Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 469 (2003) 111–115
www.elsevier.com/locate/ejphar

Effects of a selective agonist and antagonist of CRF_2 receptors on cardiovascular function in the rat

Kenneth B. Mackay*, Theodore H. Stiefel, Nick Ling, Alan C. Foster

Neurocrine Biosciences Inc., 10555 Science Center Drive, San Diego, CA 92121-1102, USA

Received 3 February 2003; received in revised form 3 April 2003; accepted 11 April 2003

Abstract

The aim of the present study was to investigate the effects of activation or blockade of the CRF_2 receptor subtype on cardiovascular function in conscious rats following systemic i.v. administration of the CRF_2 receptor peptide agonist urocortin 2 given alone and the selective CRF_2 receptor peptide antagonist antisauvagine-30 given alone. Urocortin 2 caused a dose-dependent reduction in mean arterial blood pressure and a dose-dependent increase in heart rate. Pretreatment with antisauvagine-30 blocked the hypotensive effect of urocortin 2. Antisauvagine-30 failed to produce any statistically significant effects on mean arterial blood pressure and heart rate at doses that completely blocked the effects of urocortin 2. These data verify the cardiovascular effects of selective CRF_2 receptor activation, but find no evidence for an endogenous CRF_2-mediated tone.

Keywords: CRF (corticotropin-releasing factor); CRF_2 receptor antagonist; Urocortin 2; Antisauvagine-30; Cardiovascular

1. Introduction

Corticotropin-releasing factor (CRF) is a 41 amino acid peptide that plays an important role in the regulation of the hypothalamic–pituitary–adrenal axis and in endocrine, behavioral, and autonomic responses to stress (Koob and Heinrichs, 1999). CRF belongs to a family of structurally related peptides that includes urocortin 1 (Vaughan et al., 1995), urocortin 2 (Reyes et al., 2001), and urocortin 3 (Lewis et al., 2001), that have been identified in humans, rodents, and other mammalian species. In addition to effects on the pituitary and the central nervous system (CNS), CRF and related peptides have been shown to modulate a range of peripheral activities in mammals including cardiovascular and gastrointestinal functions and inflammatory processes.

CRF-related peptides are known to be the endogenous mammalian ligands for two G-protein coupled receptor subtypes, CRF_1 and CRF_2, that are positively coupled to adenylate cyclase (Perrin and Vale, 1999). The CRF_2 receptor subtype has two functional splice variants in rodents, $CRF_{2(a)}$ and $CRF_{2(b)}$, which are approximately 70% identical in amino acid sequence (Lovenberg et al., 1995; Perrin and Vale, 1999). The two CRF receptors differ in their pharmacological profiles. Urocortin 1, urocortin 2, and urocortin 3 bind to the CRF_2 subtype with much greater affinity than CRF itself (Lewis et al., 2001; Perrin and Vale, 1999; Reyes et al., 2001). In contrast, urocortin 2 and urocortin 3 show no appreciable binding to the CRF_1 subtype (Lewis et al., 2001; Reyes et al., 2001), whereas CRF and urocortin 1 bind to the CRF_1 receptor with similar potency (Perrin and Vale, 1999; Vaughan et al., 1995).

Urocortin 2 is a selective CRF_2 receptor peptide agonist with a K_i value of 0.66 nM for inhibition of radiolabeled sauvagine binding to recombinant mouse $CRF_{2(b)}$ receptors and >100 nM for the recombinant human CRF_1 receptor (Reyes et al., 2001). Antisauvagine-30 is a selective CRF_2 receptor peptide antagonist, with reported K_i values for inhibition of radiolabeled sauvagine binding to recombinant mouse $CRF_{2(b)}$ and rat CRF_1 receptors of 1.4 and 154 nM, respectively, at room temperature (Rühmann et al., 1998) and a K_d value for radiolabeled antisauvagine-30 binding to recombinant $CRF_{2(a)}$ receptors of 0.125 nM with no specific binding detected to the recombinant human CRF_1 receptor (Higelin et al., 2001). The discovery of urocortin 2 and the advent of the selective CRF_2 receptor antagonist antisauvagine-30 have provided the pharmacological tools to explore

* Corresponding author. Tel.: +1-858-658-7727; fax: +1-858-658-7696.
E-mail address: kmackay@neurocrine.com (K.B. Mackay).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01725-4

the physiological relevance of the CRF_2 receptor subtype in vivo (Pelleymounter et al., 2002).

There is evidence available to suggest that modulation of CRF_2 receptors can have effects on cardiovascular function in rodents (Coste et al., 2002). The CRF_2 receptor subtype is localized in cardiac tissue and in the peripheral vasculature of normal animals (Baigent and Lowry, 2000; Kishimoto et al., 1995; Lovenberg et al., 1995). Systemic administration of urocortin 1 has been shown to induce a marked and persistent reduction in blood pressure, putatively through activation of peripheral CRF_2 receptors (Lawrence et al., 2002; Spina et al., 1996; Vaughan et al., 1995). In addition, CRF_2-deficient mice show elevated mean arterial blood pressure and diastolic pressure compared with wild-type littermates (Bale et al., 2000; Coste et al., 2000). Moreover, the levels of mRNA for the CRF_2 receptor in the cardiac tissue of spontaneously hypertensive rats are significantly higher than those in normotensive animals (Makino et al., 1998). Collectively, these data suggest that the CRF_2 receptor subtype may play a role in modulation of the cardiovascular system. In order to more precisely address this question, we have investigated the effects of systemic administration of the selective CRF_2 receptor peptide agonist urocortin 2 and the selective CRF_2 receptor peptide antagonist antisauvagine-30 on mean arterial blood pressure and heart rate in conscious, freely moving rats.

2. Materials and methods

2.1. Animals

Adult ($n = 50$; 220–240 g upon arrival) male Sprague–Dawley rats (Charles River, Hollister, CA) were housed 2 per cage in a standard 12 h light/12 h dark cycle, humidity and temperature controlled animal facility with free access to food and water. Rats were allowed at least one week to acclimate to the animal facility prior to the start of the studies. Surgical and experimental procedures were carried out in strict adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication no. 85-23, revised 1996) and approved by the Institutional Care and Use Committee of Neurocrine Biosciences.

2.2. Surgical preparation

All rats were anesthetized with halothane (5% induction, 1.5% maintenance) in a nitrous oxide/oxygen gas mixture (70:30). Core temperature was monitored throughout the surgical procedure by a rectal thermometer, and the animals maintained normothermic (37 ± 1 °C) via a heating blanket controlled by the thermometer. Polyethylene cannulae filled with heparinized saline (10 IU ml^{-1}) were introduced into the left carotid artery and left jugular vein during a period of brief halothane anesthesia (< 15 min). Both cannulae were passed under the skin to the dorsal neck region where they were exteriorised between the scapulae. All incision sites were then infiltrated with a local anesthetic gel (xylocaine, 2%) and sutured closed. The animals were then placed in individual rodent swivel and tethering devices (Harvard Apparatus, Holliston, MA) allowing free movement during drug/vehicle administration. The anesthetic gas mixture was withdrawn, and the rats placed in their cages where the swivel was attached to an overhead support stand and clamp (Harvard Apparatus). The intraarterial cannula was then connected to a blood pressure transducer for continuous monitoring of cardiovascular function using a computer-assisted data acquisition system (AD Instruments, Mountain View, CA). The animals were allowed to recover from the effects of anesthesia for at least 2 h before any further manipulations were performed.

2.3. Peptides

Murine urocortin 2 and antisauvagine-30 were synthesized by the Department of Peptide Chemistry at Neurocrine Biosciences using the solid-phase method of Merrifield, a methylbenzhydryl amine resin, and the Boc strategy as described in detail previously (Pelleymounter et al., 2002; Reyes et al., 2001). Urocortin 2 and antisauvagine-30 were dissolved in a vehicle of double-distilled water (1 ml kg^{-1}) immediately prior to use.

2.4. Experimental groups

The following studies were carried out at the end of the minimum 2 h recovery period. First, the cumulative dose–response relationships for urocortin 2 (0.001–1 mg kg^{-1}) and antisauvagine-30 (0.03–10 mg kg^{-1}) on mean arterial blood pressure and heart rate were examined, where urocortin 2 ($n = 4$) or antisauvagine-30 ($n = 4$) were administered by i.v. bolus injections (1 ml kg^{-1}) every 20 min for the duration of the monitoring period. Mean arterial blood pressure and heart rate values were recorded for quantitation at 10-min postinjection for both urocortin 2 and antisauvagine-30. Second, the temporal profiles of the dose-dependent effects of urocortin 2 (0.01, 0.03, or 0.1 mg kg^{-1}, i.v.) on mean arterial blood pressure and heart rate were assessed over a 30-min recording period. Third, the effect of antisauvagine-30 pretreatment (1, 3, or 10 mg kg^{-1}, i.v.) on urocortin 2-mediated (0.1 mg kg^{-1}, i.v.) cardiovascular responses was determined, where antisauvagine-30 was administered 30 min prior to urocortin 2.

2.5. Statistical analysis

The data are expressed as mean ± S.E.M. Statistical significance in mean arterial blood pressure and heart rate between experimental groups over time was determined by two-factor analysis of variance (ANOVA), with drug treatment doses as the between groups factor and time as the

repeated measure, followed by Tukey's individual comparisons of the means. $P < 0.05$ was considered significant.

3. Results

The i.v. administration of urocortin 2 reduced the mean arterial blood pressure in a dose-dependent manner (Fig. 1). The ED_{50} for the urocortin 2 cumulative dose–response effect on mean arterial blood pressure was 0.014 ± 0.004 mg kg^{-1}. Urocortin 2 administration had no statistically significant effects on heart rate in the same animals (Fig. 1). Antisauvagine-30, at doses up to 10 mg kg^{-1}, had no cumulative dose–response effects on mean arterial blood pressure or heart rate (Fig. 1).

The temporal profiles of the dose-dependent effects of urocortin 2 on mean arterial blood pressure and heart rate are shown in Fig. 2. Urocortin 2 produced a statistically significant reduction in mean arterial blood pressure as compared with vehicle administration [main treatment effect: $F(3,12) = 15.85$, $P < 0.001$; treatment × time interaction: $F(18,72) = 9.66$, $P < 0.00001$]. Urocortin 2 also induced a significant dose-dependent increase in heart rate in the same animals [treatment × time interaction: $F(18,72) = 2.97$, $P < 0.001$]. Administration of urocortin 2 at the two highest doses tested (0.03 and 0.1 mg kg^{-1}) evoked the most marked and significant effects on mean arterial blood pressure and heart rate relative to vehicle-treated animals ($P < 0.05$). The hypotensive and modest tachycardic responses were still present at 30 min postinjection although there was evidence of a gradual return toward baseline values.

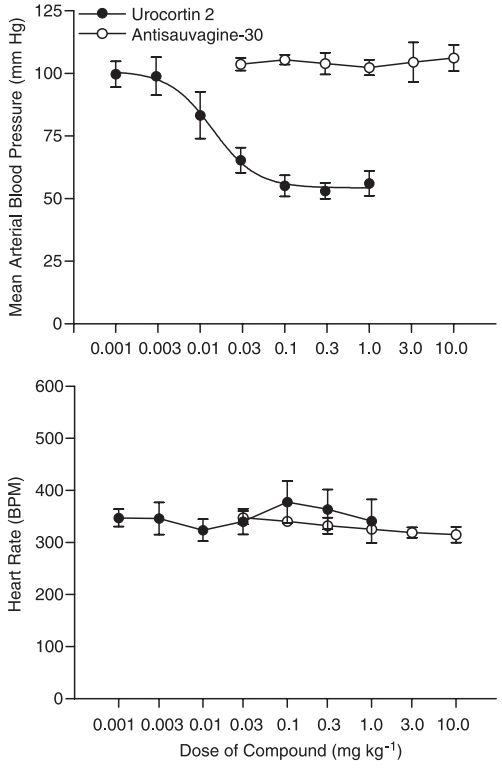

Fig. 1. Cumulative dose–response curves for the effects of urocortin 2 and antisauvagine-30 on mean arterial blood pressure (top) and heart rate (bottom) in conscious rats. Data are presented as the mean ± S.E.M. ($n = 4$ per treatment group).

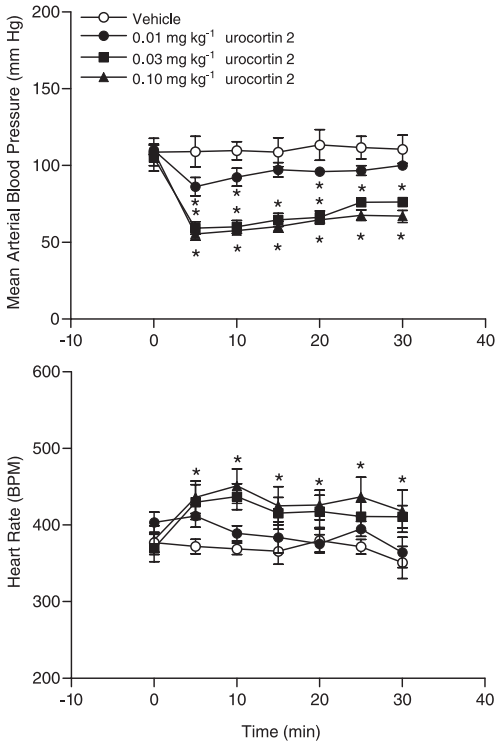

Fig. 2. Temporal profiles of the dose–response effects of urocortin 2 on mean arterial blood pressure (top) and heart rate (bottom). Data are presented as mean ± S.E.M. ($n = 4$ per treatment group). * $P < 0.05$ for all urocortin 2 groups compared with vehicle-treated control animals.

The temporal data for the effect of antisauvagine-30 pretreatment on urocortin 2 (0.1 mg kg^{-1}) evoked changes in mean arterial blood pressure are shown in Fig. 3. The

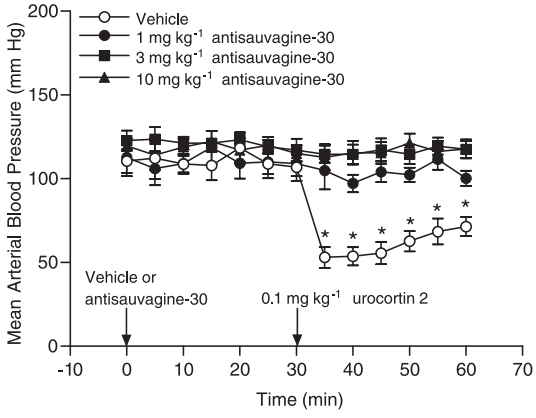

Fig. 3. Temporal data for the effects of antisauvagine-30 pretreatment on urocortin 2 mediated changes in mean arterial blood pressure. Vehicle (water, 1 ml kg^{-1}) or antisauvagine-30 (1–10 mg kg^{-1}) were administered by i.v. bolus injection at time 0, and urocortin 2 (0.1 mg kg^{-1}, i.v.) was administered at 30 min. Data are presented as mean ± S.E.M. ($n = 4$ per treatment group). * $P < 0.05$ compared with all antisauvagine-30-pretreated groups.

systemic administration of urocortin 2 (0.1 mg kg^{-1}) at 30 min produced a significant reduction in mean arterial blood pressure when compared to vehicle treatment [main treatment effect: $F(3,12) = 5.96$, $P < 0.01$; treatment × time interaction: $F(36,144) = 10.99$, $P < 0.00001$]. The reduction in mean arterial blood pressure observed following urocortin 2 treatment was blocked by pretreatment with antisauvagine-30 at time 0. Administration of antisauvagine-30 had no significant effect on mean arterial blood pressure at any of the doses investigated. Antisauvagine-30 pretreatment failed to produce any statistically significant effects on heart rate in the same animals (data not shown).

4. Discussion

The results of the current study demonstrate that the selective CRF_2 receptor peptide agonist urocortin 2 produced a dose-dependent reduction in mean arterial blood pressure, an effect that was blocked by pretreatment with the selective CRF_2 receptor peptide antagonist antisauvagine-30. Urocortin 2 also caused a concomitant dose-dependent increase in heart rate. Systemic administration of antisauvagine-30 alone had no effect on mean arterial blood pressure or heart rate at any of the doses examined.

The anatomical localization of the CRF_2 receptor may explain, at least in part, the effects of urocortin 2 on cardiovascular function observed in the present study. In the rodent, the CRF_2 receptor subtype has two functional splice variants, $CRF_{2(a)}$ and $CRF_{2(b)}$, which are differentially distributed between peripheral tissues and the CNS. The mRNA for $CRF_{2(a)}$ is found almost exclusively in the brain (Lovenberg et al., 1995). In contrast, $CRF_{2(b)}$ mRNA is found predominantly in the periphery, where high levels of expression are found in the heart (aorta and myocardium, epicardium and arterioles of the atrium and ventricles) and skeletal muscle, with lower levels in the lungs and gastrointestinal tract (Baigent and Lowry, 2000; Kishimoto et al., 1995; Lovenberg et al., 1995). $CRF_{2(b)}$ mRNA is also expressed in limited regions of the brain (Lovenberg et al., 1995). The observations that CRF_2 mRNA is expressed in cardiac arterioles (Lovenberg et al., 1995) and also in a vascular smooth muscle cell line (Kageyama et al., 2000) suggests that the hypotensive effect of systemically administered urocortin 2 in the present study may be attributed to a marked fall in resistance in response to CRF_2 activation by urocortin 2 in the peripheral vasculature, an effect that is blocked by pretreatment with the selective CRF_2 receptor antagonist antisauvagine-30. The modest tachycardia observed may be a reflex response to the reduction in mean arterial blood pressure or a direct effect of urocortin 2 on CRF_2 receptors in the heart. It is interesting to note that urocortin 2 did not significantly change heart rate in the cumulative dose–response study, but did significantly increase heart rate in the temporal profile study despite a comparable fall in mean arterial blood pressure. The reason for this difference is unclear, but one possible explanation may be that if the tachycardia is reflex in origin as suggested then it could be that it has subsided by the time the readings are taken in the cumulative dose–response study.

CRF and urocortin 1 produce marked effects on the cardiovascular system when administered both i.v. or directly into the CNS (Parkes et al., 2001). Systemic i.v administration of CRF and urocortin 1 cause a marked and long-lasting reduction in mean arterial blood pressure in rats (Briscoe et al., 2000; Lawrence et al., 2002; Spina et al., 1996; Vaughan et al., 1995). The hypotensive effects of systemic CRF treatment are blocked by pretreatment with the mixed CRF_1/CRF_2 receptor antagonist α-helical CRF_{9-41}, but not by the selective CRF_1 receptor antagonist antalarmin (Briscoe et al., 2000), suggesting that the reduction in mean arterial blood pressure is mediated by peripheral CRF_2 receptors. Moreover, it has been shown that the hypotensive response produced following systemic urocortin 1 administration is prevented following pretreatment with the selective CRF_2 receptor antagonist K41498, an analogue of antisauvagine-30 (Lawrence et al., 2002). The present results demonstrating potent effects of the selective CRF_2 receptor agonist, urocortin 2, and their reversal by a selective CRF_2 receptor antagonist, antisauvagine-30, have verified the pharmacological role of the CRF_2 receptor in the cardiovascular system.

Evidence for the involvement of the CRF_2 receptor subtype in the modulation of cardiovascular function has come from mice where the CRF_2 receptor gene has been deleted during development. Systemic administration of urocortin 1 has no effect on mean arterial blood pressure in CRF_2-deficient mice, whereas wild-type mice showed a marked and sustained hypotensive response (Bale et al., 2000; Coste et al., 2000); heart rate was not significantly affected (Coste et al., 2000). The observation that CRF_2-deficient mice exhibit a modest increase in basal blood pressure compared with wild-type littermates (Bale et al., 2000; Coste et al., 2000) could suggest that activation of the CRF_2 receptor by an endogenous agonist is important for tonic cardiovascular regulation. However, the present results have demonstrated that acute administration of the antagonist antisauvagine-30 at doses in excess of those required for pharmacological blockade of vascular CRF_2 receptors do not alter mean arterial blood pressure or heart rate. This would indicate that under the present experimental conditions, no endogenous CRF_2 tone exists. Moreover, it could also be argued that the observed phenotype of the CRF_2 knockout mice (modest hypertension) was the result of developmental compensation for the deletion of the CRF_2 receptor.

In conclusion, we have demonstrated that selective activation of presumed vascular CRF_2 receptors can influence cardiovascular function in the conscious rat, but find no evidence for an endogenous CRF_2-mediated tone. As a cautionary note, investigators who seek to probe the function of CRF receptors using peripheral administration of potent CRF_2 receptor agonists should consider the impact

that profound changes on the cardiovascular system can have on their physiological endpoints.

References

Baigent, S.M., Lowry, P.J., 2000. mRNA expression profiles for corticotropin releasing factor (CRF), urocortin, CRF receptors and CRF-binding protein in peripheral rat tissues. J. Mol. Endocrinol. 25, 43–52.

Bale, T.L., Contarino, A., Smith, G.W., Chan, R., Gold, L.H.,Sawchemko, P.E., Koob, G.F., Vale, W.W., Lee, K.-F., 2000. Mice deficient for corticotropin-releasing hormone receptor-2 display anxiety-like behaviour and are hypersensitive to stress. Nat. Genet. 24, 410–414.

Briscoe, R.J., Cabrera, C.L., Baird, T.J., Rice, K.C., Woods, J.H., 2000. Antalarmin blockade of corticotropin releasing hormone-induced hypertension in rats. Brain Res. 881, 204–207.

Coste, S.C., Kesterson, R.A., Heldwein, K.A., Stevens, S.L., Heard, A.D., Hollis, J.H., Murray, S.E., Hill, J.K., Pantley, G.A., Hohimer, A.R., Hatton, D.C., Phillips, T.J., Finn, D.A., Low, M.J., Rittenberg, M.B., Stenzel, P., Stenzel-Poore, M.P., 2000. Abnormal adaptations to stress and impaired cardiovascular function in mice lacking corticotropin-releasing hormone receptor-2. Nat. Genet. 24, 403–409.

Coste, S.C., Quintos, R.F., Stenzel-Poore, M.P., 2002. Corticotropin-releasing hormone-related peptides and receptors: emergent regulators of cardiovascular adaptations to stress. Trends Cardiovasc. Med. 12, 176–182.

Higelin, J., Py-Lang, G., Paternoster, C., Ellis, G.J., Patel, A., Dautzenberg, F.M., 2001. ^{125}I-antisauvagine-30: a novel and specific high-affinity radioligand for the characterization of corticotropin-releasing factor type 2 receptors. Neuropharmacology 40, 114–122.

Kageyama, K., Gaudriault, G.E., Bradbury, M.J., Vale, W.W., 2000. Regulation of corticotropin-releasing factor type 2β messenger ribonucleic acid in the rat cardiovascular system by urocortin, glucocorticoids, and cytokines. Endocrinology 141, 2285–2293.

Kishimoto, T., Pearse, R.V., Lin, C.R., Rosenfeld, M.G., 1995. A sauvagine/corticotropin-releasing factor receptor expressed in heart and skeletal muscle. Proc. Natl. Acad. Sci. U. S. A. 92, 1108–1112.

Koob, G.F., Heinrichs, S.C., 1999. A role for corticotropin releasing factor and urocortin in behavioral responses to stressors. Brain Res. 848, 141–152.

Lawrence, A.J., Krstew, E.V., Dautzenberg, F.M., Rühmann, A., 2002. The highly selective CRF2 receptor antagonist K41498 binds to preseynaptic CRF2 receptors in rat brain. Br. J. Pharmacol. 136, 896–904.

Lewis, K., Li, C., Perrin, M.H., Blount, A., Kunitake, K., Donaldson, C., Vaughan, J., Reyes, T.M., Gulyas, J., Fischer, W., Bilezikjian, L., Rivier, J., Sawchenko, P.E., Vale, W.W., 2001. Identification of urocortin III, an additional member of the corticotropin-releasing factor (CRF) family with high affinity for the CRF_2 receptor. Proc. Natl. Acad. Sci. U. S. A. 98, 7570–7575.

Lovenberg, T.W., Chalmers, D.T., Liu, C., De Souza, E.B., 1995. CRF_2 alpha and CRF_2 beta receptor mRNAs are differentially distributed between the rat central nervous system and peripheral tissues. Endocrinology 136, 4139–4142.

Makino, S., Asaba, K., Takao, T., Hashimoto, K., 1998. Type 2 corticotropin-releasing hormone receptor mRNA expression in the heart in hypertensive rats. Life Sci. 62, 515–523.

Parkes, D.G., Weisinger, R.S., May, C.N., 2001. Cardiovascular actions of CRH and urocortin: an update. Peptides 22, 821–827.

Pelleymounter, M.A., Joppa, M., Ling, N., Foster, A.C., 2002. Pharmacological evidence supporting a role for central corticotropin-releasing factor2 receptors in behavioral, but not endocrine, responses to environmental stress. J. Pharmacol. Exp. Ther. 302, 145–152.

Perrin, M.H., Vale, W.W., 1999. Corticotropin releasing factor receptors and their ligand family. Ann. N.Y. Acad. Sci. 885, 312–328.

Reyes, T.M., Lewis, K., Perrin, M.H., Kunitake, K.S., Vaughan, J., Arias, C.A., Hogenesch, J.B., Gulyas, J., Rivier, J., Vale, W.W., Sawchenko, P.E., 2001. Urocortin II: a member of the corticotropin-releasing factor (CRF) neuropeptide family that is selectively bound by type 2 CRF receptors. Proc. Natl. Acad. Sci. U. S. A. 98, 2843–2848.

Rühmann, A., Bonk, I., Lin, C.R., Rosenfeld, M.G., Spiess, J., 1998. Structural requirements for peptidic antagonists of the corticotropin-releasing factor receptor (CRFR): development of CRFR2beta-selective antisauvagine-30. Proc. Natl. Acad. Sci. U. S. A. 95, 15264–15269.

Spina, M., Merlo-Pich, E., Chan, R.K.W., Basso, A.M., Rivier, J., Vale, W., Koob, G.F., 1996. Appetite-suppressing effects of urocortin, a CRF-related neuropeptide. Science 273, 1561–1564.

Vaughan, J., Donaldson, C., Bittencourt, J., Perrin, M.H., Lewis, K., Sutton, S., Chan, R., Turnbull, A.V., Lovejoy, D., Rivier, C., Rivier, J., Sawchenko, P.E., Vale, W., 1995. Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. Nature 378, 287–292.