ELSEVIER

European Journal of Pharmacology 440 (2002) 189–197

ejp

www.elsevier.com/locate/ejphar

The corticotropin-releasing factor family of peptides and CRF receptors: their roles in the regulation of energy balance

Denis Richard*, Qin Lin, Elena Timofeeva

D.B. Brown Chair on Obesity Research and Laval Hospital Research Center, Laval University, Quebec (Qué), Canada G1K 7P4

Received 17 December 2001; accepted 7 January 2002

Abstract

The corticotropin-releasing factor (CRF) system could play a significant role in the regulation of energy balance. This system, which includes CRF, CRF-related peptides and CRF receptors, is part of a huge network of cells connected to central and peripheral pathways modulating energy metabolism. CRF and CRF-related peptides, which elicit their effects through G-protein-coupled receptors known in mammals as CRF_1 receptor and CRF_2 receptor, are capable of strong anorectic and thermogenic effects. Also supporting a role for the CRF system in the regulation of energy balance are findings demonstrating alterations in this system in obese and food-deprived animals that concur to facilitate energy deposition. In recent years, great progress has been made in understanding the specific physiological roles of the CRF system. In that respect, the discovery of urocortins II and III, two endogenous ligands of the CRF_2 receptor, and the development of selective and long-acting antagonists for the CRF receptors, have led to a better comprehension of the role of the CRF system in the regulation of energy balance. Although there are still important unresolved issues in the field of CRF research, the progress made recently warrants investigations aimed at evaluating the CRF system as a potential target for anti-obesity drugs. © 2002 Published by Elsevier Science B.V.

Keywords: CRF receptor; Peptide; Energy balance

1. Introduction

Evidence has accumulated throughout the years to suggest the involvement of the corticotropin-releasing factor (CRF) system in the regulation of energy balance (Richard, 1998, 1999). The mammalian CRF system consists of CRF, at least two different CRF receptor subtypes, a CRF-binding protein and endogenous CRF receptor ligands such as the urocortins. Members of the CRF family of peptides are capable of strong anorectic and thermogenic actions that appear coordinated to maximize energy losses. This short review addresses the importance of the CRF system in the regulation of energy balance and the potential of this system as a target for anti-obesity drugs.

2. The CRF family of peptides

The CRF family of peptides comprises the mammalian peptides CRF (Vale et al., 1981), urocortin (Vaughan et al., 1995), urocortin II (Hsu and Hsueh, 2001; Reyes et al., 2001), urocortin III (Hsu and Hsueh, 2001; Lewis et al., 2001), piscine urotensin I (Lederis et al., 1982) and amphibian sauvagine (Montecucchi et al., 1980). All these peptides are homologous and exert their actions through receptors, referred to as the CRF_1 receptor (Perrin et al., 1993), the CRF_2 receptor (Lovenberg et al., 1995a) and the CRF_3 receptor (Arai et al., 2001). The latter has recently been identified in the catfish.

2.1. CRF

CRF is a 41-amino acid peptide abundantly expressed in the paraventricular hypothalamic nucleus neurons that project to the median eminence to stimulate the secretion of adrenocorticotropic hormone. CRF represents the major controller of the basal and stress-induced activation of the pituitary–adrenal axis, which is in keeping with the observation that the only remarkable phenotype of the CRF

* Corresponding author. Mailing address: Département d'Anatomie et de Physiologie, Faculté de Médecine, Université Laval, Québec (Qué), Canada G1K 7P4. Tel.: +1-418-656-3348; fax: +1-418-656-7898.

E-mail address: denis.richard@phs.ulaval.ca (D. Richard).

0014-2999/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
PII: S0014-2999(02)01428-0

knockout mouse is a pituitary–adrenal hyporeactivity (Muglia et al., 2000).

CRF is also widely expressed throughout the brain (Merchenthaler et al., 1982; Sawchenko and Swanson, 1990) and in peripheral tissues. In the mammalian brain, it is significantly expressed in hypothalamic and extrahypothalamic regions, including the olfactory bulb, bed nucleus of the stria terminalis, medial preoptic area, paraventricular hypothalamic nucleus, lateral hypothalamus, central nucleus of amygdala, geniculate nucleus, Barington's nucleus, dorsal motor complex and inferior olive. The broad distribution of CRF neurons conforms to the many expected functions of the peptide (Turnbull and Rivier, 1997). When injected centrally, CRF evokes autonomic responses (Brown and Fisher, 1990; Heinrichs and Tache, 2001), a widespread arousal (Koob et al., 1990) and anxiety-like behaviors (Heinrichs and Tache, 2001; Krysiak et al., 2000). It respectively activates and inhibits the sympathetic and parasympathetic branches of the autonomic nervous system; it stimulates cardiorespiratory functions (Fisher et al., 1982) as well as brown adipose tissue thermogenesis (LeFeuvre et al., 1987) and inhibits the digestive activity (Taché et al., 1990). CRF also blunts the activity of the reproductive system (Rivest and Rivier, 1995) and induces anorexia (Heinrichs and Richard, 1999). The view that CRF orchestrates the multiple facets of the whole animal response to stress has dominated the research area on CRF since the discovery of the peptide.

2.2. The urocortins

CRF receptors also bind urocortin (Vaughan et al., 1995), urocortin II (Hsu and Hsueh, 2001; Reyes et al., 2001), and urocortin III (Hsu and Hsueh, 2001; Lewis et al., 2001). Urocortins are homologous to CRF and urotensin I and show a particularly high specificity for CRF_2 receptor.

Urocortin is a 40-amino acid peptide expressed in the mammalian brain as well as in peripheral tissues. The urocortin designation was selected because of the homology of sequence shared by this peptide with urotensin I (63%) and CRF (45%) (Vaughan et al., 1995). In the mammalian brain, urocortin mRNA is highly expressed in the Edinger–Westphal nucleus (Bittencourt et al., 1999; Vaughan et al., 1995). It is also found in the supraoptic and hypoglossal nuclei. Urocortin-immunoreactive neuron fibers are particularly abundant in the lateral septum, which noticeably expresses the mRNA encoding the CRF_2 receptor (Bittencourt et al., 1999).

Recently, two peptides, namely urocortin II and urocortin III, were added to the urocortin subfamily of peptides. Murine urocortin II (Reyes et al., 2001) is a 38-amino-acid peptide that selectively binds to the CRF_2 receptor with no appreciable affinity for the CRF_1 receptor. In the mouse brain, urocortin II is expressed in restricted areas, including the magnocellular division of the paraventricular hypothalamic nucleus, the arcuate nucleus and the locus coeruleus (Reyes et al., 2001). There are still uncertainties surrounding the exact chemical nature of the human ortholog of murine urocortin II (Hsu and Hsueh, 2001; Lewis et al., 2001). Urocortin III (Lewis et al., 2001), also referred to in humans as stresscopin (Hsu and Hsueh, 2001), has been described as a putative 38 amino acid peptide that, similar to urocortin II, selectively binds to the CRF_2 receptor. In rats and mice, urocortin III mRNA is expressed in discrete brain areas including the bed nucleus of the stria terminalis, the medial nucleus of amygdala, the hypothalamus and the brainstem (Lewis et al., 2001). In the hypothalamus, urocortin III is expressed in the median preoptic nucleus, in the rostral perifornical region and in an ill-defined region lateral to the paraventricular hypothalamic nucleus.

Urocortins are not likely to be physiologically involved in either the basal or stress-induced pituitary activation. In fact, urocortin II and urocortin III are not selective for the CRF_1 receptor, which mediates the hypophysiotropic effect within the CRF system (Smith et al., 1998). Additionally, the neurons expressing the urocortins do not project to the anterior pituitary. However, a central infusion of urocortin, which also binds to the CRF_1 receptor with a high affinity (Vaughan et al., 1995), activates the pituitary–adrenal axis (Vaughan et al., 1995) and elicits anxiety-like behaviors (Moreau et al., 1997).

Because of their selectivity for the CRF_2 receptor, urocortins II and III have been described as 'stress-coping' peptides (Hsu and Hsueh, 2001). Both peptides are capable of reducing anxiety, blood pressure and arousal. The ability of urocortin II to increase exploration of the elevated plus maze (Valdez et al., 2001) strongly suggests a role for the CRF_2 receptor in mediating anxiolytic effects.

Recent investigations have demonstrated the ability of stressors such as acute pain, ether stress and immobilization to stimulate the expression of Fos in urocortin-immunoreactive neurons of the Edinger–Westphal nucleus (Kozicz et al., 2001; Weninger et al., 2000). The physiological modulation of the brain synthesis of urocortin II and urocortin III has yet to be documented.

2.3. Urotensin I and sauvagine

Urotensin I is a 41-amino acid peptide isolated from the caudal spinal cord and urophysis of teleost fishes (Ichikawa et al., 1982; Lederis et al., 1983). Sauvagine is a 40-amino acid peptide isolated from the frog skin (Montecucchi et al., 1980). When given to mammals, urotensin I and sauvagine exhibit CRF/urocortin-like effects such as adrenocorticotropic hormone secretion (Rivier et al., 1983), gastric stasis (Kosoyan et al., 1999) as well as anorectic and thermogenic effects (LeFeuvre et al., 1989).

3. The corticotropin-releasing factor receptors

The CRF_1 receptor (Perrin et al., 1993) and the CRF_2 receptor (Lovenberg et al., 1995a) together with the newly

discovered non-mammalian CRF_3 receptor (Arai et al., 2001) are G-protein-coupled receptor types that mediate the effects of the CRF family of peptides. In addition to binding to two receptors, CRF and its related peptides also bind to CRF-binding protein (Behan et al., 1989; Orth and Mount, 1987; Potter et al., 1991).

3.1. The CRF_1 receptor

The CRF_1 receptor 1 binds with high affinity CRF (in particular ovine CRF) and urocortin as well as urotensin I and sauvagine (Dieterich et al., 1997; Vaughan et al., 1995). CRF_1 receptor mRNA is broadly distributed in the brain with high densities of expression observed in cortical, hypothalamic, limbic and cerebellar regions (Potter et al., 1994; Wong et al., 1994). It is also markedly expressed in the pituitary (Potter et al., 1994) and in other peripheral tissues (Dieterich et al., 1997). Within the paraventricular hypothalamic nucleus, CRF_1 receptor mRNA is not detected under basal conditions but can be acutely induced by stressful stimuli (Richard et al., 1996; Rivest et al., 1995; Timofeeva and Richard, 1997). CRF and glucocorticoids have been reported to repress the expression of the CRF_1 receptor in anterior pituitary cell cultures (Pozzoli et al., 1996). The pituitary and PVN are among the few regions for which modulatory effects on the CRF_1 receptor gene have been reported. These modulatory effects are consistent with the critical role played by the CRF_1 receptor in mediating the hypophysiotropic action of CRF (Smith et al., 1998).

Recent reviews (Heinrichs and Tache, 2001; Sarnyai et al., 2001) have emphasized the involvement of the CRF_1 receptor in anxiogenic behaviors, depression, drug seeking and withdrawal, anorexia and bulimia, and seizure.

3.2. The CRF_2 receptor

The CRF_2 receptor gene expresses three anatomically distinct splice variants, known as the $CRF_{2\alpha}$ receptor, the $CRF_{2\beta}$ receptor and the $CRF_{2\gamma}$ receptor. The $CRF_{2\alpha}$ receptor is mainly, though not uniquely, a brain receptor. In humans, mononuclear cells found in colon also expressed the $CRF_{2\alpha}$ receptor isoform (Muramatsu et al., 2000). The $CRF_{2\beta}$ receptor is not expressed in the brain parenchyma. In the rat, $CRF_{2\beta}$ receptor mRNA is found in the heart, the skeletal muscle and the gastrointestinal tract as well as on cerebral arterioles and in the choroid plexus (Lovenberg et al., 1995b). The $CRF_{2\gamma}$ receptor has solely been found in human brain (Kostich et al., 1998). The CRF_2 receptor has more affinity for the urocortins, urotensin I and sauvagine than for CRF itself (Dieterich et al., 1997; Vaughan et al., 1995). Urocortins II and III constitute two specific endogenous ligands for the CRF_2 receptor. In rat and mouse brains, CRF_2 receptor was restrictedly expressed in the olfactory bulb, lateral septum, ventromedial hypothalamic nucleus, medial and posterior cortical nuclei of the amygdala, mesensephalic raphe nucleus, nucleus of the solitary tract and area postrema (Lovenberg et al., 1995b; Van Pett et al., 2000).

There is evidence that the CRF_2 receptor mediates 'stress-coping' responses such as anxiolysis, 'dearousal' and hypotension (Hashimoto et al., 2001; Heinrichs and Tache, 2001). CRF_2 receptor-ablated mice tend to exhibit an increased HPA activity following restraint stress (Bale et al., 2000; Coste et al., 2000). In addition, the use of the CRF_2 receptor antagonist, antisauvagine-30, has recently revealed a negative role for the CRF_2 receptor located in the lateral intermediate septum on learning (Radulovic et al., 1999). In the periphery, the CRF_2 receptor mediates the inhibitory effect of CRF receptor agonists on gastric emptying (Chen et al., 2001; Heinrichs and Tache, 2001; Million et al., 2001). The novel long-acting and selective CRF_2 receptor antagonists completely blocks the delayed gastric emptying caused by stress, CRF or urocortin II (Chen et al., 2001; Million et al., 2001).

3.3. Additional CRF receptors

In mammals, the existence of CRF receptors other than CRF_1 receptor and CRF_2 receptor has yet to be described. However, the observation that CRF can produce effects at *loci* not expressing the CRF_1 receptor or CRF_2 receptor, including the locus coeruleus and central nucleus of amygdala, insinuates the presence of additional CRF receptors (Van Pett et al., 2000). Recently, three complementary DNA clones encoding subtypes of CRF receptors were isolated in the diploid catfish species, *Ameiurus nebulosus* (Arai et al., 2001). The first two clones encoded distinct proteins highly homologous to the murine CRF_1 receptor and CRF_2 receptor. The third full-length complementary DNA clone encoded a 428-amino acid receptor structurally related to catfish the CRF_1 receptor (85%) and CRF_2 receptor (80%). This novel receptor, which binds CRF with a 5-fold higher affinity than urotensin I and sauvagine and which is expressed in the pituitary gland, urophysis and brain, has been labeled catfish CRF_3 (Arai et al., 2001). The role of this receptor remains to be determined.

3.4. CRF-binding protein

In addition to binding to two receptors, CRF and its related peptides also bind to CRF-binding protein, which has a high affinity for rat/human CRF, urotensin I and urocortin (Ardati et al., 1998; Jahn et al., 2001). In humans, liver synthesis and blood levels of CRF-binding protein raised during pregnancy in parallel to an increased release of placental CRF, suggesting that CRF-binding protein can reduce the availability of circulating CRF to prevent an excessive pituitary stimulation during pregnancy (Linton et al., 1993; Linton et al., 1988). A late gestational fall in CRF-binding protein has been linked to the onset of parturition. However, recent data suggest that serum concentrations of CRF and CRF-binding protein are poor predictors of pre-

term delivery among women with symptoms of preterm labor (Coleman et al., 2000).

Similar to CRF and the CRF receptors, CRF-binding protein is widely distributed throughout the brain (Potter et al., 1991), where it is expressed in the cortex, amygdala and hypothalamus. In the rat, CRF-binding protein is expressed solely in the brain (Baigent and Lowry, 2000). Several brain regions of CRF-binding protein expression are also sites of CRF synthesis or release (Potter et al., 1992), supporting the view that CRF-binding protein could increase the availability of CRF or urocortins for the CRF receptors. Interestingly, CRF-(6–33), a CRF fragment inactive at CRF receptors but apparently capable of displacing CRF from CRF-binding protein (Behan et al., 1996; Behan et al., 1995), does not activate brain sites expressing CRF receptors (Chan et al., 2000). It rather stimulates brain regions expressing CRF-binding protein, which could be indicative of an unexpected role for this binding-protein in signaling by CRF-related peptides.

4. The CRF system and the regulation of energy balance

4.1. CRF family of peptides and the control of energy intake and energy expenditure

Peptides of the CRF family, including urotensin I, sauvagine, urocortin, urocortin II (Hsu and Hsueh, 2001; Reyes et al., 2001), and urocortin III (Hsu and Hsueh, 2001; Lewis et al., 2001) are anorectic agents (Heinrichs and Richard, 1999). They diminish food intake in rodents such as mice, rats and guinea pigs, in birds, in fishes and in ungulate species such as sheeps and pigs. Long-term appetite suppression is observed with chronic administration of CRF and urocortin although functional tolerance does develop over time (Krahn et al., 1990). The anorectic effect of CRF and urocortin, in particular, is seen at doses lower than those producing measurable anxiety or conditioned taste aversion (Benoit et al., 2000; Krahn et al., 1988). The anorectic effects of CRF (Heinrichs and Richard, 1999) and urocortin (Currie et al., 2001a) are not prevented by the orexigenic peptide neuropeptide Y. CRF, urotensin I, sauvagine and urocortin also stimulate energy expenditure through likely stimulating the sympathetic nervous system (LeFeuvre et al., 1987; Rothwell, 1990; Laberge and Richard, unpublished observations).

The CRF system could play a physiological role in energy balance regulation. CRF lowers the body weight threshold at which food-deprived rats start to hoard food in a home cage environment (Cabanac and Richard, 1995). Additionally, CRF receptor antagonists block the anorexia associated with conditions known to activate the CRF system, including treadmill running (Rivest and Richard, 1990), restraint stress (Shibasaki et al., 1988), estradiol (Dagnault et al., 1993), and caffeine (Racotta et al., 1994). The observation that CRF-deficient mice exhibit normal body weight gain and food intake relative to their wild-type littermates, when allowed ad libitum access to food (Weninger et al., 1999), does not necessarily invalidate the role of the CRF system in the regulation of energy balance. Similarly, the observation that CRF-knockout and wild-type mice exhibit indistinguishable anorectic responses to restraint stress (Swiergiel and Dunn, 1999) could merely emphasize the importance of other (possibly as yet undiscovered) CRF endogenous agonists such as the urocortins in the control of food intake. A physiological role of the CRF system in energy balance regulation is also consistent with the observations that chronically administered CRF (Rohner-Jeanrenaud et al., 1989) and CRF-(6–33) (Heinrichs et al., 1996) prevent weight gain in obese Zucker rats with little effect in lean animals.

The sites of the anorectic and thermogenic actions of the peptides from the CRF family have yet to be fully delineated. There is evidence that the urocortins can act either centrally (Spina et al., 1996) or peripherally (Million et al., 2001) to elicit anorectic effects. In the brain, PVN has been reported as one of the potential sites for the anorectic effects of CRF (Krahn et al., 1988) and urocortin (Currie et al., 2001b; Wang et al., 2001). There is also evidence that urocortin could also evoke anorexia when injected in the lateral septum (Bakshi et al., 2001) and that the parabrachial nucleus could be the locus of the anorectic action of CRF induced by dehydration (Kelly and Watts, 1998; Watts et al., 1999). The medial preoptic area has been reported as a site for the thermogenic action of CRF (Egawa et al., 1990).

Recent series of experiments (Kelly and Watts, 1998; Lin et al., 2001; Watts et al., 1999) indicate that the perifornical area of the lateral hypothalamus and the Edinger–Westphal nucleus could be the brain source of CRF and urocortin neurons potentially involved in the regulation of energy balance.

4.2. The CRF system in obesity and food deprivation

That chronically administered CRF (Rohner-Jeanrenaud et al., 1989) or CRF-(6–33) (Heinrichs et al., 1996) prevents weight gain in obese Zucker rats with little effect in lean animals suggests that the CRF system tone could be diminished in obesity, a view entirely consistent with the anorectic and thermogenic properties of CRF. However, investigations carried out in obese rodents do not invariably support the suggestion of a reduced CRF activity in obesity. In fact, obese rodents readily react to stressful stimuli (Guillaume-Gentil et al., 1990) and food-deprivation (Timofeeva and Richard, 1997, 2001), which can even induce, in genetically obese animals, a neurogenic-stress-like response (Timofeeva and Richard, 1997) that strongly stimulates the CRF system (Timofeeva and Richard, 1997). Nonetheless, there are adaptations of the CRF system in obesity (or following food deprivation) that could concur to reduce the CRF tone. For instance, CRF-binding protein mRNA levels in the medial preoptic area and the basolateral

complex of the amygdala have been reported to be higher in obese and food-deprived rats than in lean and fed animals (Timofeeva et al., 1997). Also, below control mRNA levels of the CRF_2 receptor in the ventromedial hypothalamic nucleus (Richard et al., 1996) and urocortin in the Edinger–Westphal (Lin et al., 2001) have been reported in obese rodents.

A functional link between leptin and the CRF system has been suggested. In obese mice, leptin down-regulates the expression of CRF in the paraventricular hypothalamic nucleus (Arvaniti et al., 2001), which is consistent with the apparent ability of leptin to reduce the hypothalamic–pituitary–adrenal axis activity (Ahima et al., 1996; Giovambattista et al., 2000; Heiman et al., 1997). Other studies have demonstrated that the anorectic effect of a central infusion of leptin could be blocked with CRF receptor antagonists (Gardner et al., 1998; Uehara et al., 1998), suggesting that leptin activates the CRF system. It remains to be demonstrated whether CRF antagonists prevents a leptin-mediated anorectic effect or whether they block a non-specific stress-related anorectic effect due to an acute central injection of a massive dose of leptin.

4.3. The CRF receptors and the CRF-binding protein in the regulation of energy balance

Evidence keeps accumulating to emphasize the importance of the CRF_2 receptor in mediating the anorectic effects of stress and CRF-related peptides (Cullen et al., 2001; Hashimoto et al., 2001; Katner et al., 2001). Recent studies have in fact demonstrated the ability of newly developed CRF_2 receptor-selective antagonists, such as antisauvagine-30 (Cullen et al., 2001) and compound 338-086-15 (Million et al., 2001), to block the effects of the urocortins and CRF on food intake. These results are consistent with the anorectic potential of urocortin II (Hsu and Hsueh, 2001; Reyes et al., 2001) and urocortin III (Hsu and Hsueh, 2001; Lewis et al., 2001), which represent specific agonists for the CRF_2 receptor. They are also in agreement with studies conducted in CRF_2 receptor-ablated mice (Contarino et al., 1999), which recover a normal food intake more rapidly than wild-type mice in response to urocortin.

The involvement of the in CRF_2 receptor in the control of food intake is also in consonance with the changes in $CRF_{2\alpha}$ mRNA levels, which inversely vary with appetite. The expression of the ventromedial hypothalamic nucleus $CRF_{2\alpha}$ receptor mRNA is reduced in obese (Richard et al., 1996; Timofeeva and Richard, 1997), diabetic (Huang and Richard, personal communications; Makino et al., 1998) and food-deprived (Timofeeva and Richard, 1997) rats. It is induced in rats intracerebroventricularly infused with leptin (Nishiyama et al., 1999; Huang and Richard, personal communications). The ventromedial hypothalamic nucleus could represent one (though not the unique) site for the CRF_2 receptor-mediated anorectic effect of the CRF-related peptides.

On the other hand, the role of the CRF_1 receptor in the feeding behavior remains unclear (Heinrichs and Richard, 1999). The observation that urocortin does not elicit an acute anorectic response in CRF_1 receptor-ablated mice could suggest that the CRF_1 receptor plays a role in the anorectic effects of the CRF-related peptides (Bradbury et al., 2000), assuming that the CRF_1 or CRF_2 receptor are the only existing CRF receptors. As previously suggested (Heinrichs and Richard, 1999), the CRF_1 receptor could mediate anorectic effects that would be non-specific and secondary to the anxiety- and fear-like behaviors triggered by non-selective CRF receptor agonists such as CRF and urocortin. Food ingestion is likely incompatible with most behaviors led to by the activation of the CRF_1 receptor. Such a view of the role of the CRF_1 receptor in food intake control is consonant with the ability of selective CRF_1 receptor antagonists to block the anorexia induced by emotional stress (Hotta et al., 1999). It is also noteworthy that the CRF_1 receptor 1 has also been positively implicated in appetitive behaviors. Exposure to stress via CRF_1 receptor activation has been reported to disinhibit consumption of rewarding drugs, such as cocaine and heroin (Heinrichs and Richard, 1999).

The possibility that the CRF_1 receptor may mediate the thermogenic effects of CRF cannot be excluded. In fact, the CRF_1 receptor is expressed in the medial preoptic area, where CRF exert thermogenic effects (Egawa et al., 1990). In addition, ovine CRF produces, among the CRF receptor agonists, one of the strongest thermogenic responses when infused in cerebral ventricles (Laberge and Richard, unpublished data). The inability of the selective CRF_2 receptor antagonist, antisauvagine-30, to totally block the effects of CRF on body weight (Cullen et al., 2001) while it blocks the anorectic effects of the peptide questions the role of the CRF_2 receptor in thermogenesis, which undoubtedly contributes to the effects of CRF in reducing fat deposition. Also, antisauvagine-30 does not prevent CRF from increasing the weight of brown adipose tissue (Cullen et al., 2001), which represents the main thermogenic effector of regulatory thermogenesis in rodents (Trayhurn, 1993). An increase in brown adipose tissue weight following a treatment reducing fat mass is soundly indicative of an increased thermogenic capacity of the tissue (LeBlanc and Villemaire, 1970).

It is also noteworthy that CRF receptors mediate effects that could unspecifically influence the regulation of energy balance. As mentioned above, anxiety- and fear-like behaviors, which are likely CRF_1 receptor-mediated behaviors, could reduce food intake because they are incompatible with food ingestion. In addition, CRF_1 receptor stimulation strongly activates the pituitary–adrenal axis and the secretion of corticosteroids, which, by contrast to CRF, promote energy deposition (Cabanac and Richard, 1996; Castonguay et al., 1986; Strack et al., 1995) and insulin resistance (Andrews and Walker, 1999). CRF treatment, in absence of the CRF CRF_2 receptor-mediated anorectic effects, leads

to an increase in insulin levels (Cullen et al., 2001) that could well be the consequence of an increase in corticosterone secretion. In addition, corticosteroids potently inhibit brown adipose tissue thermogenesis (Arvaniti et al., 1998; Galpin et al., 1983; Strack et al., 1995). This effect opposes that of CRF system activation, which stimulates thermogenesis from brown adipose tissue (LeFeuvre et al., 1991) through a sympathetically mediated process. Finally, the peripheral administration of CRF_2 receptor agonists could also diminish food intake through the inhibition of gastric emptying (Heinrichs and Tache, 2001).

CRF-(6–33), a fragment that preferentially binds to CRF-binding protein and inhibits its action, efficiently reduces weight gain in obese *fa/fa* Zucker rats (Heinrichs et al., 1996), suggesting a role for this binding protein in the regulation of energy balance. A single infusion of CRF-(6–33) has been reported to increase nonshivering thermogenesis in lean and obese rats (Heinrichs et al., 2001a) and to decrease food intake in obese *ob/ob* mice (Heinrichs et al., 2001b). The exact mechanism of action through which CRF-(6–33) affects the regulation of energy balance remains, however, obscure.

4.4. The CRF system, a potential drug target for obesity

The progress made in recent years tends to corroborate the potential of the CRF system as a target for anti-obesity drugs. The CRF system has long been only reluctantly regarded as a potential target for anti-obesity drugs, this mainly due to the fact that unspecific activation of CRF circuitries produces anxiogenic, hypophysiotropic and other potentially undesirable actions. The new synthetic and endogenous selective ligands for the CRF_2 and CRF_1 receptor could offer new possibilities for developing molecules that could, through the CRF system, elicit effects specifically related to the regulation of energy balance.

However, there are still important unresolved issues in the field of CRF research, which currently prevent the CRF system from being fully appreciated as a potential target for anti-obesity drugs. These issues include the lack of a thorough profile of the physiological roles of the CRF_2 receptor, CRF_1 receptor and CRF-binding protein. This profile is essential to ultimately appraise the potential of the CRF system as a target for anti-obesity drugs. In addition, some mismatches between the brain activation led to by CRF and CRF-related peptides and the distribution of the CRF receptors need to be clarified. The possibility that the effects of CRF and CRF-related peptides on energy balance, in particular those on thermogenesis, could be mediated through an undiscovered CRF receptor cannot be excluded.

5. Conclusion

The involvement of the CRF system in the regulation of energy balance has been reviewed. This system comprises peptides capable of strong anorectic and thermogenic actions and is anatomically configured in an extensive web of cells connected with central and peripheral pathways modulating energy metabolism. In recent years, great progress has been made in understanding the specific physiological roles of the CRF receptors. The discovery of urocortins II and III, two endogenous ligands of the CRF_2 receptor, as well as the development of potent, selective and long-acting antagonists for the CRF receptors, have led to a better comprehension of the selectivity of the CRF receptors in mediating anorectic and thermogenic effects. Although there are still important unresolved issues in the field of CRF research, the progress made recently warrants investigations aimed at evaluating the CRF system as a potential target for anti-obesity drugs.

References

Ahima, R.S., Prabakaran, D., Mantzoros, C., Qu, D.Q., Lowell, B., Maratos-Flier, E., Flier, J.S., 1996. Role of leptin in the neuroendocrine response to fasting. Nature 382, 250–252.

Andrews, R.C., Walker, B.R., 1999. Glucocorticoids and insulin resistance: old hormones, new targets. Clin. Sci. 96, 513–523.

Arai, M., Assil, I.Q., Abou-Samra, A.B., 2001. Characterization of three corticotropin-releasing factor receptors in catfish: a novel third receptor is predominantly expressed in pituitary and urophysis. Endocrinology 142, 446–454.

Ardati, A., Gottowik, J., Henriot, S., Clerc, R.G., Kilpatrick, G.J., 1998. Pharmacological characterisation of the recombinant human CRF binding protein using a simple assay. J. Neurosci. Methods 80, 99–105.

Arvaniti, K., Ricquier, D., Champigny, O., Richard, D., 1998. Leptin and corticosterone have opposite effects on food intake and the expression of UCP1 mRNA in brown adipose tissue of lep^{ob}/lep^{ob} mice. Endocrinology 139, 4000–4004.

Arvaniti, K., Huang, O.L., Richard, D., 2001. Effects of leptin and corticosterone on the expression of corticotropin-releasing hormone, agouti-related protein, and proopiomelanocortin in the brain of ob/ob mouse. Neuroendocrinology 73, 227–236.

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

Bakshi, V.P., Newman, H.C., Weinberg, L.E., Smith-Roe, S.L., Kalin, N.H., 2001. Urocortin infusion into lateral septum increases grooming and decreases ingestive behaviors. Soc. Neurosci. Abstr. 27, 414.414.

Bale, T.L., Contarino, A., Smith, G.W., Chan, R., Gold, L.H., Sawchenko, 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.

Behan, D.P., Linton, E.A., Lowry, P.J., 1989. Isolation of the human plasma corticotrophin-releasing factor-binding protein. J. Endocrinol. 122, 23–31.

Behan, D.P., Heinrichs, S.C., Troncoso, J.C., Liu, X.J., Kawas, C.H., Ling, N., Desouza, E.B., 1995. Displacement of corticotropin-releasing factor from its binding protein as a possible treatment for Alzheimer's disease. Nature 378, 284–287.

Behan, D.P., Grigoriadis, D.E., Lovenberg, T., Chalmers, D., Heinrichs, S., Liaw, C., De Souza, E.B., 1996. Neurobiology of corticotropin releasing factor (CRF) receptors and CRF-binding protein: implications for the treatment of CNS disorders. Mol. Psychiatry 1, 265–277.

Benoit, S.C., Thiele, T.E., Heinrichs, S.C., Rushing, P.A., Blake, K.A., Steeley, R.J., 2000. Comparison of central administration of corticotro-

pin-releasing hormone and urocortin on food intake, conditioned taste aversion, and c-Fos expression. Peptides 21, 345–351.

Bittencourt, J.C., Vaughan, J., Arias, C., Rissman, R.A., Vale, W.W., Sawchenko, P.E., 1999. Urocortin expression in rat brain: evidence against a pervasive relationship of urocortin-containing projections with targets bearing type 2 CRF receptors. J. Comp. Neurol. 415, 285–312.

Bradbury, M.J., McBurnie, M.I., Denton, D.A., Lee, K.F., Vale, W.W., 2000. Modulation of urocortin-induced hypophagia and weight loss by corticotropin-releasing factor receptor 1 deficiency in mice. Endocrinology 141, 2715–2724.

Brown, M.R., Fisher, L.A., 1990. Regulation of the autonomic nervous system by corticotropin-releasing factor. In: De Souza, E.B., Nemeroff, C.B. (Eds.), Corticotropin-Releasing Factor: Basic and Clinical Studies of a Neuropeptide. CRC, Boca Raton, pp. 291–298.

Cabanac, M., Richard, D., 1995. Acute intra-ventricular CRF lowers the hoarding threshold in male rats. Physiol. Behav. 57, 705–710.

Cabanac, M., Richard, D., 1996. The nature of the ponderostat: Hervey's hypothesis revived. Appetite 26, 45–54.

Castonguay, T.W., Dallman, M.F., Stern, J.S., 1986. Some metabolic and behavioral effects of adrenalectomy on obese Zucker rats. Am. J. Physiol. 251, R923–R933.

Chan, R.K., Vale, W.W., Sawchenko, P.E., 2000. Paradoxical activational effects of a corticotropin-releasing factor-binding protein "ligand inhibitor" in rat brain. Neuroscience 101, 115–129.

Chen, C.Y., Million, M., Adelson, D.W., Kosoyan, H.P., Rivier, J., Tache, Y., 2001. Compound 338-086-15, a novel peptidergic CRF receptor subtype 2 antagonist, selectively blocks urocortin-induced inhibition of gastric contractility in anesthesized rats. Soc. Neurosci. Abstr. 27, 839.821.

Coleman, M.A., France, J.T., Schellenberg, J.C., Ananiev, V., Townend, K., Keelan, J.A., Groome, N.P., McCowan, L.M., 2000. Corticotropin-releasing hormone, corticotropin-releasing hormone-binding protein, and activin A in maternal serum: prediction of preterm delivery and response to glucocorticoids in women with symptoms of preterm labor. Am. J. Obstet. Gynecol. 183, 643–648.

Contarino, A., Heinrichs, S.C., Gold, L.H., 1999. Understanding corticotropin releasing factor neurobiology: contributions from mutant mice. Neuropeptides 33, 1–12.

Coste, S.C., Kesterson, R.A., Heldwein, K.A., Stevens, S.L., Heard, A.D., Hollis, J.H., Murray, S.E., Hill, J.K., Pantely, 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.

Cullen, M.J., Ling, N., Foster, A.C., Pelleymounter, M.A., 2001. Urocortin, corticotropin releasing factor-2 receptors and energy balance. Endocrinology 142, 992–999.

Currie, P.E., Taylor, K.M., Coiro, C., Niyomchai, T., Tai, N., 2001a. Hypothalamic urocortin (UCN): suppression of food intake, neuropeptide Y (NPY) stimulated eating and metabolism. Soc. Neurosci. Abstr. 27, 422.417.

Currie, P.J., Coscina, D.V., Bishop, C., Coiro, C.D., Koob, G.F., Rivier, J., Vale, W., 2001b. Hypothalamic paraventricular nucleus injections of urocortin alter food intake and respiratory quotient. Brain Res. 916, 222–228.

Dagnault, A., Ouerghi, D., Richard, D., 1993. Treatment with α-helical-CRF-$_{(9-41)}$ prevents the anorectic effect of 17-β-estradiol. Brain Res. Bull. 32, 689–692.

Dieterich, K.D., Lehnert, H., De Souza, E.B., 1997. Corticotropin-releasing factor receptors: an overview. Exp. Clin. Endocrinol. Diabetes 105, 65–82.

Egawa, M., Yoshimatsu, H., Bray, G.A., 1990. Preoptic area injection of corticotropin-releasing hormone stimulates sympathetic activity. Am. J. Physiol. 259, R799–R806.

Fisher, L.A., Rivier, J., Rivier, C., Spiess, J., Vale, W., Brown, M.R., 1982. Corticotropin-releasing factor (CRF): central effects on mean arterial pressure and heart rate in rats. Endocrinology 110, 2222–2224.

Galpin, K.S., Henderson, R.G., James, W.P.T., Trayhurn, P., 1983. GDP binding to brown-adipose-tissue mitochondria of mice treated chronically with corticosterone. Biochem. J. 214, 265–268.

Gardner, J.D., Rothwell, N.J., Luheshi, G.N., 1998. Leptin affects food intake via CRF-receptor-mediated pathways. Nat. Neurosci. 1, 103.

Giovambattista, A., Chisari, A.N., Gaillard, R.C., Spinedi, E., 2000. Food intake-induced leptin secretion modulates hypothalamo–pituitary–adrenal axis response and hypothalamic Ob–Rb expression to insulin administration. Neuroendocrinology 72, 341–349.

Guillaume-Gentil, C., Rohner-Jeanrenaud, F., Abramo, F., Bestetti, G.E., Rossi, G.L., Jeanrenaud, B., 1990. Abnormal regulation of the hypothalamo–pituitary–adrenal axis in the genetically obese fa/fa rat. Endocrinology 126, 1873–1879.

Hashimoto, K., Makino, S., Asaba, K., Nishiyama, M., 2001. Physiological roles of corticotropin-releasing hormone receptor type 2. Endocr. J. 48, 1–9.

Heiman, M.L., Ahima, R.S., Craft, L.S., Schoner, B., Stephens, T.W., Flier, J.S., 1997. Leptin inhibition of the hypothalamic–pituitary–adrenal axis in response to stress. Endocrinology 138, 3859–3863.

Heinrichs, S.C., Richard, D., 1999. The role of corticotropin-releasing factor and urocortin in the modulation of ingestive behavior. Neuropeptides 33, 350–359.

Heinrichs, S.C., Tache, Y., 2001. Therapeutic potential of CRF receptor antagonists: a gut–brain perspective. Expert Opin. Invest. Drugs 10, 647–659.

Heinrichs, S.C., Lapsansky, J., Behan, D.P., Chan, R.K.W., Sawchenko, P.E., Lorang, M., Ling, N., Vale, W.W., De Souza, E.B., 1996. Corticotropin-releasing factor-binding protein ligand inhibitor blunts excessive weight gain in genetically obese Zucker rats and rats during nicotine withdrawal. Proc. Natl. Acad. Sci. U. S. A. 93, 15475–15480.

Heinrichs, S.C., Joppa, M., Lapsansky, J., Jeske, K., Nelson, R., De Souza, E., 2001a. Selective stimulatory actions of corticotropin-releasing factor ligands on correlates of energy balance. Physiol. Behav. 74, 5–13.

Heinrichs, S.C., Li, D.L., Iyengar, S., 2001b. Corticotropin-releasing factor (CRF) or CRF binding-protein ligand inhibitor administration suppresses food intake in mice and elevates body temperature in rats. Brain Res. 900, 177–185.

Hotta, M., Shibasaki, T., Arai, K., Demura, H., 1999. Corticotropin-releasing factor receptor type 1 mediates emotional stress-induced inhibition of food intake and behavioral changes in rats. Brain Res. 823, 221–225.

Hsu, S.Y., Hsueh, A.J.W., 2001. Human stresscopin and stresscopin-related peptide are selective ligands for the type 2 corticotropin-releasing hormone receptor. Nat. Med. 7, 605–611.

Ichikawa, T., McMaster, D., Lederis, K., Kobayashi, H., 1982. Isolation and amino acid sequence of urotensin I, a vasoactive and ACTH-releasing neuropeptide, from the carp (*Cyprinus carpio*) urophysis. Peptides 3, 859–867.

Jahn, O., Eckart, K., Sydow, S., Hofmann, B.A., Spiess, J., 2001. Pharmacological characterization of recombinant rat corticotropin releasing factor binding protein using different sauvagine analogs. Peptides 22, 47–56.

Katner, J.S., Li, D.L., Grigoriadis, D.E., Ling, N., Iyengar, S., 2001. Urocortin modulates food consumption and body weight via CRF2α receptor. Soc. Neurosci. Abstr. 27, 477.410.

Kelly, A.B., Watts, A.G., 1998. The region of the pontine parabrachial nucleus is a major target of dehydration-sensitive CRH neurons in the rat lateral hypothlamic area. J. Comp. Neurol. 394, 48–63.

Koob, G.F., Cole, B.J., Swerdlow, N.R., Le Moal, M., Britton, K.T., 1990. Stress, performance, and arousal: focus on CRF. NIDA Res. Monogr. 97, 163–176.

Kosoyan, H.P., Wei, J.Y., Tache, Y., 1999. Intracisternal sauvagine is more potent than corticotropin-releasing factor to decrease gastric vagal efferent activity in rats. Peptides 20, 851–858.

Kostich, W.A., Chen, A.R., Sperle, K., Largent, B.L., 1998. Molecular identification and analysis of a novel human corticotropin-releasing factor (CRF) receptor: The CRF2 gamma receptor. Mol. Endocrinol. 12, 1077–1085.

Kozicz, T., Gaszner, B., Arimura, A., 2001. The activation of urocortin immunoreactive neurons in the Edinger–Westhphal nucleus following various acute stressors. Soc. Neurosci. Abstr. 27, 414.412.

Krahn, D.D., Gosnell, B.A., Levine, A.S., Morley, J.E., 1988. Behavioral effects of corticotropin-releasing factor: localization and characterization of central effects. Brain Res. 443, 63–69.

Krahn, D.D., Gosnell, B.A., Majchrzak, M.J., 1990. The anorectic effects of CRH and restraint stress decrease with repeated exposures. Biol. Psychiatry 27, 1094–1102.

Krysiak, R., Obuchowicz, E., Herman, Z.S., 2000. Role of corticotropin-releasing factor (CRF) in anxiety. Pol. J. Pharmacol. 52, 15–25.

LeBlanc, J., Villemaire, A., 1970. Thyroxine and noradrenaline on noradrenaline sensitivity, cold resistance, and brown fat. Am. J. Physiol. 218, 1742–1745.

Lederis, K., Vale, W., Rivier, J., MacCannell, K.L., McMaster, D., Kobayashi, Y., Suess, U., Lawrence, J., 1982. Urotensin I—a novel CRF-like peptide in Catostomus commersoni urophysis. Proc. West. Pharmacol. Soc. 25, 223–227.

Lederis, K., Letter, A., McMaster, D., Ichikawa, T., MacCannell, K.L., Kobayashi, Y., Rivier, J., Rivier, C., Vale, W., Fryer, J., 1983. Isolation, analysis of structure, synthesis, and biological actions of urotensin I neuropeptides. Can. J. Biochem. Cell Biol. 61, 602–614.

LeFeuvre, R.A., Rothwell, N.J., Stock, M.J., 1987. Activation of brown fat thermogenesis in response to central injection of corticotropin releasing hormone in the rat. Neuropharmacology 26, 1217–1221.

LeFeuvre, R.A., Rothwell, N.J., White, A., 1989. A comparison of the thermogenic effects of CRF, sauvagine and urotensin-I in the rat. Horm. Metab. Res. 21, 525–526.

LeFeuvre, R.A., Aisenthal, L., Rothwell, N.J., 1991. Involvement of corticotrophin-releasing factor (CRF) in the thermogenic and anorexic actions of serotonin (5-HT) and related compounds. Brain Res. 555, 245–250.

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 CRF2 receptor. Proc. Natl. Acad. Sci. U. S. A. 98, 7570–7575.

Lin, Q., Huang, Q., Picard, F., Deshaies, Y., Richard, D., 2001. Urocortin mRNA levels in the Edinger–Westphal nucleus is down-regulated in several rodent models of obesity and by urocortin. Soc. Neurosci. Abstr. 27, 636.631.

Linton, E.A., Wolfe, C.D., Behan, D.P., Lowry, P.J., 1988. A specific carrier substance for human corticotrophin releasing factor in late gestational maternal plasma which could mask the ACTH-releasing activity. Clin. Endocrinol. (Oxford) 28, 315–324.

Linton, E.A., Perkins, A.V., Woods, R.J., Eben, F., Wolfe, C.D., Behan, D.P., Potter, E., Vale, W.W., Lowry, P.J., 1993. Corticotropin releasing hormone-binding protein (CRH-BP): plasma levels decrease during the third trimester of normal human pregnancy. J. Clin. Endocrinol. Metab. 76, 260–262.

Lovenberg, T.M., Liaw, C.W., Grigoriadis, D.F., Clevenger, W., Chalmers, D.T., Desouza, E.B., Olsterdorf, T., 1995a. Cloning and characterization of functionally distinct corticotropin-releasing factor receptor subtype from rat brain. Proc. Natl. Acad. Sci. U. S. A. 92, 836–840.

Lovenberg, T.W., Chalmers, D.T., Liu, C., De Souza, E.B., 1995b. $CRF_{2\alpha}$ and $CRF_{2\beta}$ receptor mRNAs are differently distributed between the rat central nervous system and peripheral tissues. Endocrinology 136, 4139–4142.

Makino, S., Nishiyama, M., Asaba, K., Gold, P.W., Hashimoto, K., 1998. Altered expression of type 2 CRH receptor mRNA in the VMH by glucocorticoids and starvation. Am. J. Physiol.: Regul., Integr. Comp. Physiol. 44, R1138–R1145.

Merchenthaler, I., Vigh, S., Petrusz, P., Schally, A.V., 1982. Immunocytochemical localization of corticotropin-releasing factor (CRF) in the rat brain. Am. J. Anat. 165, 385–396.

Million, M., Saunders, P., Rivier, J., Vale, W., Tache, Y., 2001. Compound 338-86-15, a novel peptide CRF-R2 antagonist, selectively blocks CRF and stress-induced delayed gastric emptying in rats. Soc. Neurosci. Abstr. 27, 839.817.

Montecucchi, P.C., Anastasi, A., de Castiglione, R., Erspamer, V., 1980. Isolation and amino acid composition of sauvagine. An active polypeptide from methanol extracts of the skin of the South American frog Phyllomedusa sauvagei. Int. J. Pept. Protein Res. 16, 191–199.

Moreau, J.L., Kilpatrick, G., Jenck, F., 1997. Urocortin, a novel neuropeptide with anxiogenic-like properties. NeuroReport 8, 1697–1701.

Muglia, L.J., Bethin, K.E., Jacobson, L., Vogt, S.K., Majzoub, J.A., 2000. Pituitary–adrenal axis regulation in CRH-deficient mice. Endocr. Res. 26, 1057–1066.

Muramatsu, Y., Fukushima, K., Iino, K., Totsune, K., Takahashi, K., Suzuki, T., Hirasawa, G., Takeyama, J., Ito, M., Nose, M., Tashiro, A., Hongo, M., Oki, Y., Nagura, H., Sasano, H., 2000. Urocortin and corticotropin-releasing factor receptor expression in the human colonic mucosa. Peptides 21, 1799–1809.

Orth, D.N., Mount, C.D., 1987. Specific high-affinity binding protein for human corticotropin-releasing hormone in normal human plasma. Biochem. Biophys. Res. Commun. 143, 411–417.

Perrin, M.H., Donaldson, C.J., Chen, R., Lewis, K.A., Vale, W.W., 1993. Cloning and functional expression of a rat brain corticotropin releasing factor (CRF) receptor. Endocrinology 133, 3058–3061.

Potter, E., Behan, D.P., Fischer, W.H., Linton, E.A., Lowry, P.J., Vale, W.W., 1991. Cloning and characterization of the cDNAs for human and rat corticotropin releasing factor-binding proteins. Nature 349, 423–426.

Potter, E., Behan, D.P., Linton, E.A., Lowry, P.J., Sawchenko, P.E., Vale, W.W., 1992. The central distribution of a corticotropin-releasing factor (CRF)-binding protein predicts multiple sites and modes of isnteraction with CRF. Proc. Natl. Acad. Sci. U. S. A. 89, 4192–4196.

Potter, E., Sutton, S., Donaldson, C., Chen, R., Perrin, M., Lewis, K., Sawchenko, P.E., Vale, W., 1994. Distribution of corticotropin-releasing factor receptor mRNA expression in the rat brain and pituitary. Proc. Natl. Acad. Sci. U. S. A. 91, 8777–8781.

Pozzoli, G., Bilezikjian, L.M., Perrin, M.H., Blount, A.L., Vale, W.W., 1996. Corticotropin-releasing factor (CRF) and glucocorticoids modulate the expression of type 1 CRF receptor messenger ribonucleic acid in rat anterior pituitary cell cultures. Endocrinology 137, 65–71.

Racotta, I.S., LeBlanc, J., Richard, D., 1994. The effect of caffeine on food intake in rats: involvement of corticotropin-releasing factor and the sympatho-adrenal system. Pharmacol. Biochem. Behav. 48, 887–892.

Radulovic, J., Ruhmann, A., Liepold, T., Spiess, J., 1999. Modulation of learning and anxiety by corticotropin-releasing factor (CRF) and stress: differential roles of CRF receptors 1 and 2. J. Neurosci. 19, 5016–5025.

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.

Richard, D., 1998. Role of the CRH system in the regulation of energy balance. M.S. Med. Sci. 14, 877–888.

Richard, D., 1999. The role of CRF in the regulation of energy balance. Curr. Opin. Endocrinol. Diabetes 6, 10–18.

Richard, D., Rivest, R., Naïmi, N., Timofeeva, E., Rivest, S., 1996. Expression of corticotropin-releasing factor and its receptors in the brain of lean and obese Zucker rats. Endocrinology 137, 4786–4795.

Rivest, S., Richard, D., 1990. Involvement of corticotropin-releasing factor in the anorexia induced by exercise. Brain Res. Bull. 25, 169–172.

Rivest, S., Rivier, C., 1995. The role of corticotropin-releasing factor and interleukin-1 in the regulation of neurons controlling reproductive functions. Endocr. Rev. 16, 177–199.

Rivest, S., Laflamme, N., Nappi, R.E., 1995. Immune challenge and immobilization stress induce transcription of the gene encoding the CRF receptor in selective nuclei of the rat hypothalamus. J. Neurosci. 15, 2680–2695.

Rivier, C., Rivier, J., Lederis, K., Vale, W., 1983. In vitro and in vivo

ACTH-releasing activity of ovine CRF, sauvagine and urotensin I. Regul. Pept. 5, 139–143.

Rohner-Jeanrenaud, F., Walker, C.D., Greco-Perotto, R., Jeanrenaud, B., 1989. Central corticotropin-releasing factor administration prevents the excessive body weight gain of genetically obese (fa/fa) rats. Endocrinology 124, 733–739.

Rothwell, N.J., 1990. Central effects of CRF on metabolism and energy balance. Neurosci. Biobehav. Rev. 14, 263–271.

Sarnyai, Z., Shaham, Y., Heinrichs, S.C., 2001. The role of corticotropin-releasing factor in drug addiction. Pharmacol. Rev. 53, 209–243.

Sawchenko, P.E., Swanson, L.W., 1990. Organization of CRF immunoreactive cells and fibers in the rat brain: immunohistochemical studies. In: De Souza, E.B., Nemeroff, C.B. (Eds.), Corticotropin-Releasing Factor: Basic and Clinical Studies of a Neuropeptide. CRC, Boca Raton, pp. 30–51.

Shibasaki, T., Yamauchi, N., Kato, Y., Masuda, A., Imaki, T., Hotta, M., Demura, H., Oono, H., Ling, N., Shizume, K., 1988. Involvement of corticotropin-releasing factor in restraint stress-induced anorexia and reversion of the anorexia by somatostatin in the rat. Life Sci. 43, 1103–1110.

Smith, G.W., Aubry, J.M., Dellu, F., Contarino, A., Bilezikjian, L.M., Gold, L.H., Chen, R., Marchuk, Y., Hauser, C., Bentley, C.A., Sawchenko, P.E., Koob, G.F., Vale, W., Lee, K.F., 1998. Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. Neuron 20, 1093–1102.

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.

Strack, A.M., Bradbury, M.J., Dallman, M.F., 1995. Corticosterone decreases nonshivering thermogenesis and increases lipid storage in brown adipose tissue. Am. J. Physiol.: Regul., Integr. Comp. Physiol. 37, R183–R191.

Swiergiel, A.H., Dunn, A.J., 1999. CRF-deficient mice respond like wild-type mice to hypophagic stimuli. Pharmacol. Biochem. Behav. 64, 59–64.

Taché, Y., Gunion, M.M., Stephens, R., 1990. CRF: central nervous system action to influence gastrointestinal function and role in the gastrointestinal response to stress. In: De Souza, E.B., Nemeroff, C.B. (Eds.), Corticotropin-releasing Factor: Basic and Clinical Studies of a Neuropeptide. CRC, Boca Raton, pp. 299–307.

Timofeeva, E., Richard, D., 1997. Functional activation of CRH neurons and expression of the genes encoding CRH and its receptors in food-deprived lean (*Fa*/?) and obese (*fa/fa*) Zucker rats. Neuroendocrinology 66, 327–340.

Timofeeva, E., Richard, D., 2001. Activation of the central nervous system in obese Zucker rats during food deprivation. J. Comp. Neurol. 441, 71–89.

Timofeeva, E., Rivest, R., Picard, F., Deshaies, Y., Richard, D., 1997. CRF-binding protein mRNA in the brain of fed and food-deprived lean and obese Zucker rats. Soc. Neurosci. Abstr. 23, 997.

Trayhurn, P., 1993. Brown adipose tissue—from thermal physiology to bioenergetics. J. Biosci. 18, 161–173.

Turnbull, A.V., Rivier, C., 1997. Corticotropin-releasing factor (CRF) and endocrine responses to stress: CRF receptors, binding protein, and related peptides. Proc. Soc. Exp. Biol. Med. 215, 1–10.

Uehara, Y., Shimizu, H., Ohtani, K., Sato, N., Mori, M., 1998. Hypothalamic corticotropin-releasing hormone is a mediator of the anorexigenic effect of leptin. Diabetes 47, 890–893.

Valdez, G.R., Inoue, K., Koob, G.F., Rivier, J.E., Vale, W.W., Zorrilla, E.P., 2001. Human urocortin II: effects of a novel selective CRF-R2 receptor agonist on anxiety and motor activation in rats. Soc. Neurosci. Abstr. 27, 320.313.

Vale, W., Spiess, J., Rivier, C., Rivier, J., 1981. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 213, 1394–1397.

Van Pett, K., Viau, V., Bittencourt, J.C., Chan, R.K., Li, H.Y., Arias, C., Prins, G.S., Perrin, M., Vale, W., Sawchenko, P.E., 2000. Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. J. Comp. Neurol. 428, 191–212.

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.

Wang, C., Mullet, M.A., Glass, M.J., Billington, C.J., Levine, A.S., Kotz, C.M., 2001. Feeding inhibition by urocortin in the rat hypothalamic paraventricular nucleus. Am. J. Physiol.: Regul., Integr. Comp. Physiol. 280, R473–R480.

Watts, A.G., Sanchez-Watts, G., Kelly, A.B., 1999. Distinct patterns of neuropeptide gene expression in the lateral hypothalamic area and arcuate nucleus are associated with dehydration-induced anorexia. J. Neurosci. 19, 6111–6121.

Weninger, S.C., Muglia, L.J., Jacobson, L., Majzoub, J.A., 1999. CRH-deficient mice have a normal anorectic response to chronic stress. Regul. Pept. 84, 69–74.

Weninger, S.C., Peters, L.L., Majzoub, J.A., 2000. Urocortin expression in the Edinger–Westphal nucleus is up-regulated by stress and corticotropin-releasing hormone deficiency. Endocrinology 141, 256–263.

Wong, M.L., Licinio, J., Pasternak, K.I., Gold, P.W., 1994. Localization of corticotropin-releasing hormone (CRH) receptor mRNA in adult rat brain by in situ hybridization histochemistry. Endocrinology 135, 2275–2278.