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REVIEW

Physiological Roles of Corticotropin-Releasing Hormone Receptor Type 2

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Introduction

Corticotropin-releasing hormone (CRH) is a key mediator of endocrine, autonomic, behavioral, and immune responses to stress. In response to stress, CRH released from the hypothalamic paraventricular nucleus (PVN) activates CRH receptor on the anterior pituitary corticotrophes, resulting in secretion of adrenocorticotropic hormone (ACTH) into the bloodstream. Two CRH receptors, CRH-receptor type 1 (CRH-R1) and type 2 (CRH-R2), have been cloned [1–4]. CRH-R1 is highly expressed in the anterior pituitary, neocortex, olfactory bulb, hippocampus, amygdala, septum and relay nuclei of the brain stem and hypothalamus [5]. CRH-R2 is expressed in more limited brain areas than is CRH-R1. In the rodent, one splice variant form of the CRH-R2, CRH-R2α is expressed mainly in the brain, especially in the hypothalamic ventromedial nucleus (VMH) and PVN, lateral septic nucleus, and medial amygdaloid nucleus [6]. An other variant form, CRH-R2β, is expressed mainly in the peripheral tissue as well as in the brain. It is expressed at high levels in the heart and skeletal muscles and at low levels in the lung and intestine [4, 6]. In contrast to rodents, human CRH-R2β is only weakly expressed in the heart and skeletal muscles, whereas CRH-R2α is predominant [7]. More recently, an additional variant of CRH-R2, CRH-R2γ, was found in human [8].

The poor correlation between the sites of CRH and CRH-R2 expression, as well as the relatively low affinity of CRH for CRH-R2, suggested the presence of another ligand for CRH-R2, paving the way for the discovery of urocortin (UCN) [9–10]. UCN is 43% homologous to CRH and has high affinity to CRH-R2. UCN is mainly found in the brain-stem Edinger-Westphal nucleus, and also found in the hippocampus, lateral septum, amygdala, neocortex, and nucleus tractus solitarius [11]. It is also found in peripheral sites such as the duodenum, uterus, and lymphocytes. Intracerebroventricular (icv) administration of UCN elicits a strong anorexic effect. When it is injected peripherally it reduces blood pressure [12]. As UCN has greater affinity to CRH-R2 than does CRH, it is assumed that UCN is an endogenous ligand to CRH-R2. It is accepted that the effects of CRH and UCN may be mediated via either CRH-R1 or CRH-R2, or both. However, CRH-R1 and CRH-R2 could be detected only weakly in brain areas such as the central amygdala, Edinger-Westphal nucleus and locus coeruleus, these sites being the major brain sources of CRH and UCN or target areas of these neuropeptides' action. Therefore, the existence of other yet undiscovered CRH-related peptides are proposed [13, 14].

Corticotropin-releasing hormone binding protein (CRH-BP), which binds CRH and UCN with affinities greater than CRH-R1 and CRH-R2, shows a broad distribution in the brain [15]. Colocalization of CRH-BP and CRH, UCN or CRH receptors has been observed in some brain areas. Therefore, free CRH and UCN, which do not bind to CRH-BP, are the active forms that play physiological roles in the brain.

Many experiments have been done to clarify which

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subtype of CRH receptors is involved in the activities of CRH and UCN. It has been relatively well established that CRH-R1 is involved in HPA axis activation [16–19] and in the stress-induced enhancement of anxiety, fear and learning [18–21]. However, the physiological roles of CRH-R2 and its endogenous ligand have not been fully elucidated. To elucidate the role of CRH-R2, many kinds of techniques have been used. The in situ hybridization method has been used to examine the regulation of VMH CRH-R2 mRNA expression. Non-selective or selective CRH-R1 and/or CRH-R2 antagonists, and antisense oligonucleotides against CRH-R1 or CRH-R2 have been administered to animals to see the way in which they prevented the actions of CRH or UCN. Very recently, CRH-R2 knockout mice have been developed to clarify the physiological role of CRH-R2. In this review, the results of these recent investigations are summarized.

Hypothalamic-pituitary-adrenal (HPA) axis regulation

As CRH-R2α showed a relatively high expression in the hypothalamic PVN, it was speculated that CRH-R2α might be involved in the autoregulation of CRH secretion in the PVN. However, CRH-R2α was revealed to localize mainly in the magnocellular part of the PVN [22], and icv injection of CRH significantly increased CRH-R1 but did not change CRH-R2 mRNA levels in the PVN [23]. Lipopolysaccharide injection (LPS) induced a significant increase in PVN CRH-R1 mRNA (Fig. 1), while CORT administration or adrenalectomy decreased CRH-R1 mRNA levels. In contrast, CRH-R2α mRNA levels in the PVN were not altered by these manipulations [23]. CRH-R2 knockout mice did not show any changes in pituitary corticotrophs and the adrenal cortex [24, 25], though CRH-R1 knockout mice showed a marked reduction in adrenal zona fasciculata [19]. These results indicate that CRH-R2 does not play an important role in HPA axis regulation. On the other hand, Smagin *et al.* [26] reported that icv administration of anti-sense oligonucleotide to CRH-R2 mRNA significantly attenuated CRH- and UCN-induced HPA activation, suggesting stimulatory effects of exogenously administrated CRH and UCN on HPA axis are at least partly mediated by CRH-R2. However, mice deficient for CRH-R2 showed hyperresponse of ACTH and CORT to restraint stress [24, 25], and it has been postulated that CRH-R2 is involved in physiological adaptation to stresses. Kishimoto *et al.* [27], however, did not detect ACTH or CORT hyperresponse to stress in the CRH-R2 deficient mice they developed. Whether CRH-R2 is actually involved in HPA axis regulation remains to be clarified.

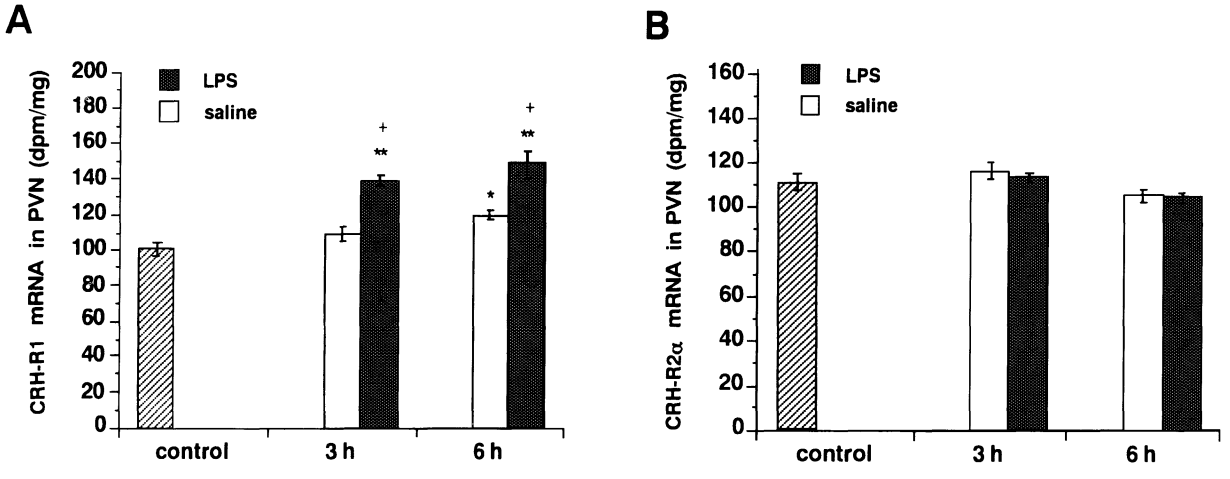

Fig. 1. CRH-R1 (A) and CRH-R2α (B) mRNA hybridization levels in the PVN after i.p. lipopolysaccharide (LPS) or saline injection. Values are mean$\pm$S.E.M. *$p<0.05$ vs. control, **$p<0.01$ vs. control, $^{+}p<1.001$ vs. saline group. (Reproduced from Ref. 23 with permission of the publisher)

Feeding and body weight regulation

Restraint-induced hypophagia and body weight loss was prevented by icv administration of non-selective CRH receptor antagonist, α-helical CRF (9–41) [28]. Hotta *et al.* [29] reported that emotional stress-induced inhibition of food intake was reversed by both icv injection of α-helical CRF (9–41) and intraperitoneal injection of a selective non-peptidic CRF-R1 antagonist, CRA 1000, and suggested that CRH-R1 mediates at least in part the emotional stress-induced reduction of feeding behavior. On the contrary, administration of a selective CRH-R1 receptor antagonist, NBI 27,914, did not affect icv CRH-induced decrease in food intake [26], and a selective CRH-R1 agonist, antalarmin, did not affect body weight, carbohydrate metabolism, or leptin expression [16]. CRH-R1 knockout mice and wild type mice showed no difference in total amount of food intake. Although there was a significant disruption in the circadian distribution of food intake, with CRH-R1-deficient mice consuming significantly more food during the light period than the dark period, the normal diurnal pattern could be completely restored by oral administration of CORT [30]. These results suggest that CRH-R1 is not likely to play a critical role in feeding behavior, although some discrepancies among reports remain to be clarified.

On the other hand, CRH-induced anorexia was significantly attenuated by the CRH-R2-selective antagonist antisauvagine-30 [31, 32]. As CRH-R2 mRNA is highly expressed in the VMH, classically referred to as the satiety center, we speculated that anorexiogenic effects of CRH or UCN are transduced via CRH-R2 in the VMH. Starvation and adrenalectomy, each of which lowered plasma insulin and leptin levels, were associated with decrements in CRH-R2 mRNA levels in VMH but not in PVH (Fig.

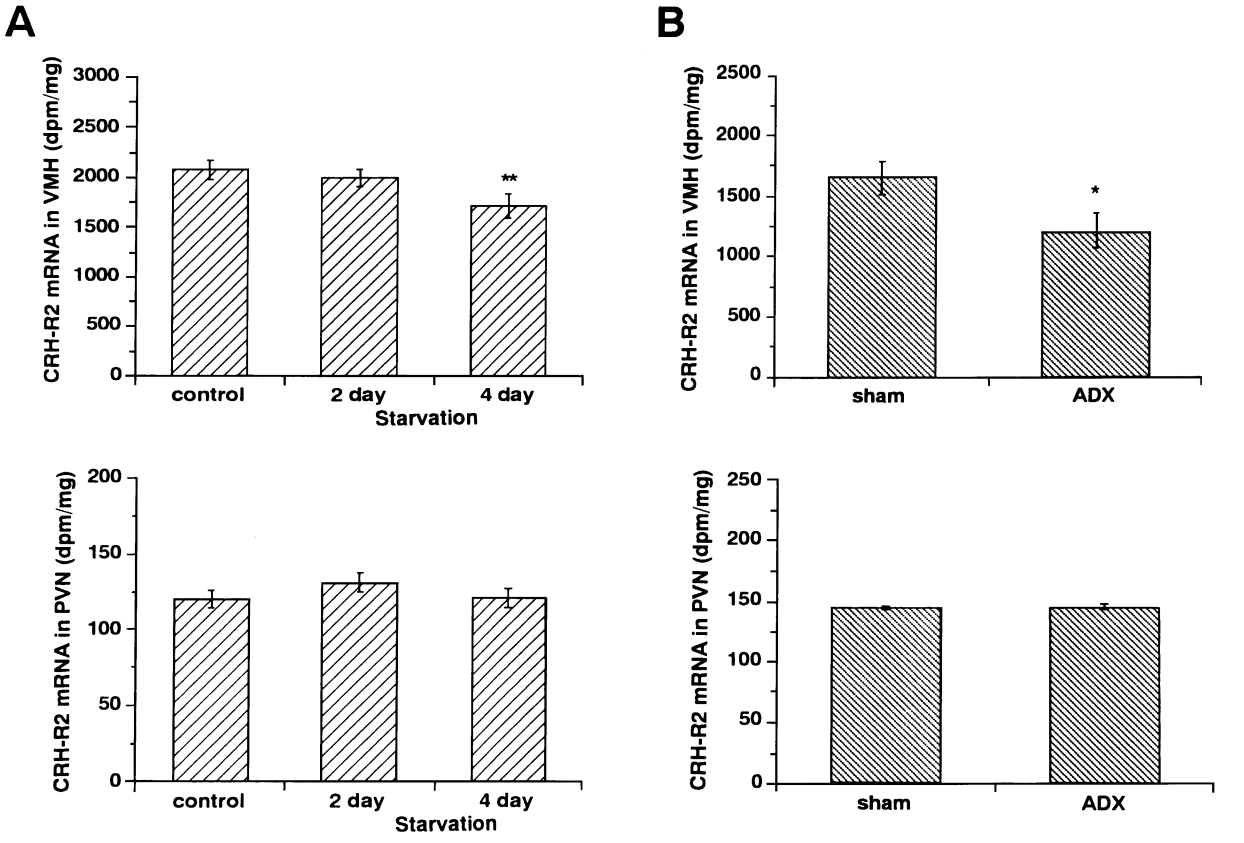

Fig. 2. Hybridization levels of CRH-R2 mRNA in the VMH and PVN after starvation for 2 or 4 days (A) and adrenalectomy (B). Values are means±SME. *$p<0.05$ vs. control group (A) or sham group (B). (Reproduced from Ref. 33 with permission of the publisher)

2) [33]. The concordance of a fall in plasma insulin and leptin levels with the fall in VMH CRH-R2 mRNA levels during starvation and adrenalectomy supports the idea that compensatory responses under the conditions of starvation and adrenalectomy include not only the disinhibiting effects of reduced insulin and leptin levels on appetite through already reported mechanisms but also via an effect of reduced leptin on VMH CRH-R2. A single intraperitoneal or continuous subcutaneous injection of leptin increased CRH-R2 mRNA levels in the rat VMH (Fig. 3) [34], suggesting that the anorexic effect of leptin may be transduced by upregulation of CRH-R2 mRNA. A large amount of CORT administration, which induces food and body weight reduction, also upregulates CRH-R2 mRNA [34]. Rats immobilized for 2 hr daily for 6 days reduced their food intake and body weight. Repeated daily immobilization increased PVN CRH mRNA and lowered plasma insulin and leptin concentrations as well as VMH CRH-R2 mRNA levels [35]. Low leptin and VMH CRH-R2 mRNA levels may also produce counter-regulatory responses against the anorexic effects of CRH or UCN. These results provide additional evidence linking plasma leptin and VMH CRH-R2 mRNA. Richard *et al.* [36] reported that expression of the CRH-R2 transcript was reduced in the VMH of obese rats.

Recently it has been reported that mice deficient for CRH-R2 express normal basal feeding and weight gain. UCN initially suppressed food intake in these mice, but they recover food intake more rapidly and completely than do wild-type mice. These results suggest that CRH-R2 is essential for sustained feeding suppression induced by UCN. Injection of anti-rat UCN rabbit γ-globulin into the bilateral VMH in freely fed rats significantly potentiated food and water intake compared with rats that received normal rabbit γ-globulin, suggesting that endogenous UCN in the VMH exert inhibitory control on ingestive behavior [37]. It is likely that VMH CRH-R2 is more important than CRH-R1 in mediating the anorexic effect of CRH, UCN, or unknown CRH-related peptides, and that stress-induced reduction of food intake is transduced by CRH-R2, though this remains to be confirmed. On the other hand, it has been reported that in the immature rat, CRH-R2 mRNA levels in VMH are governed primarily by maternal or suckling-derived sensory input rather than by food intake or peripheral stress hormones [38].

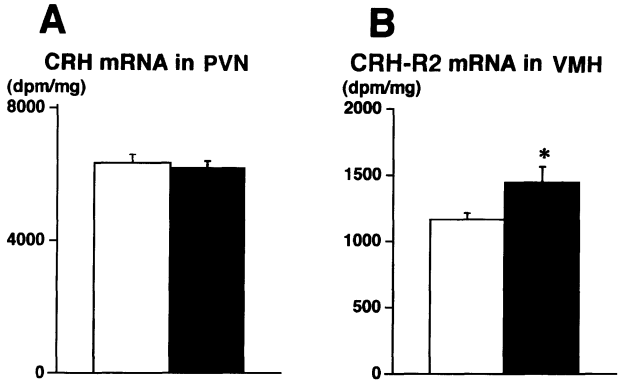

Fig. 3. Hybridization levels of CRH mRNA in the PVN (A) and CRH-R2 mRNA in the VMH (B) of vehicle- and leptin-(1.2 mg/kg/day) treated rats following a continuous 5-day infusion via an osmotic minipump. Values are means±SEM. *$p<0.05$, **$p< 0.01$ vs. vehicle group. (Reproduced from Ref. 34 with permission of the publisher)

Anxiolytic role

It has been suggested that CRH mediates behavioral responses caused by stress. CRH increased anxiety [39, 40], startle response [41], and grooming [42], and decreased explorative behavior [43], though it has not been fully clarified which subtype of receptors is involved in these responses. A selective non-peptide CRH-R1 antagonist, antalarmin, impaired both the induction and expression of conditioned fear [17, 44]. Other selective nonpeptide CRH-R1 antagonists CP-154,526 [45, 46], CRA 1000, and CRA 1001 [47], also showed anxiolytic- and antidepressant-like properties in various experimental models. Chronic infusion of CRH-R1 antisense oligonucleotide into the rat brain also caused an anxiolytic-like effect [20]. CRH-R1 deficient mice displayed markedly reduced anxiety [18, 19]. These results all suggest that CRH-R1 plays a key role in mediating anxiety-related behavior. However, Radulovic *et al.* [21] observed by using a selective CRH-R2 antagonist, anti-sauvagine 30, that a high dose of CRH induced anxiety in rats probably via septal CRH-R2, which remains to be clarified.

In contrast, CRH-R2 knockout mice exhibited enhanced anxiety-like behavior in several anxiety tests, such as dark-light emergence task test and plus-maze

test [25, 27]. The enhanced anxiety of CRH-R2-deficient mice was not due to changes in hypothalamic-pituitary-adrenal axis activity, but rather reflects impaired responses in specific brain regions [27]. Neither was it due to altered locomotor activity, because CRH-R2-deficient mice or mice given CRH-R2 antisense oligonucleotide did not show changes in overall locomotor activity [20, 25, 27]. These results suggest that CRH-R2 predominantly mediates a central anxiolytic response, opposing the anxiogenic effect of CRH mediated by CRH-R1. CRH-R2 seems to play a role counterregulatory to that of CRH-R1.

Learning and memory

The modulation of learning and memory seems to be one of the major roles of CRH in the brain. Injection of CRH into the dorsal hippocampus enhanced learning before and after training, and this effect was prevented by the local injection of the nonselective CRH-R1 antagonist astressin but not by the CRH-R2 specific antagonist antisauvagine-30 [21]. Therefore, hippocampal CRH-R1 may mediate stress-induced enhancement of learning. In contrast, injection of CRH into the lateral intermediate septum impaired learning, which was blocked by antisauvagine-30. When antisauvagine-30 was injected alone into the lateral intermediate septum, learning was enhanced. These results suggest that CRH-R2 in the lateral intermediate septum plays a part in the impairment of learning. The existence of two receptors that mediate opposite effects provides the CRH system with high flexibility and a dynamic role in the adaptation of the CNS to environmental challenge [21].

Cardiovascular regulation

Icv administration of CRH mimics stress-induced elevation of blood pressure and heart rate as well as the elevation of plasma norepinephrine (NE) and epinephrine (E) levels [48]. Although the CRH-induced cardiovascular effect was attenuated by pretreatment with a nonselective CRH-R antagonist, a-helical CRF (9–41) [49], it was unclear which type of CRH receptor was responsible for the CRH-induced autonomic responses. Icv treatment of a selective CRH-R1 antagonist, CP-154526 did not affect baseline heart rate, plasma NE and E levels, whereas it partially blocked the CRH-induced increase in heart rates and plasma NE and E levels, indicating that CRH activates the sympathetic nervous system at least in part via CRH-R1 [50].

In the rodent, CRH-R2 mRNA is found in peripheral tissues, especially in the heart and skeletal muscle, while CRH-R1 mRNA is undetectable in the heart. CRH-R2 mRNA expression in the heart is regulated by systemic administration of UCN, CORT, and cytokines [51, 52]. Systemic injection of LPS markedly downregulated CRH-R2 mRNA levels in the heart in a dose- and time-dependent manner, while CRH-R2 mRNA levels in skeletal muscle increased following exposure to endotoxin, suggesting that CRH-R2 may be differentially regulated in cardiac tissue and skeletal muscle [53].

CRH-R2 mRNA levels in spontaneously hypertensive rats (SHR) were significantly higher than those in normotensive controls. In contrast, CRH-R2 mRNA levels in the hearts of deoxycorticosterone acetate (DOCA)-salt hypertensive rats were significantly lower than that of sham-operated controls after 6 weeks of treatment (Fig. 4) [54]. Although the mechanisms governing the changes of CRH-R2 mRNA may differ in these hypertensive models, these results also suggest some roles of CRH-R2 in blood pressure regulation. CRH-R2-deficient mice showed elevated mean arterial pressure (MAP) and diastolic pressure compared with wild-type mice [24]. Systemic UCN administration decreases MAP in intact rats [10], but not in CRH-R2-deficient rats [24, 25]. These results suggest that peripheral CRH-R2 mediates the hypotensive effect of systemically administered UCN.

UCN stimulated atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) secretions from neonatal rat cardiomyocytes [55]. It also stimulated leucine uptake into neonatal rat cardiomyocytes and potentiated the endothelin-induced increase of leucine uptake. CRH-R2 may be involved in UCN-induced ANP and BNP secretions. However, the physiological role of CRH-R2 in the heart remains to be clarified.

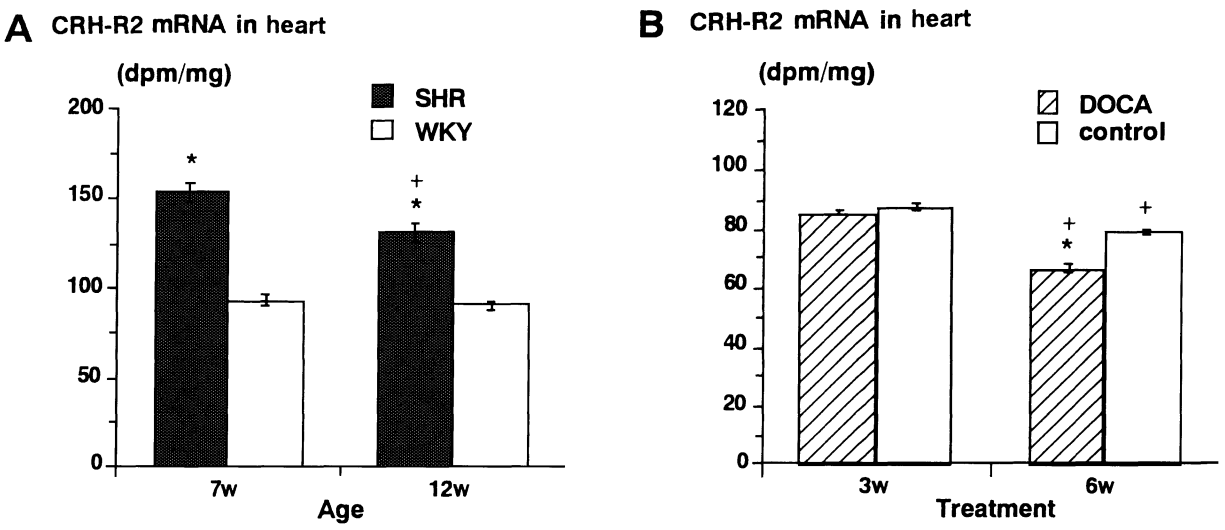

Fig. 4. Hybridization levels in the heart of SHR (A) and DOCA-salt hypertensive rats (B). Values are means ± SEM. *$p < 0.01$ vs. WKY (A) or control (B). $^{+}p < 0.01$ vs. 7-week-old SHR (A) or 3-week-treatment. (Reproduced from Ref. 54 with permission of the publisher)

Other roles of CRH-R2

Intravenous administration of CRH and UCN causes an inhibition of gastric emptying, which is fully reversed by the non-selective CRH receptor antagonist, astressin [56]. Astressin also completely prevented abdominal surgery-induced inhibition of gastric emptying, while the selective nonpeptide CRH-R1 antagonists antalarmin and NBI-27914 did not prevent CRH- and UCN-induced delay of gastric emptying. These results suggest that peripheral CRH-R2 is involved in intravenous CRH-, UCN- and abdominal surgery-induced gastric stasis. CRH-R2 is also involved in the central CRH-induced delay of gastric emptying [57].

Intradermal UCN at a concentration as low as 10 nM induced skin mast cell degranulation and increased vascular permeability in rats [58]. Both the selective nonpeptide CRH-R1 antagonist, antalarmin, and the nonselective peptide antagonist, astressin, reduced UCN-induced vascular permeability, but not completely, suggesting that UCN-induced skin mast cell degranulation and subsequent vascular permeability in rodents may involve a CRH receptor other than the known CRH-R1, CRH-R2α, and CRH-R2β subtypes. A possible candidate may be the CRH-R2γ, which has been identified in human brain tissue [8].

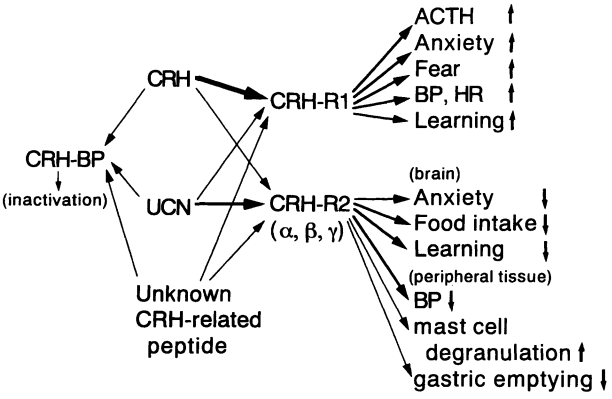

Fig. 5. Relationship among CRH-related peptides, their receptors and their physiological roles. CRH-BP: corticotropin-releasing hormone binding protein, UCN: urocortin, CRH-R1: CRH receptor type 1, BP: blood pressure, HR: heart rate.

Summary

Recent investigations of the physiological roles of CRH-R2 are reviewed and summarized in Fig. 5. VMH CRH-R2 is more important than CRH-R1 in mediating anorexic effect of CRH or urocortin (UCN) and stress-induced reduction of food intake. CRH-R2 mediates a central anxiolytic response, opposing the anxiogenic effect of CRH mediated by CRH-R1. Hippocampal CRH-R1 mediates stress-induced enhancement of learning, while CRH-R2 in

the lateral intermediate septum may act to impair learning. CRH-R1 mediates CRH-induced blood pressure elevation, while peripheral CRH-R2 mediates the hypotensive effect of systemically administered UCN and CRH. It is likely that CRH-R2 does not play an important role in hypothalamic-pituitary adrenal axis regulation, though it has been reported that CRH-R2-deficient mice showed hyperresponse of ACTH and corticosterone. Peripheral CRH-R2 mediates UCN-induced mast cell degranulation, vascular permeability, and abdominal surgery-induced gastric stasis. These recent investigations have revealed that the existence of two CRH receptors, which mediate some opposite effects, provides the CRH and UCN systems a high flexibility and dynamic role in the adaptation of the body to environmental challenge.

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