

Cardiovascular Effects of Long-Term Central and Peripheral Administration of Urocortin, Corticotropin-Releasing Factor, and Adrenocorticotropin in Sheep

R. S. WEISINGER, J. R. BLAIR-WEST, P. BURNS, D. A. DENTON, B. PURCELL, W. VALE, J. RIVIER, H. S. WEISINGER, AND C. N. MAY

Howard Florey Institute of Experimental Physiology and Medicine (R.S.W., P.B., D.A.D., B.P., C.N.M.) and Departments of Physiology (J.R.B.-W.) and Optometry and Vision Science (H.S.W.), University of Melbourne, Parkville, Australia 3010; and The Clayton Foundation Laboratories for Peptide Biology (W.V., J.R.), The Salk Institute, La Jolla, California

The neuroendocrine hormones ACTH and corticotropin-releasing factor (CRF), which are involved in the stress response, have acute effects on arterial pressure. New evidence indicates that urocortin (UCN), the putative agonist for the CRF type 2 receptor, has selective cardiovascular actions. The responses to long-term infusions of these hormones, both peripherally and centrally, in conscious animals have not been studied. Knowledge of the long-term effects is important because they may differ considerably from their acute actions, and stress is frequently a chronic stimulus. The present experiments investigated the cardiovascular effects of CRF, UCN, and ACTH in conscious sheep. Infusions were made either into the lateral cerebral ventricles (icv) or iv over 4 d at

5 $\mu\text{g}/\text{h}$. UCN infused icv or iv caused a prolonged increase in heart rate (HR) ($P < 0.01$) and a small increase in mean arterial pressure (MAP) ($P < 0.05$). CRF infused icv or iv progressively increased MAP ($P < 0.05$) but had no effect on HR. Central administration of ACTH had no effect, whereas systemic infusion increased MAP and HR ($P < 0.001$). In conclusion, long-term administration of these three peptides associated with the stress response had prolonged, selective cardiovascular actions. The striking finding was the large and sustained increase in HR with icv and iv infusions of UCN. These responses are probably mediated by CRF type 2 receptors because they were not reproduced by infusions of CRF. (*Endocrinology* 145: 5598–5604, 2004)

CORTICOTROPIN-RELEASING hormone (CRF) is a key regulator of the hypothalamo-pituitary-adrenal axis and a major factor responsible for the coordination of the behavioral and neuroendocrine responses to stress (1–3). CRF, administered centrally, increases blood pressure by actions on the autonomic nervous system and by stimulating ACTH release, which increases blood pressure secondary to secretion of mineralocorticoids and glucocorticoids (2, 4–12). In contrast, CRF administered into the circulation has a vasodilator action and reduces blood pressure in rats (5, 6, 13–15) but has little effect on blood pressure in sheep (16).

Urocortin (UCN) is a newly discovered mammalian member of the CRF family that was isolated from rat midbrain (17). UCN has 45% homology with CRF and binds with high affinity to CRF type 1 and CRF type 2 receptors but has an almost 40-fold greater affinity for type 2 receptors than CRF (17). CRF type 2 receptors are localized at distinct central and peripheral sites that are involved in cardiovascular regulation (18–20). In rodents there are two functional splice variants of the CRF type 2 receptors, CRF 2 α is found primarily in the brain, whereas CRF R2 β is predominantly expressed in heart, gastrointestinal tract, arterioles, and muscles (19, 21). The cardiovascular actions of central UCN have been

examined in only one study in which bolus injection caused a small increase in blood pressure in rats (22). Peripheral administration of UCN decreased blood pressure in rats (23) and mice (24–26). In sheep, bolus iv administration of UCN caused a prolonged increase in cardiac contractility and heart rate, which increased cardiac output and arterial pressure (16). These effects of UCN were blocked by a low dose of α -helical CRF (9–41) that had no effect when given alone (16). In mice, iv administration of UCN caused increases in heart rate and cardiac contractility and vasodilation, actions absent in mice deficient for CRF type 2 receptors (25, 26). In rats iv administration of urocortin 2, which is selective for the CRF type 2 receptor, caused a reduction in arterial pressure and an increase in heart rate (27)]. These data indicate that the actions of UCN, in mice, rats and sheep, depend on stimulation of CRF type 2 receptors.

In most of the previous studies, peptides have been administered as bolus doses, yet stress is not necessarily a brief experience and prolonged infusions of neuropeptides have shown physiological and behavioral responses that have taken up to 24 h to develop (28, 29). In the present study, therefore, we determined the effects of icv infusion of UCN for 4 d, and the responses have been compared with those of CRF. Because both CRF and UCN cause ACTH release and increased release of adrenocorticosteroids by actions on CRF type 1 receptors (7–10), the responses to ACTH have also been studied. To determine the long-term responses to systemic administration of these peptides, we also examined the effects of 4-d iv infusion of the same doses.

In a previous report in which we examined the effects of

Abbreviations: aCSF, Artificial cerebrospinal fluid; CRF, corticotropin-releasing factor; HR, heart rate; MAP, mean arterial pressure; UCN, urocortin.

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4 d of iv and icv infusion of these peptides at 5 $\mu\text{g}/\text{h}$ on ingestive behavior, we found that the increase in NaCl intake induced by iv ACTH was inhibited by iv infusion of UCN (27). We have, therefore, also investigated whether there is any interaction between the cardiovascular actions of UCN and ACTH, specifically whether the effects of UCN were to inhibit the effects of ACTH as was observed in the previous study (27). The same dose of peptides was used here in view of the previous experience of effectiveness.

Materials and Methods

Animals

Experiments were performed on 15 Merino ewes. Before the studies, the sheep underwent two aseptic surgical procedures, separated by 2 wk. Anesthesia was induced with iv sodium thiopental (15 mg/kg) and after intubation was maintained with 1.5–2.0% isoflurane- O_2 . In the first procedure, sheep were oophorectomized and prepared with bilateral carotid arterial loops. In the second procedure, guide tubes were placed above the lateral cerebral ventricles. Sheep had continuous access to water and 0.5 M NaCl to drink and were fed 800 g oaten-lucerne chaff (Na^+ content 90–100 mmol/kg, K^+ content 200–300 mmol/kg) daily. Experimental procedures were approved by the Animal Experimentation Ethics Committee of the Howard Florey Institute under guidelines laid down by the National Health and Medical Research Council of Australia.

Peptides

The infusates used were: ovine/rat UCN (MW 4707.3, provided by J. Rivier, The Salk Institute, La Jolla, CA); human CRF (molecular weight 4758, Auspep, Parkville, Victoria, Australia); ACTH (1–24, MW 2934, Novartis, Basel, Switzerland). For icv infusion, peptides were dissolved in artificial cerebrospinal fluid [aCSF, 30 = 151 mM, Cl = 157.5 mM, K = 2.8 mM, Ca = 1.1 mM, Mg = 0.9 mM, and $\text{HPO}_4 = 0.5$ mM] and for iv infusion peptides were dissolved in normal saline.

Infusion procedures

In all three studies, at least 14 d recovery was allowed between treatments, which was sufficient to prevent any carry over effects. The order of the treatments was randomized.

For icv infusion, a probe (20-gauge needle), connected to a polyethylene cannula filled with aCSF, was inserted through the guide tube into a lateral brain ventricle. Sheep were infused with ovine/rat UCN ($n = 7$); human CRF ($n = 7$); ACTH ($n = 6$), or aCSF ($n = 12$, 0.2 ml/h). Peptides were infused at 5 $\mu\text{g}/\text{h}$ (0.2 ml/h) from 1130 h on d 0 to 1400 h on d 4.

For iv infusion, a polyethylene cannula was inserted into the jugular vein (10–12 cm toward the heart) 1–2 d before the experiment. Sheep were infused with UCN ($n = 6$), CRF ($n = 8$), ACTH ($n = 9$), or vehicle (normal saline, $n = 6$, 0.2 ml/h) from 1130 h on d 0 to 1400 h on d 4. Peptides were infused at 5 $\mu\text{g}/\text{h}$ (0.2 ml/h) from 1130 h on d 0 to 1400 h on d 4.

For combined infusion, sheep were treated with iv infusion of ACTH concurrently with either icv ($n = 5$) or iv ($n = 5$) infusion of UCN. Peptides were infused at 5 $\mu\text{g}/\text{h}$ (0.2 ml/h) from 1130 h on d 0 to 1400 h on d 4.

Blood sampling and arterial blood pressure measurements

Arterial pressure was measured from the carotid artery via an 18-gauge needle and polyethylene cannula filled with heparinized saline (50 IU/ml), using a pressure transducer (345-931-009, COBE, Arvada, CO). This was connected to a pressure amplifier (RK8, JRACK, Sydney, Australia), and arterial pressure was recorded on a chart recorder [GRAPHTEC Thermal Arraycorder (GRAPHTEC, Yokohama, Japan), model WR7700]. Mean arterial pressure (MAP) was measured at 1000 h on d 0, before the start of infusion, and on the subsequent 4 d of infusion. MAP and heart rate (HR) were read from the trace, and the mean over

20 min was calculated. Carotid arterial blood samples (10 ml) were collected at the end of each measurement period.

Plasma analyses

Plasma (Na^+ and K^+) was measured using a Synchron CX-5 clinical system (Beckman, Brea, CA). Cortisol concentration in plasma was analyzed by RIA (31).

Statistical analysis

Unless otherwise specified, a two-way ANOVA, repeated measures on one variable (*e.g.* days) and subsequent least significant differences tests (Statistica, StatSoft, Tulsa, OK) were used to compare the baseline value (*i.e.* the average value obtained on the day before each infusion for each animal) to the value(s) obtained during or after each of the treatments or to compare various treatment values. Data are presented as means \pm SEM.

Results

Effects of icv infusions of UCN, CRF and ACTH

Intracerebroventricular infusion of UCN and CRF increased MAP by 5–10 mm Hg over the 4 d of infusion ($P < 0.05$) (Fig. 1). The infusions of ACTH or vehicle did not alter MAP. Only the infusion of UCN altered HR, which progressively increased from 65 ± 2 to 90 ± 9 beats/min over the 4 d of infusion ($P < 0.001$).

Plasma cortisol concentration was increased on d 2–4 of infusion of UCN and CRF ($P < 0.01$). Infusion of ACTH and CSF had no effect on plasma cortisol levels. Plasma (Na^+ and K^+) was not altered by any of the icv infusions.

Effect of iv infusions of UCN, CRF, and ACTH

ACTH infusion increased MAP by 20–25 mm Hg throughout the 4-d infusion period ($P < 0.001$) (Fig. 2). CRF and UCN infusions increased MAP by approximately 5 mm Hg ($P < 0.05$). The ACTH-induced increases in MAP were greater ($P < 0.001$) on each day than the increases with CRF or UCN. Infusion of normal saline had no effect.

The infusions of UCN and ACTH increased HR on each infusion day. The increases in HR due to UCN were greater ($P < 0.05$) than those due to ACTH on d 3 and 4 of infusion. There were no changes in HR during CRF or normal saline infusions.

Plasma cortisol concentration was increased during each day of UCN, CRF, and ACTH infusion. On d 2, the response to ACTH was greater than the responses to UCN or CRF ($P < 0.01$). During infusion of ACTH plasma (Na^+) increased from 144.2 ± 0.8 to a peak of 147.8 ± 1.2 mmol/liter on d 2 ($P < 0.01$) and plasma (K^+) decreased from 4.5 ± 0.2 to a minimum of 3.5 ± 0.2 mmol/liter on that day ($P < 0.001$). These changes continued to the end of the infusion. With iv infusion of CRF, there were no significant changes in plasma (Na^+ or K^+), and with iv infusion of UCN, there was a transient increase in Na^+ to 146.6 ± 3.2 mmol/liter on d 2 and no change in plasma (K^+).

Interaction of icv or iv UCN with iv infusion of ACTH

The increase in MAP in response to iv infusion of ACTH was not altered by concurrent icv infusion of UCN (Fig. 3). The hypertensive action of iv ACTH was, however, reduced on d 2 and 3 by concurrent iv infusion of UCN ($P < 0.05$),

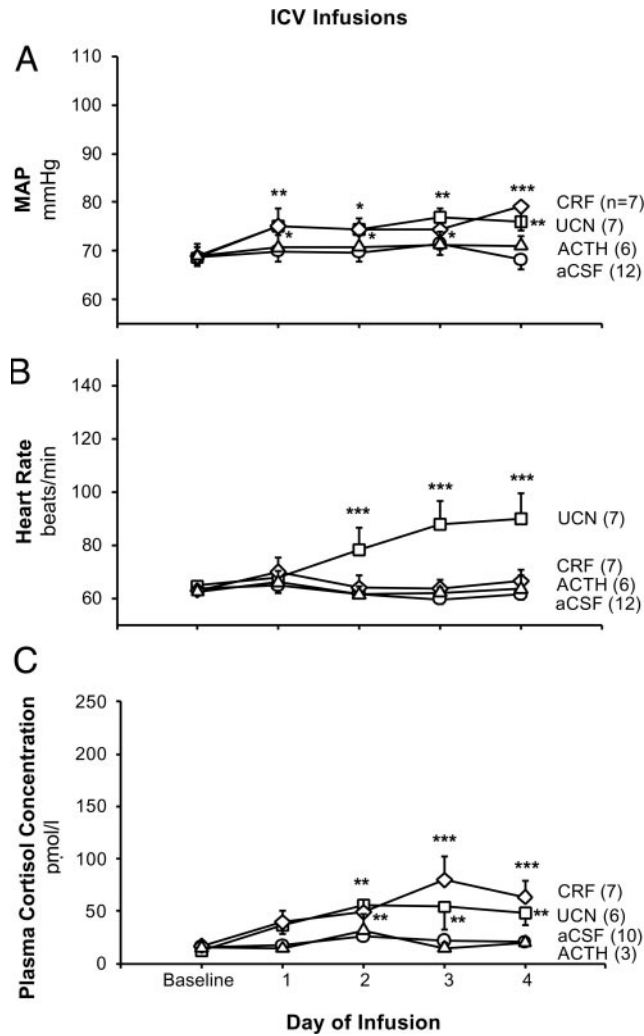


FIG. 1. Effect of a 4-d icv infusion of aCSF (0.2 ml/h, open circle), UCN (5 μ g/h, open square), CRF (5 μ g/h, open diamond), or ACTH (5 μ g/h, open triangle) on MAP (A), HR (B), and plasma cortisol concentration (C) of sheep. Statistical analysis as described in text vs. baseline: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

suggesting an inhibitory action of UCN on the effect of ACTH.

The increase in HR in response to concurrent infusion of iv UCN with iv ACTH was significantly greater than the response to ACTH alone ($P < 0.05$) and was at least additive. In contrast, when icv infusion of UCN was combined with iv ACTH, the HR response was not significantly different from that with ACTH alone, *i.e.* the iv administration of ACTH appeared to abolish the chronotropic action of icv UCN.

The increase in plasma cortisol in response to iv ACTH was equaled or exceeded during concurrent icv or iv infusion of UCN. The responses to the combinations on infusion d 1 were significantly ($P < 0.01$) greater than the responses to ACTH alone. The hypernatremia due to ACTH (147.8 ± 1.2 mmol/liter) was significantly reduced by concurrent infusion of icv UCN (143.4 ± 1.2 mmol/liter, $P < 0.01$) and iv UCN (145.2 ± 0.9 mmol/liter, $P < 0.05$). In contrast, the ACTH-induced hypokalemia was sustained.

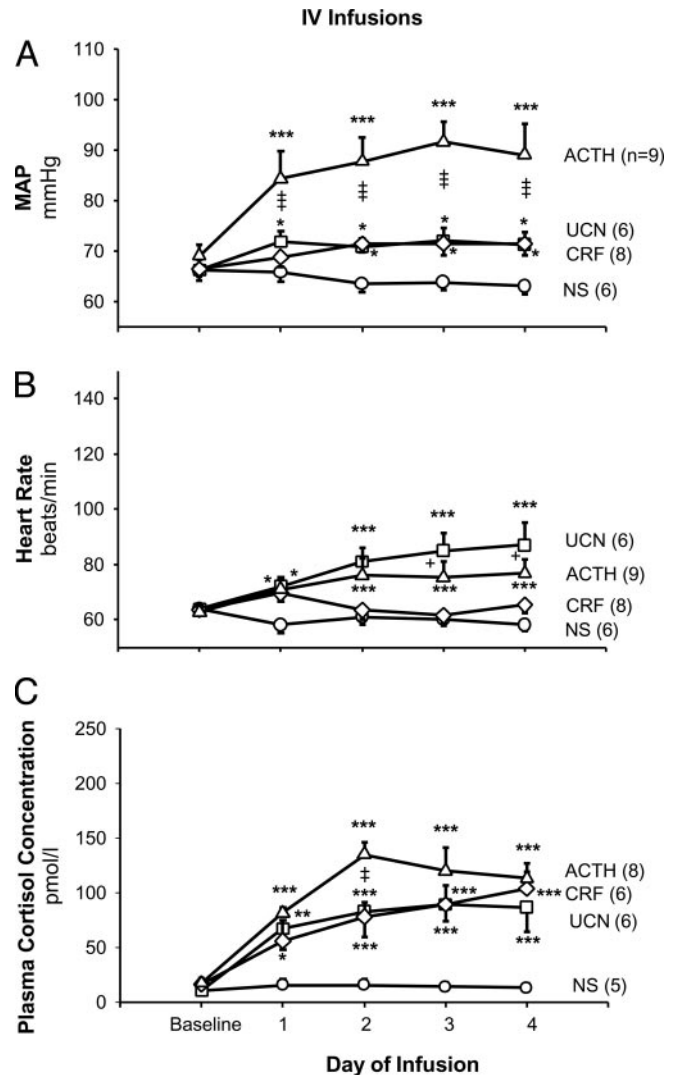


FIG. 2. Effect of a 4-d iv infusion of 0.9% saline (0.2 ml/h, open circle), UCN (5 μ g/h, open square), CRF (5 μ g/h, open diamond), or ACTH (5 μ g/h, open triangle) on MAP (A), HR (B), and plasma cortisol concentration (C) of sheep. Statistical analysis as described in text vs. baseline: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; vs. ACTH: +, $P < 0.05$; ++, $P < 0.01$; +++, $P < 0.001$.

Discussion

This is the first study to examine the cardiovascular effects of long-term central and peripheral administration of the recently discovered peptide UCN. In addition, the responses were compared with those of CRF and ACTH, other peptides involved in the stress response. The main findings were that long-term ICV infusions of UCN and CRF, and IV infusions of UCN, CRF, and ACTH caused sustained increases in MAP. UCN infused icv or iv also increased HR, as did iv infusion of ACTH. These effects were maintained throughout the 4 d of infusion, indicating that release of these peptides, either centrally or peripherally in response to stress, can result in sustained cardiovascular responses.

Systemic administration

In the present study, long-term iv administration of UCN caused a sustained increase in arterial pressure, which prob-

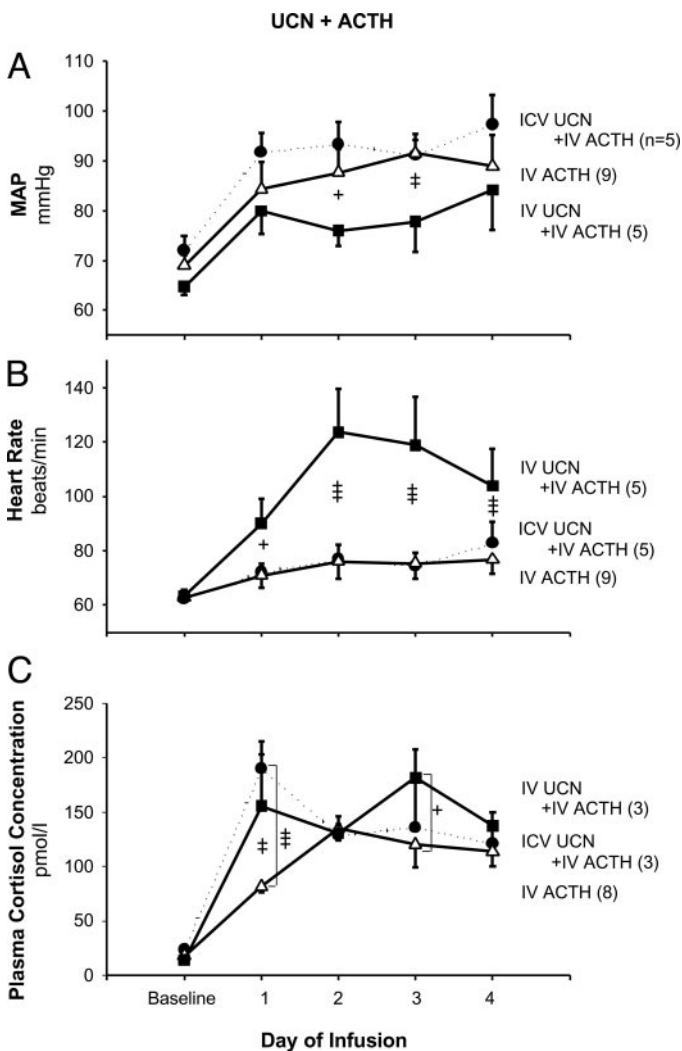


FIG. 3. Effect of a 4-d iv infusion of ACTH (5 μ g/h, open triangle) alone or ACTH (5 μ g/h) combined with icv (filled circle, dotted line) or iv (filled square) infusion of UCN (5 μ g/h) on MAP (A), HR (B), and plasma cortisol concentration (C) of sheep. Statistical analysis as described in text vs. ACTH alone: +, $P < 0.05$; ++, $P < 0.01$; +++, $P < 0.001$.

ably resulted partly from an increase in cardiac output due to chronotropic and inotropic actions. We demonstrated that this is the mechanism by which acute administration of UCN increases MAP (16). In the present study, in which UCN was infused iv for 4 d, the chronotropic action was probably a response to stimulation of CRF type 2 receptors because infusion of CRF did not increase HR. This is supported by our previous findings that the acute cardiac actions of iv UCN were blocked by a low dose of α -helical CRF (9–41) and were not mimicked by a similar dose of CRF, indicating that these actions were mediated by CRF type 2 receptors (16). Systemic UCN has also been shown to increase cardiac contractility and heart rate in mice, and these effects are abolished in mice deficient for the CRF type 2 receptor (25). It has been hypothesized that UCN is the endogenous ligand for the CRF type 2 receptor, and these findings, together with those of the present study, provide evidence that this receptor subtype mediates the chronotropic and inotropic actions of UCN. The

presence of high levels of CRF type 2 receptor mRNA within the cardiac atria and ventricles (myocardium, epicardium, and arterioles) provides the substrate for a direct action of UCN on the heart (32, 33). These authors also demonstrated the presence of UCN in both myocytes and blood vessels of the heart, with the highest concentrations in the left ventricle. UCN expression is markedly increased in the ventricles of patients with cardiomyopathy (34, 35), and a beneficial action of UCN has been demonstrated in an ovine model of heart failure (36), suggesting that UCN has a role in the pathology of heart failure.

ACTH, administered into the circulation, causes increased blood pressure in rats (37–39), humans (40–43), mice (44), sheep (45, 46), and baboons (28). The mechanism by which circulating ACTH increases MAP has been thoroughly investigated and shown to depend on the release of adrenocortical steroids (47). Because there were similar increases in cortisol with systemic administration of ACTH, UCN, and CRF, it is possible that the mild hypertension induced by iv infusion of these peptides was partly due to release of adrenocortical steroids. The significantly larger increase in MAP with ACTH than with CRF or UCN is probably because ACTH causes a greater increase in aldosterone release, as indicated by the persistent hypernatremia and hypokalemia.

Central administration

In the present study, icv administration of UCN increased MAP and HR. These changes were similar to those with systemic administration of UCN, so it cannot be confirmed that these responses to icv administration resulted from a central action. A central site of action of UCN is evidently possible considering that UCN-induced cFos expression is localized in areas of the brain that influence cardiac rate, such as the area postrema, nucleus tractus solitarius, and ventrolateral medulla (48). The lack of a similar chronotropic effect after infusion of CRF suggests that these chronotropic actions of icv UCN were mediated by CRF type 2 receptors in sheep. Several studies have reported the presence of CRF type 2 receptors in distinct areas of the brain (19, 20). In rats, icv administration of UCN (10 μ g) induced cFos expression, which occurred at sites containing both CRF type 1 and 2 receptors (17, 48). Furthermore, expression of cFos in response to UCN was observed in cell groups involved in central autonomic control that express neither type of CRF receptor, including the central amygdaloid and paraventricular hypothalamic nuclei and brain stem catecholaminergic cell groups, indicating recruitment of central autonomic structures secondary to effects directly on cell groups that contain CRF receptors (17, 48). In rats, acute icv administration of UCN (10 μ g) caused a mild increase in blood pressure but no change in cardiac contractility (22). A similar dose given systemically decreased blood pressure, indicating that the pressor response to icv UCN in rats resulted from a central action (22). In the present study, the tachycardia in response to icv UCN was most likely due to adrenaline release or increased cardiac sympathetic nerve activity together with vagal withdrawal. It is unlikely that the chronotropic effect of icv UCN depended on the UCN-induced increase in cortisol release because icv infusion of CRF had

similar effects to UCN on cortisol but did not influence heart rate. Moreover, iv infusion of cortisol has no effect on HR in sheep (49).

Previous studies in a number of species have found that icv infusion of high doses of CRF increases blood pressure, *e.g.* rats (5, 50); dogs (51); rabbits (52); and heart rate (53–55). In sheep, icv infusion of CRF (10–100 $\mu\text{g}/\text{h}$, but not 1 $\mu\text{g}/\text{h}$, over 24 h) increased blood pressure (56, 57), and the smaller response in the present study probably reflects the low dose of CRF infused. This response to CRF was probably due to an action on brain CRF type 1 receptors, for which CRF has a high affinity (58). The increase in blood pressure caused by icv administration of CRF in rats and rabbits has been shown to be dependent on activation of the sympathetic nervous system (5, 52, 59, 60). This activation is probably selective to the sympathetic vasomotor nerves because there was no increase in heart rate in sheep, suggesting that there was no increase in circulating epinephrine or cardiac sympathetic nerve activity. In the present study, icv ACTH (5 $\mu\text{g}/\text{h}$) did not increase MAP, which is in contrast to a previous report that central administration of ACTH (0.8 $\mu\text{g}/\text{h}$ over 48 h) increased blood pressure in sheep (56). It is unclear why the responses differ between these two studies.

Peptide interactions

In a previous study, which also employed a 4-d infusion protocol, we demonstrated that iv UCN inhibited the increase in sodium intake induced by iv infusion of ACTH in sheep (29). The present study delineated further anomalous interactions between these two peptides. The most striking interaction was the finding that although both icv and iv UCN caused similar increases in heart rate, when infused together with iv ACTH, the response to iv UCN, but not icv UCN, was enhanced. The chronotropic response to combined iv UCN and iv ACTH was more than additive, but the response to combined icv UCN with iv ACTH was similar to the response to ACTH alone and less than the response to UCN alone. Our finding that the chronotropic action of iv UCN was enhanced in the presence of iv ACTH, which would increase circulating glucocorticoids, suggests that in our experiment the cardiac actions of UCN were not being modulated by glucocorticoid-induced down-regulation of the CRF2 β receptors on the heart (61). Furthermore, it appears that UCN can be either excitatory or inhibitory, depending on the variable under study (MAP or HR) and the route of administration (icv or iv). The chronotropic effect of systemic UCN is probably by a direct action on cardiac CRF type 2 receptors. The ability of central UCN to increase HR may be by an action on autonomic pathways, causing an increase in cardiac nerve activity, a reduction in vagal tone or an increase in adrenaline release. With our present knowledge of the actions of these hormones, it is unclear how systemic infusion of ACTH would affect these mechanisms.

The second interaction we observed was that the pressor action of iv ACTH was reduced by iv UCN, although not by icv UCN. In the sister study on ingestive responses (27), we found a similar interaction in that iv UCN abolished the large and sustained increase of NaCl and hypernatremia induced by iv ACTH. These effects of ACTH, together with the ac-

companying hyperkalemia, have been ascribed to mineralocorticoid secretion (37, 54, 55), but it seems unlikely that this is the mechanism by which iv UCN reduces the hypertensive action of ACTH because during the combined infusion of ACTH and UCN, the hypokalemia persisted. A possible mechanism for the inhibition of the pressor action of ACTH is via UCN-induced release of natriuretic peptides because UCN has been demonstrated to stimulate release of natriuretic peptides from isolated cardiomyocytes (62).

In conclusion, these studies demonstrate that long-term infusion of UCN and CRF, both centrally and peripherally, and ACTH, systemically, had sustained effects on MAP and HR. The findings indicate that the cardiovascular actions of these peptides would be maintained during extended periods of stress. Because the chronotropic responses to UCN were not mimicked by CRF, we propose that the effect of UCN is via an action on CRF type 2 receptors, in agreement with the hypothesis that UCN is the endogenous ligand for CRF type 2 receptors. Although evidence suggests that UCN is increased in stressful situations (63, 64), the physiological role of UCN still remains to be defined. However, it is becoming apparent that UCN is one of a group of hormones that mediates the cardiovascular and other autonomic and behavioral responses to stress. There is also evidence that UCN has important pathophysiological roles; it is cardioprotective (65–67), and it has beneficial actions in heart failure (36).

Acknowledgments

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Address all correspondence and requests for reprints to: Dr. R. S. Weisinger, Howard Florey Institute, University of Melbourne, Victoria 3010, Australia. E-mail: rsw@hfi.unimelb.edu.au.

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