values are means $\pm$ S.E.M. Significant differences were observed for plasma arginine vasopressin ($P=0.009$), adrenocorticotrophin (ACTH) ($P=0.012$), aldosterone ($P=0.001$) and cortisol ($P=0.014$). Individual time points significantly different from time-matched control (Fisher's protected LSD) are indicated by * $P<0.05$ and † $P<0.01$.

Figure 3 Plasma atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), cyclic GMP and cyclic AMP response to i.v. bolus injections of apelin (●) or vehicle control (○) in ten sheep. Values are means $\pm$ S.E.M. Significant differences were observed for plasma ANP ($P=0.036$), BNP ($P<0.001$), cyclic GMP ($P=0.003$) and cyclic AMP ($P=0.002$). Individual time points significantly different from time-matched control (Fisher's protected LSD) are indicated by * $P<0.05$ and † $P<0.01$.

Figure 4 Renal response to i.v. bolus injections of apelin (hatched bars) or vehicle control (open bars) in ten sheep. Values are means $\pm$ S.E.M.

Thus we report the haemodynamic, hormonal and renal effects of apelin in normal conscious sheep. A high i.v. bolus dose (1 mg) of apelin induced a biphasic haemodynamic response characterised by an acute (peak effect 2 min post-bolus) fall in arterial pressure and rise in heart rate followed immediately by a rise in arterial pressure and

fall in heart rate. The secondary hypertensive phase was associated with a fall in cardiac output and significant rises in CTPR and RAP. Changes in all haemodynamic indices were short-lived having returned to vehicle control levels by 15–20 min post-bolus. ECG changes were also observed in four of ten sheep, most notably varying

degrees of AV block. At this dose, apelin also induced significant activation of the HPA axis with increased plasma AVP, ACTH, aldosterone and cortisol levels peaking by 15 min post-bolus (first blood sampling time-point). ANP, BNP, cyclic GMP and cyclic AMP levels were also increased but there was no effect on the other plasma hormones measured or on renal indices.

Previous reports of the effect of apelin on blood pressure are conflicting. Whilst several studies show that apelin lowers arterial pressure in rats (Lee *et al.* 2000, Tatemoto *et al.* 2001, Cheng *et al.* 2003, El Messari *et al.* 2004), other authors have shown that apelin increases blood pressure (Kagiyama *et al.* 2005). The current study is the first to report a biphasic response of arterial pressure to apelin. Of interest, similar biphasic responses of MAP (immediate hypotension followed by more prolonged hypertension) have been noted for other vasoactive peptides such as endothelin (King *et al.* 1990, Rohmeiss *et al.* 1990) and urotensin II (Gardiner *et al.* 2004). The initial hypotensive response to the 1 mg bolus of apelin was rapid in onset and peaked at 2 min. This is consistent with other reports of blood pressure lowering by apelin where the peak effect was observed 0.5–1 min post injection and pressures had returned to baseline by 2–4 min (Lee *et al.* 2000, 2005, Tatemoto *et al.* 2001), and may occur via a nitric oxide dependent mechanism (Tatemoto *et al.* 2001). By contrast, the secondary hypertensive response might be attributed to the activation of hydrogen/sodium and sodium/calcium exchangers in the vasculature as previously reported for apelin in the myocardial sarcolemma (Szokodi *et al.* 2002). Clearly, further studies examining underlying mechanisms are required.

The reasons for the different reported haemodynamic responses are unclear but may relate to species, dose and the structure of apelin administered. Prior studies were performed in rats, either anaesthetised (Lee *et al.* 2000, 2005, Tatemoto *et al.* 2001, El Messari *et al.* 2004) or conscious (Cheng *et al.* 2003). To our knowledge, this is the first report of the effect of apelin on MAP in a large animal model (conscious sheep). In the present study, the 10 and 100 µg doses of apelin had no effect and only the 1 mg dose induced the biphasic response on MAP and heart rate. A 1 mg bolus in sheep is equivalent to approximately 20 µg/kg. Of note, doses employed in the studies that showed only a fall in MAP were generally lower being 3–15 µg/kg (Lee *et al.* 2000, 2005, Tatemoto *et al.* 2001, El Messari *et al.* 2004). The authors who reported that i.v. apelin increased MAP used markedly higher doses than those employed in the present study (approximately 100 and 250 µg/kg); however they presented no information on the time-course of effects making it unclear as to whether the hypertensive response was preceded by a fall in MAP (Kagiyama *et al.* 2005). Cheng *et al.* (2003) employed doses spanning those effective in sheep (15–60 µg/kg) and reported only a fall in MAP, but again with no time-course information

reported. Thus, it is possible that the effects of apelin on MAP are dose- and time-dependent and careful consideration should be paid to the time-course of effects in future studies.

Another possible explanation for differences in haemodynamic responsiveness to apelin may be related to the structure of the apelin administered. Mature apelin is likely derived from the C-terminal region of the pro-peptide as either a 36 or a 13 amino acid peptide. A wide range of apelin peptides has been administered in previous studies producing variable results. Tatemoto *et al.* (2001) found that the rank order of potency of three different apelin peptides for lowering MAP in rats was apelin-12 > apelin-13 > apelin-36. By contrast, Lee *et al.* (2005) reported that apelin-13 lowers MAP in spontaneously hypertensive rats with more potency than apelin-12. Others have shown that apelin-17 is more potent than apelin-13 at lowering MAP in rats (El Messari *et al.* 2004). Thus, it is possible that different length apelin peptides have differing affinities for the APJ receptor. Moreover, it is also possible that peptide effects differ depending on species and/or vascular bed.

Previous studies reported that hypotensive actions of apelin were associated with either no change (Tatemoto *et al.* 2001, Lee *et al.* 2005) or a rise in heart rate (Lee *et al.* 2000, Cheng *et al.* 2003, El Messari *et al.* 2004). In the present study, changes in MAP were associated with mirror-image changes in heart rate, namely an acute rise in heart rate during the hypotensive phase (2 min post-bolus) followed by a fall in heart rate during the ensuing hypertensive phase. Given the similar biphasic time-course but directionally opposite changes in MAP and heart rate, it seems likely that changes in heart rate were baroreceptor mediated. This concurs with data showing that apelin-induced increases in heart rate are abolished in ganglion-blocked rats (Cheng *et al.* 2003). Cheng *et al.* (2003) also showed in ganglion-blocked rats that mean circulatory filling pressure is reduced by apelin, suggesting that it is a venodilator. In contrast, apelin has been shown to be a potent constrictor of isolated human saphenous veins (Katugampola *et al.* 2001). Results from the present study give no evidence that apelin is a venodilator, but rather, the 1 mg dose induced a consistent, immediate rise in RAP. Methodology (thermodilution) did not allow rapid (within 1–2 min) measurement of cardiac output in the present study with the first measurements being made at 5-min intervals following bolus administration. Thus, cardiac output was not determined during the acute hypotensive phase (2 min) but was significantly reduced during the hypertensive phase (5 min post-bolus). This coincided with a significant rise in CTPR, suggesting that a rise in cardiac afterload contributed to the decline in cardiac output. Clearly, mechanisms underlying the effects of apelin on cardiac output are complex and require further study. Results from the current study clearly show that apelin can have an effect on cardiac

electrophysiology. Whether apelin causes this effect by acting directly on the heart at the level of the sinus node and/or AV node, or whether its effects are mediated via the central nervous system is uncertain. To our knowledge there are no other reports of such an effect for apelin.

Contrasting reports on the effect of apelin on AVP have been published, with apelin inducing an increase in AVP secretion from hypothalamic explants (Taheri *et al.* 2002) but a reduction in AVP release reported *in vivo* in response to i.c.v. apelin (Reaux *et al.* 2001, De Mota *et al.* 2004). In the present study, the 1 mg apelin bolus elevated plasma AVP along with an increase in circulating levels of the other HPA hormones, namely plasma ACTH, aldosterone and cortisol. These results are consistent with previous reports showing apelin increases ACTH (Taheri *et al.* 2002) and corticosterone (Taheri *et al.* 2002, Jaszberenyi *et al.* 2004) release *in vivo*. It is likely that the observed rise in aldosterone levels was secondary to the ACTH rise, as PRA levels did not change. Plasma ANP and BNP levels were also raised in response to apelin. Interestingly, plasma BNP levels were significantly higher than time-matched controls during the 30 min preceding the 1 mg dose and were further augmented in response to the high dose. There was a similar trend for plasma ANP levels to rise before the 1 mg dose, but this rise did not achieve statistical significance. Thus, it is possible that the threshold for apelin's effect on the natriuretic peptides is lower than that for its other biological actions. To our knowledge, there have been no other reports of apelin affecting the natriuretic peptides. Cyclic GMP is the intra-cellular second messenger for the natriuretic peptides so it seems likely, given the same time-course of response, that increased cyclic GMP levels are secondary to changes in ANP and BNP. Of note, plasma cyclic GMP levels were positively correlated with plasma ANP levels in the present study ($P < 0.001$). Cyclic AMP levels were also significantly raised following the 1 mg apelin bolus. This is in contrast to previous reports showing that apelin inhibits cyclic AMP production (Reaux *et al.* 2001, El Messari *et al.* 2004). We cannot discount the possibility that rises in cyclic AMP observed in the present study were secondary to rises in other hormones, such as ACTH, for which there is a positive correlation in the present study ($P < 0.001$). Despite apelin being reported to have a diuretic effect, consistent with its presence in the hypothalamus where it seems to inhibit vasopressin-releasing neurons (De Mota *et al.* 2004), apelin had no significant effect on any renal indices measured in the present study.

In conclusion, high dose (1 mg bolus) administration of apelin to normal conscious sheep induces a significant biphasic response in MAP and heart rate, namely immediate hypotension with tachycardia followed by hypertension and bradycardia. This biphasic response in MAP and heart rate was associated with rises in RAP and CTPR and a fall in cardiac output during the hypertensive phase. This dose of apelin was also associated with activation

of the HPA axis (possibly secondary to haemodynamic effects) and activation of the natriuretic peptides. Taken together, these results suggest a potential role for apelin in pressure/volume homeostasis. Further studies are required with careful attention being given to structure/activity relationships and dose responses in different species.

Acknowledgements

We are grateful to the staff of the Christchurch School of Medicine and Health Sciences Animal Laboratory for assistance with animal studies and the Christchurch Cardioendocrine Laboratory staff for hormone assays. Support was provided through grants from the National Heart Foundation of New Zealand and the Health Research Council of New Zealand. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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Received in final form 26 February 2006

Accepted 6 March 2006

Made available online as an Accepted Preprint

13 March 2006