

Research report

# Central urocortin activation of sympathetic-regulated energy metabolism in Wistar rats

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## Abstract

The corticotropin-releasing hormone (CRH) system, including CRH and urocortin (UCN), is implicated in the central control of appetite and energy metabolism. Urocortin, a recently isolated neuropeptide closely related to CRH is involved in the central signaling cascade that inhibits energy intake. When administered intracerebroventricularly and intra-hypothalamically, UCN potently decreases food intake. Receptors for UCN, while widely distributed, are expressed in hypothalamic nuclei. As the hypothalamus is involved in modulating autonomic outflow, UCN may also act as a catabolic neuropeptide to facilitate energy expenditure through sympathetic-regulated thermogenesis. To test the hypothesis that UCN also enhances regulatory energy expenditure via the activation of the sympathetic nervous system, we examined whole body oxygen consumption ( $\text{VO}_2$ ) and colonic temperature in male Wistar rats in response to central UCN administration. That is, the intracerebroventricular injection of 1.0  $\mu\text{g}$  of UCN in male Wistar rats ( $n=10$ ) significantly increased whole body oxygen consumption compared to PBS control. In addition, colonic temperature was significantly increased ( $\Delta 0.7 \pm 0.08$  °C) in UCN- vs. PBS-administered rats, which was prevented by pretreatment with the ganglionic blocker chlorisondamine. These studies suggest that UCN acutely increased whole body oxygen consumption and body temperature via central activation of sympathetic outflow. © 2002 Elsevier Science B.V. All rights reserved.

*Theme:* Neurotransmitters, modulators, transporters, and receptors

*Topic:* Behavioral pharmacology

*Keywords:* Energy expenditure; Thermogenesis; Autonomic nervous system

## 1. Introduction

Physiological mechanisms involved in regulating food intake and body weight are comprised of numerous neurotransmitters and a complex neural network. The corticotropin-releasing hormone system, including CRH and UCN, is considered an integral component of this circuitry. Central (intracerebroventricular and intra-hypothalamic) administration of CRH decreases food intake [1], while antagonists against CRH increase feeding [7]. Urocortin, a neuropeptide closely related to CRH, sharing 45% sequence identity [21] also decreases food intake when intracerebroventricularly and intra-hypothalamically administered [12,18]. The anorectic effect of the central

injection of UCN, however, is more potent than that of CRH [18].

Receptors for CRH and UCN are expressed in the paraventricular (CRH-R1) and ventromedial hypothalamic nuclei (CRH-R2) [4] overlapping with the distribution of UCN immunoreactivity [10]. While the CRH-R1 receptors display similar affinity for UCN and CRH, the CRH-R2 receptors exhibit over a 10-fold higher affinity for UCN than CRH [14]. The CRH-R2 form has been found to be widely distributed with high levels of CRH-R2 mRNA present in the brain, including autonomic regulatory centers in the hypothalamus and brainstem, as well as in peripheral tissues such as the heart, skeletal muscle, lung, and intestine [4,11,20]. In addition, the CRH-R2 receptors expressed in the ventromedial hypothalamus (VMH) activate neurons involved in appetite control, which are thought to transduce the anorectic effects of CRH and UCN [8]. As these VMH CRH-R2-containing neurons are

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also involved in modulating autonomic outflow [19], UCN may also act as a catabolic neuropeptide to promote energy expenditure through sympathetic stimulation of thermogenesis. Interestingly, central injections of CRH also increase sympathetic nervous system activity resulting in enhanced firing rate of nerves to brown adipose tissue, increased whole body oxygen consumption and plasma norepinephrine levels [1,6].

Urocortin is involved in the central signaling cascade that inhibits energy intake and the CRH-R2 receptors are expressed in discrete hypothalamic areas involved in the modulation of the autonomic nervous system. We, therefore, hypothesized that UCN also enhances regulatory energy expenditure via the activation of the sympathetic nervous system. To test this, we examined whole body oxygen consumption ( $\text{VO}_2$ ) and colonic temperature in male Wistar rats in response to intracerebroventricular (i.c.v.) administration of UCN.

## 2. Materials and methods

### 2.1. Animals

Adult male Wistar rats ( $n=10$ ; 250–325 g) from Harlan-Teklad (Barcelona, Spain) were individually housed in suspended metabolic cages in a temperature-controlled room ( $23\pm 1^\circ\text{C}$ ) on a 12:12-h light–dark schedule (lights on at 08:00 h). Unless otherwise specified, animals were given free access to ground rat chow (Harlan-Teklad) and water at all times. All procedures were performed according to national and institutional guidelines of the Animal Care and Use Committee at the University of Navarra.

### 2.2. Surgery

The rats were anesthetized with 75 mg/kg of a ketamine HCl plus 10 mg/kg xylazine and placed in a stereotaxic instrument (Kopf Instruments, Tujunga, CA). A 24-gauge guide cannula (316GC, Plastics One, Roanoke, VA) was implanted into the right lateral cerebroventricle and anchored to the skull with three stainless steel machine screws (Small Parts Inc, Miami Lakes, FL) and cranioplastic cement (Dentsply, Detrey). The stereotaxic coordinates used were: AP,  $-0.8$  mm, ML, 1.2 mm with respect to bregma, and DV,  $-3.5$  mm from the skull surface [13]. A 31-gauge stylet (316DC, Plastics One) kept the guide cannula clear when the rat was not receiving injections. A recovery period following surgery extended 1 week before rats were subjected to an angiotensin II (ANG II) drinking test. ANG II (Sigma, St. Louis, MO) was dissolved in sterile phosphate buffered saline+calcium ( $\text{PBS}^+$ ) at a concentration of 20 ng/ $\mu\text{l}$ . An increased drinking response of at least 3 ml of water within 20 min following an i.c.v.

injection of 100 ng ANG II confirmed correct cannula placement. Cannula placement was verified again by the same drinking response to ANG II after the completion of each individual experiment.

### 2.3. Experimental protocol

The treatments were randomly assigned to the i.c.v. cannulated rats that responded positively to ANG II. The treatments were 0 ( $\text{PBS}^+$ ) and 1.0  $\mu\text{g}$  UCN injected into the lateral ventricle. Each rat received one of the treatments on a given day followed by 2 days of recovery. Food was removed from the animal cages at 09:00 h and all injections were performed 2 h after the beginning of the light cycle (between 10:00 and 11:00 h). Animals were injected in the lateral ventricle with either 2  $\mu\text{l}$  of sterile  $\text{PBS}^+$  or an equal volume of rat UCN (American Peptide, Sunnyvale, CA) in  $\text{PBS}^+$  using an injector cannula (C316I, Plastics One) attached by polyethylene tubing (PE-10) to a 25- $\mu\text{l}$  glass Hamilton syringe. For each trial infusate was delivered with a CMA/100 pump (CMA/Microdialysis) at 1.0  $\mu\text{l}/\text{min}$ , after which the injector cannula was left in place for an additional 1 min to allow for diffusion away from the injection site, before being replaced by the stylet.

Measurement of colonic temperature was accomplished using a petroleum jelly covered YSI 400 series rectal probe thermocouple thermometer (Panlab S.L., Barcelona, Spain) inserted a standardized distance (6 cm) until a stable temperature reading was obtained. Baseline temperature was measured over 60 min prior to administration of experimental treatments and every 15 min over 120 min post-treatment. In order to eliminate stress-related elevations in temperature, rats were previously habituated to the rectal probe insertion. The ganglionic blocker, chlorisondamine diiodide (Tocris Cookson, Bristol, UK), was dissolved in sterile water and was given intraperitoneally (3 mg/kg) 2 h prior to UCN administration.

Whole body oxygen consumption was measured via indirect calorimetry. A computer-controlled open circuit system (Datex, Deltatrac II) was used and oxygen consumption was measured for each rat at 1-min intervals. Rats were acclimated to the 1.5-l respiration chambers over a 7-day period (1 week after the temperature study). Prior to treatment, all rats had a positive drinking response to ANG II.

### 2.4. Data analysis

Data are shown as means $\pm$ S.E.M. Statistical analyses were performed by two-way analyses of variance (ANOVA) for repeated measures. Fisher's protected least significance difference test was used for comparisons between some specific means. A  $P<0.05$  was taken to be statistically significant.

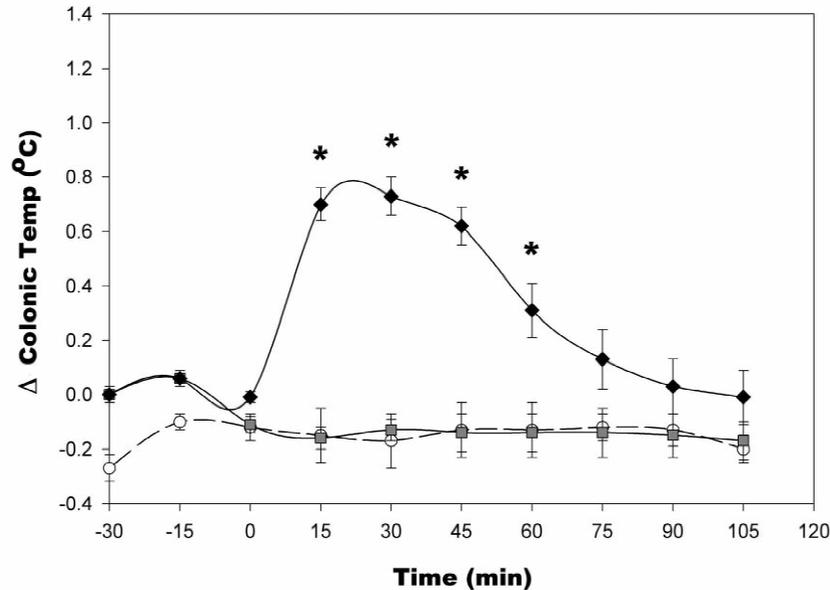


Fig. 1. Effects of central UCN on body temperature in male Wistar rats ( $n=10$ /group). Mean colonic temperature 30 min prior to and 1.5 h following i.c.v. administration of either 1.0  $\mu\text{g}$  UCN (◆), PBS (■), or UCN+chlorisondamine (○). Rats were used as own controls and values are expressed as means  $\pm$  S.E.M. \*Denotes significant difference from PBS ( $P<0.05$ ).

### 3. Results

Our results demonstrate a UCN-induced increase in parameters associated with energy expenditure. That is, the i.c.v. administration of 1.0  $\mu\text{g}$  of UCN in male Wistar rats ( $n=10$ ) significantly increased colonic temperature ( $\Delta 0.7 \pm 0.08$  °C). The colonic temperature results (Fig. 1) are expressed as net change (i.e. difference in which average baseline measurements were subtracted from the post-injection measurements for each rat). While each rat served as its own control, the average baseline colonic temperature used for difference score calculation was  $37.8 \pm 0.12$  °C. In addition, the UCN-induced increase in colonic temperature was prevented by the pretreatment with the ganglionic blocker chlorisondamine. While i.p. chlorisondamine induced an initial hypothermic response ( $-1.0 \pm 0.10$  °C) lasting  $\sim 60$  min, the colonic temperature returned to a stable pre-injection baseline prior to the UCN administration.

Whole body oxygen consumption as measured by indirect calorimetry (Fig. 2) was significantly increased in UCN- vs. PBS-administered rats. After subtracting out the increase attributed to the stress of handling/injection, this enhanced expenditure averaged 35% above the baseline.

### 4. Discussion

A complex array of molecules influences the regulation of appetite and energy metabolism. Urocortin, a recently characterized 40-amino acid peptide and member of the

CRH family [21], acts as an endogenous ligand for the CRH-R2 receptor and is implicated in the regulation of feeding, energy balance, and may also be involved in the etiology of obesity. Central administration of UCN results in a potent suppression of food intake in rats and is less anxiogenic than CRH [3,22]. Our present results suggest a further role for UCN in the brain as a signal to promote regulatory energy expenditure. We demonstrated that acute administrations of UCN increased whole body oxygen consumption and body temperature, apparently as a result of sympathetic activation.

While our study utilized i.c.v. injections distributing UCN throughout the brain, CRH-R2 receptors located in the hypothalamus, namely the ventromedial and paraventricular nuclei, appear to be responsible for the effects of CRH and UCN on food intake and energy metabolism [17]. Hypothalamic levels of CRH-R2 mRNA have been found to change in relation to peripheral variables related to energy homeostasis (plasma leptin levels) [23], while obese animals resistant to leptin are accompanied by reduced VMH CRH-R2 mRNA levels [15].

Urocortin appears to have a much higher affinity for the CRH-R2 receptors than does CRH [21] and centrally administered antagonists selective for CRH-R2 (but not CRH-R1) receptors attenuate both CRH- and UCN-induced anorexia [17]. UCN reduces food intake and promotes weight loss at concentrations that do not activate the stress response [18]. Consistent with the focus on appetite regulation, considerable evidence supports the hypothesis that UCN plays an important part in the regulation of energy balance. Exogenous UCN also increases mean

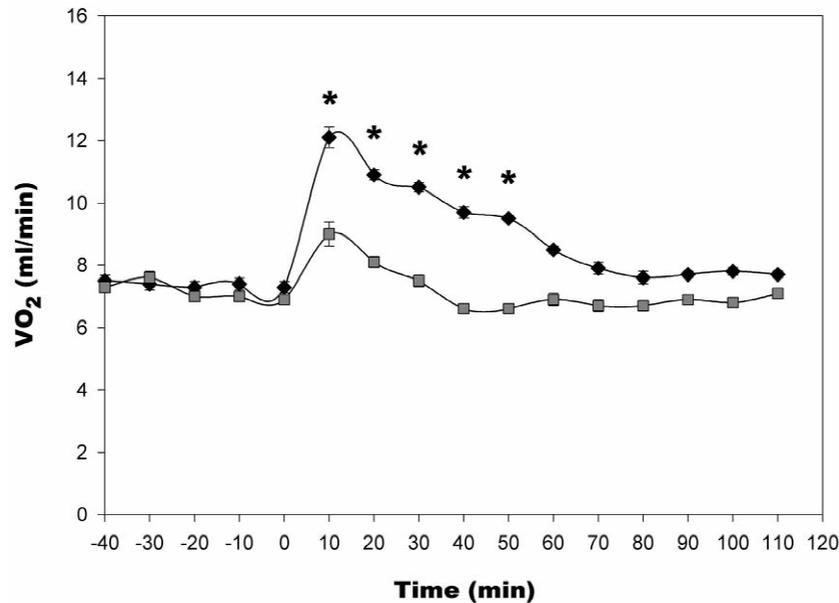


Fig. 2. Effects of central UCN on whole body oxygen consumption in male Wistar rats ( $n=9$ /group). Mean oxygen consumption 1 h prior to and 2 h following i.c.v administration of either 1.0  $\mu$ g UCN (◆) or PBS (■). Rats were used as own controls and values are expressed as means  $\pm$  S.E.M. \*Denotes significant difference from PBS ( $P<0.05$ ).

arterial pressure [18] and decreases gastroduodenal motor activity via the vagus [9]. This apparent reduction in parasympathetic tone is supportive of a general function for UCN in the activation of nuclei involved in the modulation of the autonomic nervous system to promote catabolic processes while diminishing activity over anabolic pathways. A recent study in mice, however, reported that UCN given peripherally decreased oxygen consumption [2], and along with similar results with i.c.v. CRH in mice [5] contrast with CRH-induced elevation of oxygen consumption in rats. Rather than being contradictory to our hypothesis, these data appear to suggest a species-specific response to CRH/UCN.

## 5. Summary and conclusions

Based on the findings that stimulation of the VMH enhances metabolism and inhibits ingestive behavior [16,19], CRH-R2 activation of the VMH would also be expected to promote negative energy balance. These studies revealed that UCN acutely increased whole body oxygen consumption and body temperature. Moreover, ganglionic blockade was able to completely prevent the UCN-induced increase in body temperature, suggesting that the observed enhancement of energy expenditure was via the central activation of the SNS. These results support a role for UCN and CRH-R2 receptors as a thermoregulatory component in the energy balance regulatory network.

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## References

- [1] K. Arase, D.A. York, H. Shimizu, N. Shargill, G.A. Bray, Effects of corticotropin-releasing factor on food intake and brown adipose tissue thermogenesis in rats, *Am. J. Physiol.* 255 (1988) E255–259.
- [2] A. Asakawa, A. Inui, N. Ueno, S. Makino, M. Fujimiya, M.A. Fujino, M. Kasuga, Urocortin reduces oxygen consumption in lean and ob/ob mice, *Int. J. Mol. Med.* 7 (2001) 539–541.
- [3] S.C. Benoit, T.E. Thiele, S.C. Heinrichs, P.A. Rushing, K.A. Blake, R.J. Steele, Comparison of central administration of corticotropin-releasing hormone and urocortin on food intake, conditioned taste aversion, and c-Fos expression, *Peptides* 21 (2000) 345–351.
- [4] D.T. Chalmers, T.W. Lovenberg, E.B. De Souza, Localization of novel corticotropin-releasing factor receptor (CRF2) mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF1 receptor mRNA expression, *J. Neurosci.* 15 (1995) 6340–6350.
- [5] V.S. Drescher, H.L. Chen, D.R. Romsos, Corticotropin-releasing hormone decreases feeding, oxygen consumption and activity of genetically obese (ob/ob) and lean mice, *J. Nutr.* 124 (1994) 524–530.
- [6] M. Egawa, H. Yoshimatsu, G.A. Bray, Preoptic area injection of corticotropin-releasing hormone stimulates sympathetic activity, *Am. J. Physiol.* 259 (1990) R799–806.
- [7] S.C. Heinrichs, B.J. Cole, E.M. Pich, F. Menzaghi, G.F. Koob, R.L. Hauger, Endogenous corticotropin-releasing factor modulates feed-

- ing induced by neuropeptide Y or a tail-pinch stressor, *Peptides* 13 (1992) 879–884.
- [8] S.C. Heinrichs, J. Lapsansky, T.W. Lovenberg, E.B. De Souza, D.T. Chalmers, Corticotropin-releasing factor CRF1, but not CRF2, receptors mediate anxiogenic-like behavior, *Regul. Pept.* 71 (1997) 15–21.
- [9] N. Kihara, M. Fujimura, I. Yamamoto, E. Itoh, A. Inui, M. Fujimiya, Effects of central and peripheral urocortin on fed and fasted gastroduodenal motor activity in conscious rats, *Am. J. Physiol. Gastrointest. Liver Physiol.* 280 (2001) G406–G419.
- [10] T. Kozicz, H. Yanaihara, A. Arimura, Distribution of urocortin-like immunoreactivity in the central nervous system of the rat, *J. Comp. Neurol.* 391 (1998) 1–10.
- [11] T.W. Lovenberg, C.W. Liaw, D.E. Grigoriadis, W. Clevenger, D.T. Chalmers, E.B. De Souza, T. Oltersdorf, Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain, *Proc. Natl. Acad. Sci. USA* 92 (1995) 836–840.
- [12] H. Ohata, K. Suzuki, Y. Oki, T. Shibasaki, Urocortin in the ventromedial hypothalamic nucleus acts as an inhibitor of feeding behavior in rats, *Brain Res.* 861 (2000) 1–7.
- [13] G. Paxinos, C. Watson, *The Rat Brain in Stereotaxic Coordinates*, Academic, San Diego, 1997.
- [14] T.M. Reyes, K. Lewis, M.H. Perrin, K.S. Kunitake, J. Vaughan, C.A. Arias, J.B. Hogenesch, J. Gulyas, J. Rivier, W.W. Vale, P.E. Sawchenko, Urocortin: a member of the corticotropin-releasing factor (CRF) neuropeptide family that is selectively bound by type 2 CRF receptors, *Proc. Natl. Acad. Sci. USA* 98 (2001) 2843–2848.
- [15] D. Richard, R. Rivest, N. Naimi, E. Timofeeva, S. Rivest, Expression of corticotropin-releasing factor and its receptors in the brain of lean and obese Zucker rats, *Endocrinology* 137 (1996) 4786–4795.
- [16] M. Ruffin, S. Nicolaidis, Electrical stimulation of the ventromedial hypothalamus enhances both fat utilization and metabolic rate that precede and parallel the inhibition of feeding behavior, *Brain Res.* 846 (1999) 23–29.
- [17] G.N. Smagin, L.A. Howell, D.H. Ryan, E.B. De Souza, R.B. Harris, The role of CRF2 receptors in corticotropin-releasing factor- and urocortin-induced anorexia, *Neuroreport* 9 (1998) 1601–1606.
- [18] M. Spina, E. Merlo-Pich, R.K. Chan, A.M. Basso, J. Rivier, W. Vale, G.F. Koob, Appetite-suppressing effects of urocortin, a CRF-related neuropeptide, *Science* 273 (1996) 1561–1564.
- [19] A.B. Steffens, A.J. Scheurink, P.G. Luiten, B. Bohus, Hypothalamic food intake regulating areas are involved in the homeostasis of blood glucose and plasma FFA levels, *Physiol. Behav.* 44 (1988) 581–589.
- [20] A.V. Turnbull, C. Rivier, Corticotropin-releasing factor (CRF) and endocrine responses to stress: CRF receptors, binding protein, and related peptides, *Proc. Soc. Exp. Biol. Med.* 215 (1997) 1–10.
- [21] J. Vaughan, C. Donaldson, J. Bittencourt, M.H. Perrin, K. Lewis, S. Sutton, R. Chan, A.V. Turnbull, D. Lovejoy, C. Rivier et al., Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor, *Nature* 378 (1995) 287–292.
- [22] C. Wang, M.A. Mullet, M.J. Glass, C.J. Billington, A.S. Levine, C.M. Kotz, Feeding inhibition by urocortin in the rat hypothalamic paraventricular nucleus, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 280 (2001) R473–480.
- [23] S. Makino, M. Nishiyama, K. Asaba, P.W. Gold, K. Hashimoto, Altered expression of type 2 CRH receptor mRNA in the VMH by glucocorticoids and starvation, *Am. J. Physiol.* 275 (1998) R1138–1145.