Urocortin 2 induces potent long-lasting inhibition of cardiac sympathetic drive despite baroreflex activation in conscious sheep

Christopher J Charles, David L Jardine, Miriam T Rademaker and A Mark Richards

Christchurch Cardioendocrine Research Group, University of Otago, Christchurch, PO Box 4345, Christchurch 8140, New Zealand

(Correspondence should be addressed to C J Charles; Email: chris.charles@otago.ac.nz)

Abstract

Emerging evidence suggests that the urocortin (UCN) peptides contribute to pressure and volume regulation with possible involvement in the pathophysiology of cardiovascular disease. We have recently reported that i.v. UCN1 potently inhibits cardiac sympathetic nerve activity (CSNA) in normal sheep. However, little is known about possible interactions between UCN2 and the sympathetic nervous system. Accordingly, we have examined the effects of i.v. UCN2 on CSNA, hemodynamics, and plasma catecholamines in normal conscious sheep. Bolus i.v. administration of UCN2 (25 and 100 µg) resulted in the expected hemodynamic actions including transient falls in arterial pressure ($P=0.016$) and more sustained rises in heart rate ($P<0.001$) and cardiac output ($P<0.001$) and falls in peripheral resistance ($P<0.001$). CSNA burst frequency showed a biphasic response ($P<0.001$) with an acute rise

followed by a more prolonged fall. CSNA burst area and incidence showed prolonged, dose-dependent falls in response to UCN2 administration (all $P<0.001$). UCN2 also induced a short-lived rise in plasma norepinephrine levels ($P=0.006$). The marked rise in heart rate in response to UCN2 is preserved in sheep undergoing pharmacological ganglionic blockade with hexamethonium. In conclusion, this is the first study to report the effects of UCN2 on SNA and indicates potent inhibition of sympathetic traffic to the heart despite a generalized baroreceptor-induced activation of sympathetic activity. These findings suggest an important role for UCN2 in cardiovascular homeostasis and warrant further investigation for potential therapeutic applications in acute myocardial injury and heart disease.

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Introduction

Emerging evidence suggests that the urocortin (UCN) peptides UCN1, UCN2, and UCN3, recently identified members of the corticotropin-releasing factor (CRF) peptide family, contribute to pressure and volume regulation with possible involvement in the pathophysiology of cardiovascular disease (as reviewed by Charles *et al.* (2004), Fekete & Zorilla (2007) and Boonprasert *et al.* (2008)). Unlike UCN1, which shows little selectivity for CRF receptor subtypes, UCN2 (and UCN3) binds selectively to the G protein-coupled CRF receptor type 2 (CRF₂; Hsu & Hsueh 2001, Lewis *et al.* 2001, Reyes *et al.* 2001). Both UCN2 and CRF₂ are widely distributed throughout the brain and peripheral tissues, with strong expression apparent in the heart and vasculature (Wiley & Davenport 2004). We have recently reported the bioactivity of UCN1 (Rademaker *et al.* 2002), UCN2 (Rademaker *et al.* 2005) and UCN3 (Rademaker *et al.* 2006) in sheep with and without pacing-induced heart failure (HF). All three peptides exhibit similar bioactivity, inducing reductions in peripheral resistance, arterial and left atrial pressures and increases in cardiac output in association with attenuation of vasoconstrictor/volume-retaining

neurohumoral systems. However, the time-course of effects of the three peptides differs markedly with UCN1 having a long half-life (19 h) with bioactivity lasting more than 24 h following i.v. bolus doses compared to a much shorter time-course of action (several hours) for UCN2 and UCN3 (Rademaker *et al.* 2002, 2005, 2006).

The sympathetic nervous system (SNS) is a pivotal element of normal cardiac and circulatory regulation. We have recently reported that UCN1 potently inhibits cardiac sympathetic nerve activity (CSNA) in normal sheep (Charles *et al.* 2008). However, little is known about possible interactions between the more recently identified UCN2 and the SNS. UCN2-induced rises in heart rate do not appear to be solely baroreflex mediated as, in normal sheep, UCN2 increases heart rate and blood pressure in parallel (Rademaker *et al.* 2005). This raises the possibility that UCN2 may modulate sympathetic outflow from the central nervous system (CNS). Studies examining the effects of UCN2 on plasma norepinephrine report either no effect (Rademaker *et al.* 2005, Davis *et al.* 2007a) or activation (Davis *et al.* 2007b) of norepinephrine, taken as an index of global activity of the SNS. To our knowledge, there have been no studies investigating the effect of UCN2 on SNA to a specific organ

as measured by direct means, such as microneurography. Accordingly, we have examined the effects of UCN2 on CSNA, hemodynamics, and plasma catecholamines in normal conscious sheep.

Materials and Methods

The Animal Ethics Committee of the University of Otago, Christchurch approved the study protocol. A total of seven Coopworth ewes weighing 53–76 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 anesthesia (induced by 17 mg/kg thiopentone sodium and maintained by a mixture of halothane, nitrous oxide, and oxygen), a left lateral thoracotomy was performed to allow up to five stainless steel needle electrodes to be inserted and glued in the thoracic cardiac nerves as previously described (Jardine *et al.* 2002). Connecting leads were sutured to the mediastinum and exteriorized dorsally through the chest wall. During the same anesthetic, via a 5-cm neck incision, a carotid artery was cannulated (16G Angiocath; Becton Dickinson, Sandy, UT, USA) for direct measurement of arterial pressure and heart rate; polyethylene catheters were placed in the jugular vein for blood sampling, 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. The animals were allowed to recover for at least 4 days before experiments.

CSNA recordings were made in fully conscious sheep from pairs of electrodes via a preamplifier with an active probe (DAM-80 pre-amplifier, World Precision Instruments Inc., Sarasota, FL, USA). The raw signal was amplified ($\times 10^6$), filtered between 300 and 3000 Hz, and integrated using a time constant of 100 ms. The integrated nerve signal was digitally converted using in-house software (sampling rate 200 Hz), and postganglionic efferent sympathetic activity was identified in all animals by the following characteristics: a) bursts were synchronised to the diastolic phase of the arterial pulse; b) bursts decreased during sympathetic blockade with hexamethonium infusion (2 mg/kg over 2 h) on day 3 postthoracotomy; and c) an inverse relationship existed between burst area and diastolic blood pressure during baroreflex tests undertaken on each recording day. Background noise was quantified as the small deviations from baseline observed during hexamethonium infusion and phenylephrine bolus. Only recordings with a signal-to-noise ratio of greater than two and coinciding with the correct part of the cardiac cycle were analyzed. Using these criteria on the first day, the best signal from all possible electrode combinations was selected and used for subsequent recordings. CSNA was quantified from the integrated nerve signal by: a) counting the number of bursts per minute

(burst frequency); b) counting the number of bursts per 100 heart beats (burst incidence); and c) measuring the area under the integrated signal per minute (burst area/min) and burst area/100 beats.

Each animal was studied on two occasions 1–3 days apart, receiving vehicle control (10 ml of 0.9% saline) and murine UCN2 (American Peptides, Sunnyvale, CA, USA) according to a balanced random order design. Ovine UCN2 has been shown to have 97% homology (one amino acid different) to mouse UCN2 (Rademaker *et al.* 2005). UCN2 was injected i.v. as two incremental bolus doses of 25 and 100 μ g given 2 h apart.

Arterial pressure recordings, using an in-house online data acquisition system, commenced 30 min before the first injection and were continued for 120 min after the high dose. Heart rate and pressures were digitally integrated in 5-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. Stroke volume was calculated as cardiac output divided by heart rate.

Venous blood was drawn at preset intervals during the study protocol. Blood was taken into chilled EDTA tubes, centrifuged, and the plasma stored at -80°C before assay for catecholamines (Goldstein *et al.* 1981).

Ganglionic blockade

Six additional sheep, surgically prepared as above but without usable nerve recording fields, were used to assess the effect of ganglionic blockade on UCN2-induced rises in heart rate. After 30-min baseline recording, the sheep received i.v. hexamethonium at a dose previously shown to inhibit CSNA (Jardine *et al.* 2002) namely 120 mg hexamethonium infused over 60 min. Thirty minutes after commencement of hexamethonium, the sheep received an i.v. bolus (25 μ g) of UCN2. Heart rate, MAP, and cardiac output were recorded at 15-min intervals from 30 min prior to ganglionic blockade to 60 min after UCN2.

Statistical analysis

Results are expressed as mean $\pm$ S.E.M. Two-way ANOVA with time as a repeated measure was used to determine time and treatment differences between UCN2 and control arms of the study. Where significant differences were identified by ANOVA, *a priori* Fisher's protected least square difference (LSD) tests were used to identify individual time-points significantly different from time-matched control data. One-way ANOVA with time as a repeated measure was used to determine significant effects of UCN2 in the hexamethonium-treated sheep. Where significant differences were identified by ANOVA, *a priori* Fisher's protected LSD tests were used to identify individual time-points significantly

different from baseline values. *T*-tests were used to determine differences in maximal hemodynamic response to UCN2 between normal sheep and sheep treated with hexamethonium. Statistical significance was assumed at $P < 0.05$.

Results

All animals successfully completed the experimental protocol, and full data collection was achieved with the exception of plasma epinephrine measurements. In samples from two animals, the epinephrine peaks from the HPLC profiles of the catecholamine assays were masked by unknown interference. Therefore, plasma epinephrine data are shown for $n=5$ sheep. CSNA burst area is an area under the curve measurement calculated in arbitrary units, and as such these data are expressed as percentage change from baseline. Significant baseline (time-points -30 to 0 min) differences were observed for CSNA burst frequency (51.9 ± 7.6 vs 63.9 ± 12.7 bursts/min for control and active days respectively, $P < 0.05$) and CSNA burst incidence (62.8 ± 6.3 vs 72.3 ± 9.2 bursts/100 beats for control and active days respectively, $P < 0.05$). Therefore, these data are also presented as percentage change from baseline.

Original arterial pressure, integrated CSNA, and ECG recordings from a representative sheep are shown in Fig. 1. Compared with baseline (top left panel), 30 min following 25 μ g bolus (top right panel), UCN2 induced a reduction in arterial pressure and increase in heart rate with modest increase in CSNA burst frequency (corresponding to the increased heart rate), but no increase in burst amplitude. In the lower panels, 90 min following 25 μ g bolus (lower left) or 100 μ g bolus (lower right), the effects of UCN2 on

arterial pressure and heart rate were no longer evident, while dose-dependent and sustained reductions in both CSNA nerve frequency and amplitude were observed.

UCN2 induced dose-dependent prompt, but short-lived reductions in MAP ($P=0.016$; Fig. 2). In response to both doses, the nadir in MAP was 30 min after the bolus with pressures returning to time-matched control by 60-min postbolus. Dose-dependent increases in heart rate ($P < 0.001$) were longer lasting, and while the peak response still occurred at 30 min after each bolus, heart rate remained above time-matched control at least 120 min after each dose (Fig. 2). UCN2 also increased cardiac output ($P < 0.001$), to a similar degree with both doses with outputs only returning to time-matched control values after 90 min following each bolus (Fig. 2). Increases in cardiac output were reciprocated by falls in CTPR ($P < 0.001$), with changes following the same time-course as observed for cardiac output (Fig. 2). Stroke volume fell following both doses of UCN2 and remained lower than time-matched control for the duration of the study ($P < 0.001$).

UCN2 induced dose-dependent changes in all indices of CSNA compared with time-matched control. CSNA burst frequency (burst/min) showed a biphasic response ($P < 0.001$) with an acute rise above vehicle control confined to the first 15–30 min after 25 μ g bolus followed by a sustained reduction below vehicle control at 90–120 min following the UCN2 bolus (Fig. 3). Administration of the 100 μ g bolus also induced a brief (15 min) rise in burst frequency followed by a sustained reduction. CSNA burst incidence (bursts/100 beats) did not show this biphasic response but rather a significant ($P < 0.001$) and progressive reduction to remain $\sim 40\%$ lower than time-matched vehicle control for 120 min following the high dose bolus of UCN2 (Fig. 3). Similarly,

Figure 1 Sample tracings from a representative sheep of arterial pressure, cardiac sympathetic nerve activity (CSNA), and ECG at baseline, 30 and 90 min following 25 μ g bolus and 90 min following 100 μ g bolus administration of urocortin 2 (UCN2).

Figure 2 Mean arterial pressure, heart rate, cardiac output, stroke volume, and calculated total peripheral resistance (CTPR) response to i.v. bolus administration of urocin 2 (●) at doses of 25 and 100 µg or vehicle control (○) in seven sheep. Values shown are mean $\pm$ S.E.M. Urocin 2 induced significant falls in mean arterial pressure ($P=0.016$), stroke work ($P<0.001$), and in CTPR ($P<0.001$) and significant rises in heart rate ($P<0.001$) and cardiac output ($P=0.03$). Individual time-points significantly different from time-matched control (Fisher's protected LSD) are indicated by * $P<0.05$ and † $P<0.01$.

both CSNA burst area/min ($P<0.001$) and burst area/100 beats ($P<0.001$) were dose-dependently reduced in response to bolus administration of UCN2 with levels being 50–80% lower than time-matched vehicle control following the 100 µg UCN2 bolus (Fig. 3).

Bolus administration of UCN2 at these doses induced significant, short-lived activation of plasma norepinephrine ($P=0.006$), with significant elevation of levels occurring 15–30 min following each UCN2 bolus administration but thereafter norepinephrine returned to time-matched control levels (Fig. 4). Plasma epinephrine levels ($n=5$) tended to follow a similar pattern but these changes did not achieve statistical significance (Fig. 4).

Ganglionic blockade

Ganglionic blockade with i.v. hexamethonium induced minimal rises in heart rate and minimal reductions in MAP, cardiac output, and stroke volume with only the effect on stroke volume being statistically significant (Fig. 5). Subsequent bolus administration of UCN2 resulted in significant rises in heart rate within 15–30 min of UCN2 bolus ($P<0.001$). UCN2 induced significant falls in MAP ($P=0.018$), stroke volume ($P<0.001$), and CTPR ($P<0.001$) but no consistent effect on cardiac output in hexamethonium-treated sheep (Fig. 5). Comparison of the maximal change in response to 25 µg bolus of UCN2 for these hemodynamic indices in normal and hexamethonium-treated sheep is shown in Fig. 6. Maximum increase in heart rate was similar between the two groups of sheep being 42.4 ± 11.5 and 52.2 ± 6.9 in normal and hexamethonium-treated sheep respectively. Cardiac output increments were significantly attenuated in the hexamethonium sheep ($P=0.02$). There was no significant difference in the response of MAP, CTPR, or stroke volume between the two groups (Fig. 6).

Discussion

The effect of UCN2 on SNA, including sympathetic traffic to the heart, has not previously been reported. Accordingly, we have examined the effects of UCN2 on CSNA, hemodynamics, and plasma catecholamines in normal conscious sheep. Bolus i.v. administration of UCN2 resulted in the expected hemodynamic actions of transient falls in arterial pressure with more sustained rises in heart rate and cardiac output and falls in CTPR. CSNA burst frequency showed a biphasic response with an acute rise followed by a more prolonged fall. All other indices of CSNA showed prolonged, dose-dependent falls in response to UCN2. UCN2 induced a short-lived activation of plasma catecholamine levels. Thus, UCN2 exhibits potent sustained inhibition of sympathetic traffic to the heart, despite an apparent short-lived generalized baroreceptor-induced activation of sympathetic activity as indicated by plasma catecholamines. The concurrent pronounced activation of heart rate suggests a direct chronotropic action for UCN2, which appears to be (at least in part) independent of actions on the autonomic nervous system.

While studies in man and sheep from our laboratory have reported plasma catecholamine levels following systemic

Figure 3 Cardiac sympathetic nerve activity (CSNA) burst frequency (bursts/min), burst area (BA/min), burst incidence (bursts/100 beats), and burst area incidence (BA/100 beats) responses to i.v. bolus administration of urocortin 2 (●) at doses of 25 and 100 µg or vehicle control (○) in seven sheep. Values shown are mean $\pm$ S.E.M. Urocortin 2 induced a significant biphasic response in CSNA bursts/min ($P < 0.001$) and consistent falls in BA/min ($P < 0.001$), bursts/100 beats ($P < 0.001$), and BA/100 beats ($P < 0.001$). Individual time-points significantly different from time-matched control (Fisher's protected LSD) are indicated by * $P < 0.05$ and † $P < 0.01$.

UCN2 administration, results have been variable. In normal and HF sheep, there were no significant changes observed in plasma norepinephrine levels, but plasma epinephrine levels were reduced following UCN2 administration in HF sheep (Rademaker *et al.* 2005). In normal humans, UCN2 induced a rise in circulating norepinephrine without change in epinephrine levels (Davis *et al.* 2007a), while identical doses of UCN2 infused in patients with stable HF resulted in no significant changes in either plasma epinephrine or norepinephrine (Davis *et al.* 2007b). Results from the present

study clearly show that UCN2 raised plasma norepinephrine levels in a manner that reciprocated a significant fall in MAP. In fact, the time-course of changes in norepinephrine and in MAP were identical, with the peak or nadir respectively occurring 15–30 min after each dose. This pattern was identical for plasma epinephrine with a trend to rise, which did not achieve statistical significance, as results could only be obtained for $n = 5$ sheep. In addition, the reciprocal changes in blood pressure and plasma norepinephrine suggest that the rise in plasma catecholamines reflect a baroreceptor-induced reflex response, probably directed at the peripheral vasculature. *In vitro* data suggest that UCN2 may regulate catecholamine synthesis and release in the adrenal medulla. In cultured PC12 cells, UCN2 induces norepinephrine release and phosphorylation of tyrosine hydroxylase through protein kinase A and protein kinase A–Erk1/2 pathways respectively (Nemoto *et al.* 2005). However, conversely, UCN2 activation of CRFR2 actually suppresses the secretion of catecholamines in dispersed rat and human adrenal chromaffin cells, whereas UCN1/CRF activation of CRFR1 induces catecholamine secretion (Dermitzaki *et al.* 2007). On balance, it would appear that plasma catecholamine responses in the present study reflect a generalized baroreceptor-induced activation of the SNS.

Despite this apparent generalized activation of the SNS, the main finding of our study is that UCN2 induces prolonged and potent inhibition of sympathetic drive directed to the heart as measured by direct microneurography. In the case of

Figure 4 Plasma norepinephrine ($n = 7$) and epinephrine ($n = 5$) response to i.v. bolus administration of urocortin 2 (●) at doses of 25 and 100 µg or vehicle control (○). Values shown are mean $\pm$ S.E.M. Urocortin 2 induced significant rises in plasma norepinephrine ($P = 0.006$). Individual time-points significantly different from time-matched control (Fisher's protected LSD) are indicated by † $P < 0.01$.

Figure 5 Hemodynamic response to i.v. bolus administration of urocortin 2 (25 µg) in six sheep that received the ganglionic blocking agent hexamethonium (120 mg over 60 min). Values shown are mean ± S.E.M. Urocortin 2 induced significant falls in mean arterial pressure ($P=0.18$), stroke volume ($P<0.001$), and calculated total peripheral resistance (CTPR; $P<0.001$) and significant rises in heart rate ($P<0.001$). Individual time-points significantly different from baseline values (Fisher's protected LSD) are indicated by * $P<0.05$, † $P<0.01$, and ‡ $P<0.001$.

CSNA burst frequency, the observed pattern of changes was biphasic, with an acute rise in burst frequency at 15–30 min following each dose of UCN2 followed by a sustained fall below time-matched control levels. All other indices of CSNA were reduced for the duration of measurements following UCN2 administration. Such falls in CSNA during concurrent activation of plasma catecholamines should not be interpreted as conflicting data, as it is important to note that there can be great selectivity of sympathetic reflex activity to different tissue beds (Ninomiya *et al.* 1971, Pagani *et al.* 1974).

This highlights the need to measure SNA efferent traffic to the organ of interest. We propose that the biphasic change in CSNA burst frequency may be explained by initial vasodilation and off-loading of baroreflex activity, contributing to the observed increase in HR and a short-lived increase in CSNA burst frequency. However, indices of CSNA burst size suggest simultaneous central inhibition of CSNA, which is of longer duration. Sympathetic nerve burst frequency reflects probability of firing of individual fibers, is gated to HR, and is regulated primarily by baroreflex responses to rapid changes in blood pressure (Sundlof & Wallin 1978, Malpas & Ninomiya 1992a). However, burst size, reflecting recruitment of postganglionic fibers, is more affected by central pathways, for example, paraventricular nucleus secretion of vasopressin (Malpas & Coote 1994) and chemoreceptor stimulation (Malpas & Ninomiya 1992b). Therefore, the temporal changes we observed in different CSNA indices may be explained by the immediate indirect effects of baroreflex unloading coupled with more persistent direct sympathoinhibitory effects of UCN2 on the CNS. Nonetheless, the initial acute activation of CSNA burst frequency observed for UCN2 in the present study is in contrast to that previously observed for UCN1, where there was also no other evidence for other activation of the SNS as measured by plasma catecholamines (Charles *et al.* 2008).

The mechanism underlying UCN2-induced inhibition of CSNA cannot be defined by the present study but presumably occurs via central pathways. Of note, UCN2 has been shown to cross the blood–brain barrier in intact form by passive diffusion, entering brain parenchyma at a moderate rate (Kastin & Akerstrom 2002). The neuroanatomy of sympathetic outflow is well known, in that the hypothalamus sends both direct and indirect projections, via the brainstem, to sympathetic preganglionic neurons in the spinal cord (Saper *et al.* 1976). Regional expression of the UCNs

Figure 6 Comparison of maximum change in hemodynamic parameters in response to 25 µg urocortin 2 in normal sheep (open bars, $n=7$) compared with ganglionic-blocked sheep (pretreatment with hexamethonium; crosshatched bars, $n=6$). Values shown are mean ± S.E.M. There was a significant difference between the response of cardiac output between the normal and hexamethonium group (* $P=0.02$).

throughout the brain suggests a neurotropic role for these peptides in the CNS (Koob & Heinrichs 1999). Furthermore, CRF₂ receptors are present at high levels in the CNS, including autonomic regulatory centers in the hypothalamus and brain stem (De Fanti & Martinez 2002). Although the above-mentioned studies have shown that pathways exist whereby both central and systemic administration of UCN peptides can activate neuronal projections throughout the hypothalamus, brainstem, and spinal cord and could thereby modulate sympathetic outflow, further studies are required to confirm such a mechanism.

In the present study, UCN2 clearly induced acute falls in MAP. This appears to be in contrast to our previously published data on UCN2 in normal sheep where we reported a subtle but overall significant rise in MAP in response to similar doses of UCN2 (Rademaker *et al.* 2005). However, careful examination of the time-course of the changes in MAP in our earlier study (Rademaker *et al.* 2005) shows that MAP was only raised above time-matched control data from 45 min following bolus administration and then only by 3–4 mmHg. In fact, the acute effect (at 15–30 min following bolus administration) was a trend (not significant) for MAP to fall, as seen in the present study. Significant falls in MAP (15–30 min following UCN2 bolus) were observed in the sheep with pacing induced HF (Rademaker *et al.* 2005). Thus, in our earlier publication (Rademaker *et al.* 2005), the overall small elevation of MAP reported for normal sheep masked an initial transient fall in MAP.

Falls in MAP in the present study were reciprocated by rises in heart rate. However, while falls in MAP were short lived (15–30 min postbolus), heart rate remained significantly above time-matched control for the duration of the study (at least 120 min postbolus). This may indicate that rises in heart rate in response to UCN2 are not merely baroreceptor mediated but that UCN2 may have direct chronotropic actions to increase heart rate independent of baroreceptor activation. Indeed, we have previously shown that UCN2 induces large dose-dependent increases in heart rate in normal sheep in the absence of any fall in arterial pressure (Rademaker *et al.* 2005). These results are in contrast to those of Gardiner *et al.* (2007) who demonstrated that UCN2 (stresscopin-related peptide) administered to conscious male Sprague–Dawley rats lowered blood pressure and increased heart rate, but that the tachycardic effects were abolished by pretreatment with atropine and propranolol. Thus, Gardiner *et al.* (2007) concluded that in conscious male rats, the tachycardic effects of UCN2 (and UCN1) are due to autonomic nervous activation mainly through baroreflex mechanisms. However, there appears to be species differences to the effects of the UCN peptides on heart rate and whether or not they are only mediated by baroreceptor activation following falls in arterial pressure. There may also be other factors contributing to the tachycardic effects of the UCN peptides (for example gender) but this requires further study. With regard to a role for the autonomic nervous system, in the case of UCN1, rises in cardiac output, heart rate, and

cardiac contractility were similar in intact sheep and in sheep undergoing ganglionic blockade with hexamethonium (Parkes & May 1999). Thus, it seems likely that, at least in conscious sheep, the UCN peptides may have direct chronotropic actions independent of baroreceptor-mediated sympathetic activation. Of note, in the six sheep that underwent ganglionic blockade (hexamethonium infusion), the rise in heart rate following a 25 µg bolus of UCN2 was ~50 bpm (Fig. 6), similar in magnitude to that observed in intact sheep. Thus, both UCN1 (Parkes & May) and UCN2 (present study) dramatically increase the heart rate similarly in both intact sheep and ganglion blocked (hexamethonium) sheep. This indicates that the actions of the UCN peptides to increase heart rate are not mediated by the autonomic nervous system, and it is likely that they are mediated by a direct action of UCN peptides on the heart. We cannot discount a contribution from an action of the UCN peptides to inhibit vagal activity, although to our knowledge no studies have addressed this question to date. However, it should be noted that vagal withdrawal is likely to induce only a modest rise in heart rate in sheep. Previous studies both from our laboratory (unpublished data) and from the literature (Hays & Webster 1971, Lumbers & Yu 1999) show that i.v. atropine or vagal section increases heart rate in sheep by ~20–30 bpm with the maximum heart rates observed following withdrawal of vagal tone being 110–120 bpm. This is much less than the maximal increments observed (80 bpm) to increase heart rate to ~130 and 180 bpm following 25 and 100 µg UCN2 respectively (Fig. 2).

In the present study, stroke volume fell following UCN2 administration. The observed reduction in stroke is likely to be secondary to the marked cardiac sympathetic drive. However, despite such dramatic cardiac sympathoinhibition, stroke volume did not fall precipitously, which may reflect some degree of inotropic action of UCN2, as has been suggested previously (Bale *et al.* 2004, Rademaker *et al.* 2005). Despite this reduction in stroke volume, cardiac output was actually increased following UCN2. Thus, the contribution of the dramatically increased heart rate to increase cardiac output more than counterbalanced the modest fall that would have been expected with this degree of stroke volume reduction. Of note, similar reductions in stroke volume but increments in cardiac output have previously been reported for UCN1 (Parkes & May 1999). In hexamethonium-infused sheep, there was no significant increase in cardiac output in response to UCN2 but, of note, trend for cardiac output to fall with hexamethonium was reversed with administration of UCN2. Nonetheless, there was a relative lack of any contribution from positive inotropic actions of UCN2 in the pharmacologically denervated sheep. Taken together, any peptide that concurrently inhibits CSNA yet has direct actions on the heart to maintain cardiac output (and coronary blood flow) is of potential therapeutic interest in cardiovascular disorders. UCN2 is reported to reduce damage in rat hearts subjected to regional ischemia/reperfusion, probably via MAPK-dependent pathway (Brar *et al.* 2004). In the intact

animal, reduced sympathetic traffic to the heart could further enhance these cardioprotective actions of UCN2 (as reviewed by Fekete & Zorilla (2007) and Boonprasert *et al.* (2008)), particularly in the setting of acute myocardial injury and may also protect against incidence and/or severity of arrhythmias.

In conclusion, this is the first report of UCN2 effects on SNA and indicates potent inhibition of sympathetic traffic to the heart, despite a generalized baroreceptor-induced activation of sympathetic activity. It also confirms a potent action of UCN2 to increase heart rate in both intact and ganglion-blocked sheep. These findings suggest an important role for UCN2 in cardiovascular homeostasis and warrant further investigation for potential therapeutic applications in acute myocardial injury and heart disease.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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