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The role of central corticotropin-releasing hormone in the anorexic and endocrine effects of the bacterial T cell superantigen, *Staphylococcal enterotoxin A*

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Abstract

Bacterial superantigens, such as the staphylococcal enterotoxins, exert a strong capacity for in vivo stimulation of T cell proliferation and cytokine production. Previously, staphylococcal enterotoxin A (SEA) was shown to induce an anorexic effect under novel contextual conditions of testing, and produced an increase in plasma ACTH and corticosterone levels in C57BL/6J mice. In the present study, the role of corticotropin releasing hormone (CRH) in promoting these effects of SEA was addressed via intracerebroventricular (icv) administration of α-helical CRH_{9-41} (αhCRH), a non-selective CRH receptor antagonist, and astressin-2B, a selective CRH receptor 2 antagonist. The efficacy of αhCRH and astressin-2B in blocking anorexic responses to CRH and urocortin under the current conditions of testing was first confirmed. Subsequently, it was found that αhCRH (20 μg icv), but not astressin-2B (10 and 25 μg icv), significantly attenuated the anorexia induced by SEA. This suggested that central CRH is involved in mediating the anorexia induced by SEA, but potentially through CRH receptor 1. Additional results revealed that plasma ACTH stimulation in response to SEA was not significantly attenuated by either antagonist administered icv. However, the plasma corticosterone elevation showed a modest, but significant, attenuation in SEA challenged mice given αhCRH. These data suggest a possible influence of central CRH on adrenocorticoid activity subsequent to SEA challenge. More importantly, it appears that central activation of CRH receptors is a consequence of SEA challenge, and this likely contributes to its anorexic effects.

1. Introduction

Corticotropin-releasing hormone (CRH) is a central and peripheral peptide that plays a variety of roles in the modification of immunological, endocrine, gastrointestinal, and behavioral functions (Dunn and Berridge, 1990; Koob and Heinrichs, 1999; Smagin and Dunn, 2000). It is widely distributed in the brain (Swanson et al., 1983; Vaughan et al., 1995), and has been shown to engage two receptor subtypes, CRH receptor 1 (CRH-R1) and CRH receptor 2 (CRH-R2) (Donaldson et al., 1996; Lovenberg et al., 1995; Perrin et al., 1995). Both receptors are found in limbic regions of the brain, although CRH-R1 is more widespread, showing not only a presence in the central and extended amygdala, but also an extensive cortical and cerebellar distribution (Steckler and Holsboer, 1999). Interestingly, CRH was shown to display more than 10-fold greater affinity for CRH-R1 than for CRH-R2 (Donaldson et al., 1996). In contrast, peptides of the CRH-related urocortin (UCN) family either bind both receptors with near equal affinity (UCN1) or are selective agonists for CRH-R2 (e.g., UCN2 and UCN3) (Donaldson et al., 1996; Vaughan et al., 1995; Zorrilla et al., 2003, 2004).

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One of the most prominent behavioral effects of intracerebroventricular CRH administration is suppression of food intake (Dunn and Berridge, 1990), while central CRH antagonism was shown to attenuate anorexia following stressor exposure (Krahn et al., 1986; Shibasaki et al., 1988). More recently, it was demonstrated that stimulation of both CRH receptor subtypes can promote anorexic behavior, although compared to CRH-R2, stimulation of CRH-R1 produces a faster rate of onset, and a shorter duration for the anorexic response (Zorrilla et al., 2003). In addition, evidence in mice and rats suggests that selective stimulation of CRH-R2 results in cessation of food intake independent of malaise and/or arousal (Zorrilla et al., 2004). Alternatively, anorexia subsequent to selective CRH-R1 stimulation is associated with anxiety-like behaviors and illness-related effects that can result in conditioned taste aversion (Zorrilla et al., 2003, 2004). Moreover, stressor-induced anorexia was reversed by CRH-R1 antagonism (Hotta et al., 1999). Therefore, while stimulation of both CRH receptors produces reduction of food intake, each appears to promote this effect under different behavioral states.

A number of studies have also attempted to characterize the endocrine functions of CRH-R1 and CRH-R2. Pituitary ACTH release appears to be mediated by stimulation of CRH-R1, since the pituitary expresses mainly CRH-R1, and the ACTH response to various stressors was blocked by pharmacological antagonism of CRH-R1, but not CRH-R2 (Ohata et al., 2002; Rivier et al., 2003). However, whether central CRH receptors are involved in the pituitary–adrenal response has not been extensively addressed.

Previous studies have implicated the CRH system in the neuroendocrine effects of immunologic stimuli, including cytokines and injection with LPS (Dunn et al., 1991; Rivier et al., 2003). On the other hand, there have been important observations challenging the role of CRH in the behavioral effects of IL-1 and LPS challenge. For example, in rats, IL-1-induced suppression of operant responding for food was unaffected by central administration of the non-selective CRH antagonist, α-helical CRH (Bluthe et al., 1992). Similarly, CRH knockout mice continued to display hypophagic responses to injections of IL-1 or LPS (Swiergiel and Dunn, 1999). These observations, therefore, suggest that the appetitive effects of cytokines derived from activation of the innate immune response may not involve central CRH activation.

While administration of LPS readily engages the innate immune system, other bacterial agents, such as the staphylococcal enterotoxins, specifically activate T lymphocytes and induce the appearance of measureable plasma levels of T cell derived IL-2 and TNFα within 1–2 h of injection (Bette et al., 1993; Rosendahl et al., 1997). Because staphylococcal enterotoxins possess such unusual properties of strong T cell activation, they have been referred to as bacterial superantigens (SAgs). We have observed that HPA axis activation in response to staphylococcal enteroxin B (SEB) is dependent on immunoneutralizable humoral CRH, and in addition, also produces a significant reduction in consumption of a novel liquid diet (Kusnecov et al., 1999). Recently, we have extended these observations to another bacterial SAg, staphylococcal enterotoxin A (SEA), which in C57BL/6J mice produces significant elevations in circulating ACTH and corticosterone (Kawashima and Kusnecov, 2002). In this latter study it was also shown that pituitary–adrenal activation was dependent on functional T cells, since RAG-1 knockout mice that lack functional lymphocytes did not display increased plasma corticosterone in response to SEA. Finally, these neuroendocrine changes were related to anorexia and augmented reactivity to a novel object. Interestingly, the anorexic response, or reduced ingestion of a liquid food diet, was most pronounced if SEA challenged animals were tested under novel contextual conditions (Kawashima et al., 2002), which was also seen in BALB/cByJ mice challenged with SEB (Kusnecov et al., 1999). This suggests a possible interaction between psychogenic stimuli and the systemic, immunologic effects of SEA.

In view of these observations, and the increased understanding in the fundamental processes by which CRH modifies behavior, the present study sought to determine whether the anorexic response to SEA challenge involved stimulation of central CRH receptors. Moreover, few studies have addressed the differential importance of CRH-R2 receptors in the behavioral effects of immune stimuli. To this end, SEA challenged animals were centrally administered either a non-selective receptor antagonist, $\alpha hCRH_{9-41}$, or the selective antagonist for CRH-R2, astressin-2B (Rivier et al., 2002). In addition, since it had previously been suggested that engagement of CRH receptors in the brain may contribute to elevated plasma levels of corticosterone following stressor exposure (Jezova et al., 1999), the pituitary–adrenal response to SEA was assessed following central administration of $\alpha hCRH_{9-41}$.

2. Materials and methods

2.1. Animals

All experiments used male C57BL 6/J mice (Jackson Laboratory, Bar Harbor, MN) aged 5–6 weeks (22–25 g) upon arrival and acclimated to a 12–12 h light–dark cycle (lights on at 07:00 h) for four weeks prior to surgery and subsequent experimentation. Mice were housed 4/cage prior to surgery, with food and water available ad libitum. Following surgery, animals were

singly housed. All procedures were approved by the Rutgers Institutional Animal Care and Use Committee.

2.2. Drugs and reagents

Staphylococcal enterotoxin A (SEA), human/rat synthetic corticotropin-releasing hormone, α-helical $CRH_{9–41}$, human urocortin (UCN), and artificial cerebrospinal fluid (CSF) were purchased from Sigma–Aldrich (St. Louis, MO). Astressin-2B was a gift from Dr. Jean Rivier (Salk Institute, La Jolla, California). Recombinant murine IL-1β was purchased from R&D Systems (Minneapolis, MN).

2.3. General experimental procedure

Four days prior to experimentation, mice underwent stereotaxic surgery to place an indwelling guide cannula into the right lateral ventricle. Mice were anesthetized with a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg) purchased from Sigma (St. Louis, MO), and a 26 gauge steel guide cannula (internal diameter 0.24 mm, outer diameter 0.46 mm; Plastics One, Roanoke, VA) was unilaterally implanted into the lateral ventricle using the following coordinates: 0.1 mm posterior to bregma, 1.0 mm lateral from the midsagittal suture, and 2.5 mm ventral. A dummy cannula was then inserted and the entire assembly secured to the skull with quick-drying cyanoacrylate glue (Plastics One, Roanoke, VA). Two and three days after surgery, mice experienced sham-infusion as habituation, and on the fourth day were subjected to experimental testing as described below. Accurate cannula placement was confirmed by infusion of methylene blue. Animals failing to show obvious diffusion of methylene blue throughout the ventricle were excluded from analysis. In all experiments, animals were completely naïve to icv treatments and behavioral testing.

2.4. Food intake testing

Food intake was tested over a 1 h period as previously described (Kawashima and Kusnecov, 2002; Rossi-George et al., 2004) using a commercial baby liquid formula (Prosobee, Mead Johnson), prepared in distilled water according to the manufacturer's instructions. Mice were not food deprived, since previous and preliminary studies (Kawashima and Kusnecov, 2002; Kusnecov et al., 1999; Rossi-George et al., 2004) have confirmed that satiated mice will readily consume this solution. For testing, animals were taken to a separate, quiet room and placed individually in a novel test cage (a regular opaque polypropylene mouse cage fitted with the tube containing Prosobee solution). After 1 h, animals were removed, infused with methylene blue (as described above), and sacrificed by decapitation.

2.5. Experiments with CRH receptor agonists (CRH and urocortin) and antagonists

Two experiments were conducted to confirm the ability of CRH antagonists to block the anorexic effects of CRH and UCN. Mice received icv infusion of either vehicle (CSF), 20 μg of α-helical $CRH_{9–41}$ [αhCRH] or 10 μg of astressin-2B in a volume of 2.5 μl. Drugs or vehicle were delivered over a period of 1 min using a Harvard Apparatus pulse-free pump (Harvard Apparatus, Holliston, MA) fitted with a 50 μl Hamilton syringe. Fifteen minutes after infusion, mice received icv infusion of either CSF, 100 ng of CRH or 100 ng of UCN in a volume of 2.5 μl. The doses of agonists and antagonists were chosen on the basis of previous studies (Berridge and Dunn, 1987; Rivier et al., 2002; Swiergiel and Dunn, 1999). Thirty minutes after the first infusion with the antagonist or vehicle (but 15 min after infusion with the agonist), mice were tested for Prosobee food intake as described above.

Testing of αhCRH against UCN infusion, and conversely, astressin-2B against CRH infusion, was not conducted, since the main intent was to ensure the functional efficacy of these antagonists. To this end, we focused on testing astressin-2B with a high affinity CRH-R2 agonist, while αhCRH was tested against CRH, which preferentially binds CRH-R1. Conceptually, there is no reason why αhCRH, which is a non-selective receptor antagonist, should not affect the anorexic effects of UCN. Indeed, this has already been confirmed (Wang and Kotz, 2002). Moreover, it has been confirmed in mice that CRH-R2 antagonism attenuates CRH-induced appetite suppression (Pelleymounter et al., 2000).

2.6. Experiments with SEA and CRH antagonists

Staphylococcal enterotoxin A (SEA) purchased from Sigma (St. Louis, MO) was injected ip in a volume of 0.2 ml, and control animals received vehicle (saline). All injections were administered between 12:00 and 15:00 h. Four days after surgery, mice received an ip injection of either 5 μg of SEA or saline, and 1 h after injection, received icv infusion of either CSF, 20 μg of αhCRH, or 10 and 25 μg astressin2-B. Thirty minutes after infusion of vehicle or antagonist (i.e., 90 min after SEA or saline injection), mice were tested for Prosobee food intake as described above. In the first experiment, which also involved testing for endocrine effects, animals were treated with SEA or saline (as described above) and each of these groups was subsequently infused with either αhCRH or CSF. At the end of testing for consumption, the animals were sacrificed by decapitation to measure plasma concentrations of corticosterone. In a second experiment, SEA and saline-injected mice received icv infusions of astressin-2B and CSF. In a third experiment, animals were injected with SEA or saline

and 1 h later were infused with αhCRH or CSF, returned to their home cage for 1 h, and then sacrificed by decapitation to collect trunk blood for assessment of plasma corticosterone and ACTH.

2.7. Endocrine assay

Trunk blood was collected by decapitation into chilled EDTA-treated tubes and centrifuged at 2000 rpm for 15 min. Plasma was stored at −70 °C prior to assay for corticosterone and ACTH. In one experiment (see Fig. 3A), corticosterone was assayed using a commercial enzyme-linked immunoassay kit (Alpco, Windham, NH), while in a second experiment (Figs. 3B and 4), corticosterone and ACTH were assayed using commercial radioimmunoassay kits (ICN Biomedicals, Costa Mesa, CA). The use of RIA enabled a smaller quantity of plasma (10 μl) to be used for measurement of corticosterone, hence allowing ACTH (which requires 100 μl plasma) to be measured. Detection of corticosterone by ELISA and RIA was comparable in that quantification of corticosterone levels did not differ significantly between the two types of assay (see Section 3). Since we did not use ELISA to measure ACTH, no comparisons were necessary.

2.8. Data analysis

Consumption and endocrine data were analyzed by two-way analysis of variance (ANOVA). Where permissible through a priori justification, unpaired *t* tests were also conducted. Differences were deemed significant at $p < .05$.

3. Results

3.1. Ingestive behavior

Fig. 1A presents the appetitive effects of CRH infusion, with or without α-helical $CRH_{9–41}$ (αhCRH), a non-selective CRH receptor antagonist. A two-way ANOVA (antagonist × peptide) showed a significant main effect of CRH infusion ($F_{(1,20)} = 10.239$, $p < .05$), due to the lower consumption of Prosobee intake in the CRH/CSF group relative to saline-injected controls. Furthermore, there was a significant interaction between antagonist and peptide ($F_{(1,20)} = 11.210$, $p < .05$). This was due to the CRH/αCRH group displaying consumption equivalent to both saline groups, but greater than the CRH/CSF group (see Fig. 1A). These data, therefore, showed that the dose of αhCRH was sufficient to block the hypophagic effects of CRH, and was subsequently used to test the effect of challenge with SEA on reduction of food intake. This second experiment is shown in Fig. 1B. Analysis revealed significant main effects of SEA ($F_{(1,20)} = 30.549$, $p < .001$) and αhCRH treatment ($F_{(1,20)} = 4.718$, $p < .05$). In addition, there

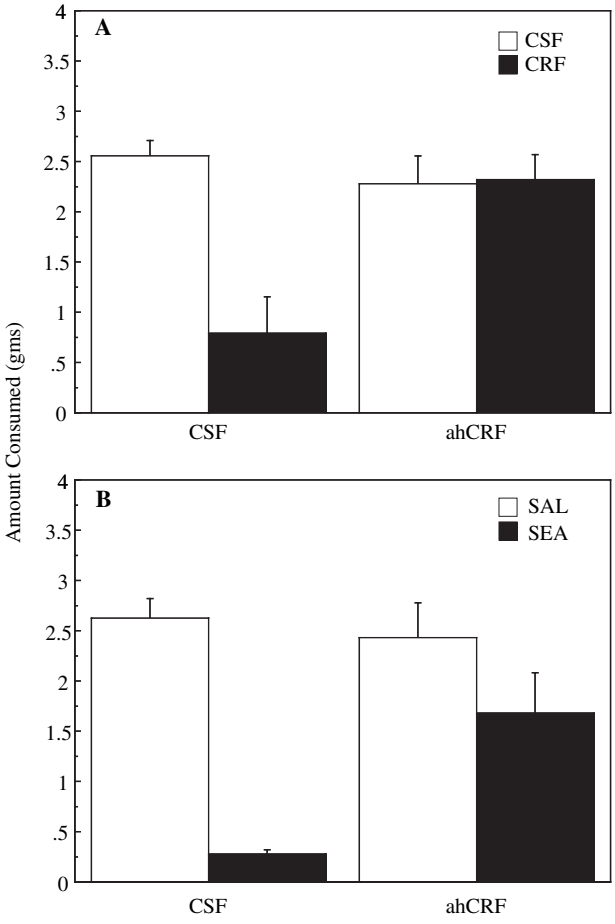

Fig. 1. Effect of icv infusion with CSF or 20 μg α-helical $CRH_{9–41}$ (αhCRH) on consumption of a novel liquid diet after icv infusion of 100 ng CRH (A) or intraperitoneal injection of 5 μg SEA (B). (A) and (B) represent separate experiments involving naïve animals, with each bar representing the mean ± SE of $n = 6$ animals.

was a significant interaction between SEA and αhCRH treatment ($F_{(1,20)} = 8.1$, $p < .01$). The latter was due to higher consumption by the SEA/αhCRH group, relative to the SEA/CSF group (see Fig. 1B).

Food intake following CRH-R2 antagonism was tested using the selective antagonist, astressin-2B. These data are shown in Fig. 2. Initially, the efficacy of astressin-2B was confirmed in an experiment involving infusion of 100 ng UCN, which has a greater affinity than CRH for CRH-R2 (Vaughan et al., 1995). As can be seen in Fig. 2A, UCN combined with CSF infusion significantly reduced food intake, which was reflected by a significant main effect of UCN treatment ($F_{(1,20)} = 11.34$, $p < .01$). Similarly, there was a significant antagonist × UCN treatment interaction ($F_{(1,20)} = 5.34$, $p < .05$), due to the increased consumption observed in animals administered UCN and 10 μg astressin-2B (see Fig. 2A). These data confirmed the efficacy of astressin-2B in reducing the anorexic effects of UCN.

Fig. 2B presents the results of the first experiment testing whether icv administration of 10 μg astressin-2B

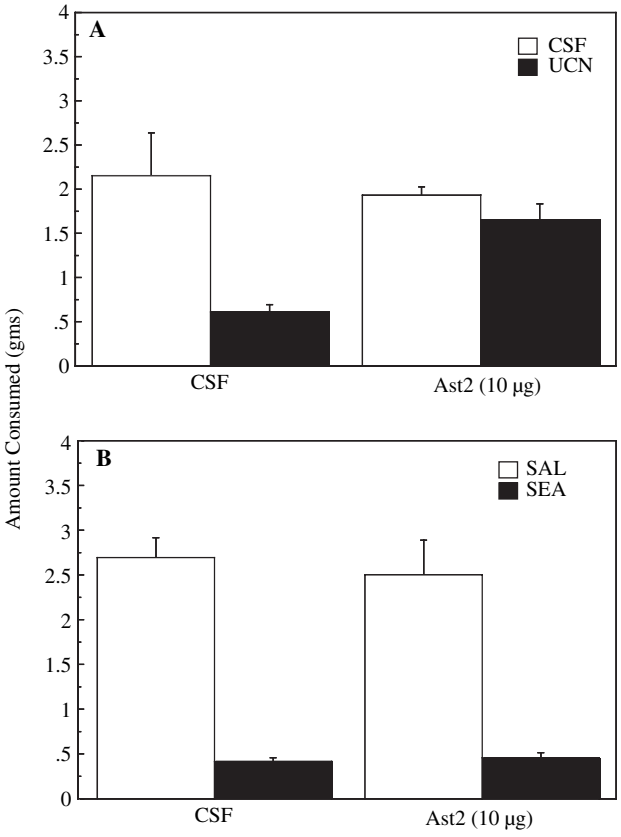

Fig. 2. Effect of icv pretreatment with CSF or 10 µg astressin-2B on consumption of a novel liquid diet after icv infusion of 100 ng urocortin (UCN) (A) or intraperitoneal injection of 5 µg SEA (B). (A) and (B) represent separate experiments involving naïve animals, with each bar representing the mean ± SE of $n = 6$ animals.

blocks the anorexic effects of SEA. These data show that in spite of a significant suppression of food intake by SEA ($F_{(1,20)} = 90.9, p < .0001$), there was no attenuation of this effect by astressin-2B. Because the immune response to SEA may have altered either the number of receptors or concentration of endogenous CRH-R2 selective ligands, a second experiment tested a higher dose of astressin-2B (25 µg icv). The results of this experiment were similar to that using 10 µg astressin-2B, in that there was no attenuating effect of the higher dose of astressin-2B on SEA-induced anorexia, although, once again there was a main effect of SEA ($F_{(1,15)} = 15.8$, $p < .01$) [grams consumed (mean ± SE): CSF/saline ($n = 5$): 2.5 ± 0.6; CSF/SEA ($n = 5$) 1.0 ± 0.3; 25 µg Ast2/saline ($n = 5$): 2.9 ± 0.5; 25 µg Ast2/SEA ($n = 4$): 0.8 ± 0.3].

3.2. Endocrine response

The effect of central αhCRH administration on the corticosterone and ACTH response to SEA was tested in two separate experiments. In the first experiment, animals were exposed to the 1 h consumption test and then sacrificed (Fig. 3A), while in the second experiment, sacrifice took place without any preceding consumption

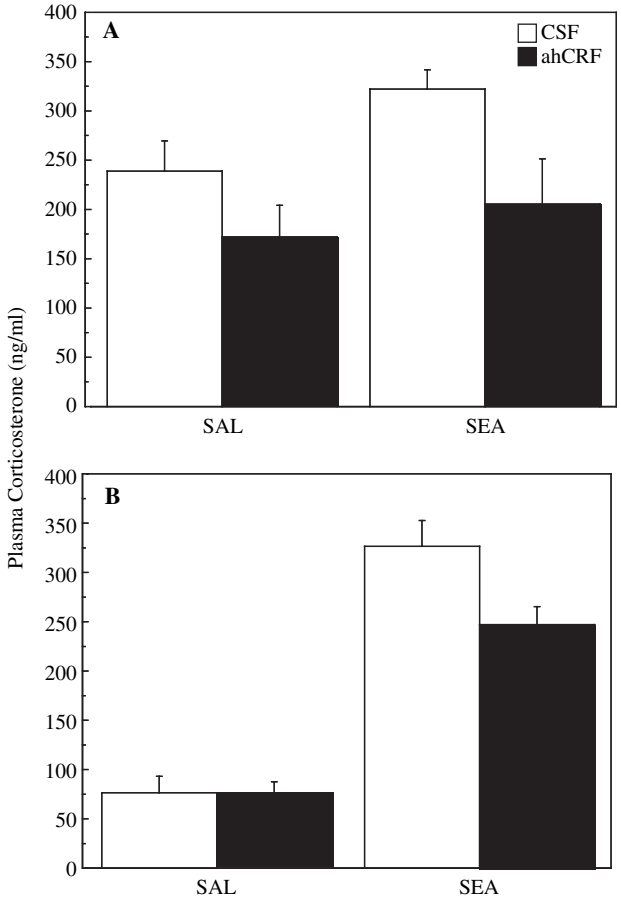

Fig. 3. Effect of icv infusion of CSF or 20 µg α-helical CRH_{9-41} (αhCRH) on plasma corticosterone levels after intraperitoneal injections of saline or 5 µg SEA. Measures were taken either at the end of a 1 h consumption test (A) or 2 h after injection without an intervening consumption test (B). Infusion of antagonist occurred 30 min prior to initiation of food exposure (A) or 60 min prior to sacrifice (B). Each bar represents the mean ± SE of $n = 6$ animals.

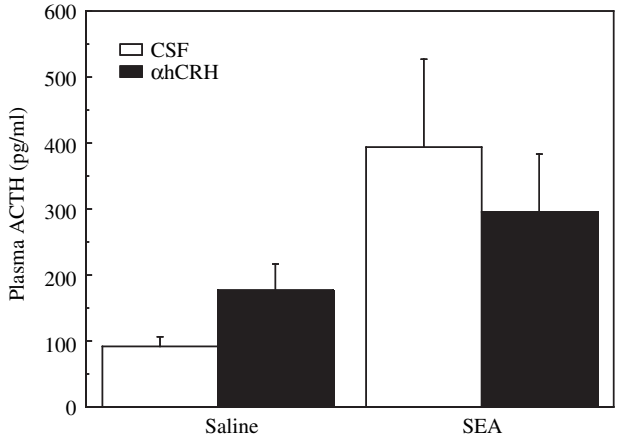

Fig. 4. Effect of icv infusion of CSF or 20 µg α-helical CRH_{9-41} (αhCRH) on plasma ACTH levels after intraperitoneal injections of saline or 5 µg SEA. Measures were taken 2 h after injection without an intervening consumption test (see also Fig. 3B). Infusion of antagonist occurred 60 min prior to sacrifice. Each bar represents the mean ± SE of $n = 6$ animals.

test (Figs. 3B and 4). For the first experiment, corticosterone was measured by ELISA, while in the second experiment, RIA was used to measure corticosterone and ACTH. Both the ELISA and RIA procedures for corticosterone were confirmed to be comparable by measurement in each assay of identical plasma from animals injected with saline ($n = 3$) or 100 ng murine IL-1β ($n = 4$) and killed 2 h later (ELISA: saline = 120.8 ± 22.0 ng/ml, IL-1β = 380.51 ± 46.0; RIA: saline = 116.87 ± 20.5 ng/ml; IL-1β = 414.63 ± 72.0). Therefore, both assays were capable of providing similar measures of corticosterone under control (viz., saline) and immunologically provoked (viz., IL-1β) conditions.

Fig. 3A shows that αhCRH-treated mice showed a lower level of corticosterone, which was confirmed by ANOVA as a significant main effect of antagonist treatment ($F_{(1,18)} = 6.788$, $p < .025$). There were no other main nor interaction effects. However, because SEA has consistently been shown to elevate plasma corticosterone (Kawashima et al., 2002), a priori justification was available for an independent comparison between the SEA and saline groups given only CSF. An unpaired t test confirmed a significant difference between these two groups ($t_{(10)} = -2.357$, $p < .05$). Therefore, given the small number of animals in each group ($n = 6$), the lack of a significant main effect of SEA treatment may have been due to a lack of statistical power.

Previous evidence had shown that consumption testing per se activates the pituitary–adrenal axis, since animals are placed in isolation and in a novel context (Kusnecov et al., 1999). Therefore, a second experiment was conducted to assess the effects of αhCRH infusion on SEA-induced plasma corticosterone and ACTH in the absence of exposure to the environmental manipulations required for implementing the consumption test. These data are presented in Figs. 3B and 4. With regard to the corticosterone data, SEA produced an elevation in corticosterone, which was confirmed by a significant main effect of toxin challenge ($F_{(1,20)} = 132.879$, $p < .05$) [see Fig. 3B]. Moreover, there was a significant interaction between toxin challenge and antagonist treatment ($F_{(1,20)} = 4.738$, $p < .05$), due to the lower corticosterone level of SEA-challenged animals administered α-helical CRH. This was supported by a significant main effect of antagonist treatment ($F_{(1,20)} = 4.792$, $p < .05$). With respect to the ACTH data, Fig. 4 shows that SEA injection elevated plasma ACTH, when compared to saline-injected control animals ($F_{(1,20)} = 6.02$, $p < .025$). However, there were no main effects of antagonist treatment, nor interaction effects with SEA.

4. Discussion

The current set of experiments tested whether the anorexic and pituitary–adrenal activating effects of the bacterial T cell superantigen, SEA (Kawashima et al., 2002; Kusnecov et al., 1999; Shurin et al., 1997), were dependent on activation of central CRH receptors. The results implicate engagement of central CRH receptors in the hypophagic effects of SEA, although additional studies are required to test whether this applies to other tests of ingestive behavior. For example, challenge with IL-1 and LPS has been shown to affect operant responding for food (De La Garza et al., 2004; Kent et al., 1996), and similar studies need to be conducted with respect to SEA.

The potential role of CRH in the behavioral effects of SEA was confirmed using αhCRH, a non-selective CRH receptor antagonist, which is well known to inhibit stress-induced anorexia and other behavioral effects (Dunn and Berridge, 1990). However, given that CRH-R2 has been suggested to mediate a pure anorexic influence independent of concomitant anxiety-related behaviors (Zorrilla et al., 2003, 2004), separate experiments administered a CRH-R2 specific antagonist, astressin-2B (Rivier et al., 2002), following SEA challenge. Interestingly, the results suggested that independent stimulation of CRH-R2 was not a significant mediating factor in the hypophagic effects of SEA, since neither 10 nor 25 μg astressin-2B affected the anorexic effects of SEA. Since, a separate experiment showed that astressin-2B blocked the hypophagic effects of the high affinity CRH-R2 agonist, UCN, it is unlikely that the drug was ineffective in blocking CRH-R2 receptors in SEA-treated mice. These results suggest that engagement of CRH-R2 receptors by endogenous ligands activated by SEA does not exert a significant anorexic influence. Similarly, a recent finding in C57BL/6 mice, showed that centrally administered astressin-2B, given at a dose of 10 μg icv, failed to block the effects of a psychogenic stressor, (viz., restraint) on gastric and colonic activity (Martinez et al., 2004). In contrast, other data using CD-1 mice showed that CRH-R2 may be involved in stress-related changes in behavior, including anorexia (Pelleymounter et al., 2000). While the present experiments suggest that CRH-R2 stimulation in response to SEA challenge may be minimally important in promoting anorexic behavior, it is possible that there may be a synergistic interaction with stimulation of CRH-R1 receptors. This was also suggested by Martinez et al. (2004) with respect to some of the gastrointestinal effects of restraint stress, although at present the precise nature of this synergism remains unknown. Still, given that SEA-induced suppression of food intake persisted in the presence of selective CRH-R2 antagonism, argues strongly for the possibility that CRH-R1 receptors mediate the observed anorexia in the present studies. However, full confirmation of whether this is the case, and that CRH-R2 receptors are not involved in SEA-induced anorexia, must await further testing with selective CRH-R1 antagonists.

The present data appear at odds with previous evidence that similar to wildtype controls, CRH deficient mice maintain suppressed food intake in response to either IL-1 or LPS injections (Swiergiel et al., 1999). However, in rats injected with IL-1 or LPS, central CRH antagonism attenuated the observed anorexic effects (Uehara et al., 1989). It is possible that CRH deficient mice develop compensatory mechanisms which allow for alternative anorexic peptides, such as the variants of UCN, to become prominent mediators of anorexia following immunologic challenge. Given that UCN can activate both CRH-R1 and CRH-R2, this is a plausible hypothesis. Finally, it is also important to note that the mechanisms of CNS activation by SEA may differ significantly from those observed in response to LPS and IL-1, and hence, this may result in differences in recruitment of CRH-related neuropeptides.

The ability of CRH to suppress food intake can involve both CRH receptors, although CRH-R1 may be prominent during conditions of stress (Zorrilla et al., 2003). If challenge with SEA induces stressor-like effects in the brain (Anisman et al., 1993; Dunn, 1993), then it can be hypothesized that suppression of food intake is a component of the general anxiety-like pattern of behaviors mediated by CRH. To further examine this possibility, future studies should test the effects of a selective CRH-R1 antagonist administered after SEA challenge. Interestingly, although SEA challenged mice exposed to the elevated plus maze, did not show overt anxiety-like patterns of behavior (Rossi-George et al., 2004), locomotor activity and number of contacts with a novel object were reduced by SEA challenge (Kawashima et al., 2002). This suggests that SEA may induce increased anxiety and/or arousal, and based on the present results, activation of central CRH may be important in mediating this effect.

Intravenous and intracerebroventricular delivery of CRH increases plasma ACTH concentrations (Donald et al., 1983), and pituitary ACTH output induced by stressors is likely to be due to CRH derived from neurosecretory cells innervating the hypophyseal portal venous system in the median eminence. However, neuronal CRH released at sites remote from the median eminence may also contribute to stressor-induced pituitary–adrenal activation (Jezova et al., 1999). This might result from synaptic input to the PVN by CRH-secreting cells, which upon releasing CRH can induce an increase in CRH and CRH-R1 mRNA (Mansi et al., 1996). In the present study, there was little evidence that the increase in plasma corticosterone and ACTH in response to SEA challenge was heavily dependent on stimulation of central CRH receptors. Nonetheless, it was notable that central αhCRH administration produced a significant, if modest, reduction in the plasma corticosterone response to SEA. Interestingly, the plasma ACTH concentration was unaffected by αhCRH treatment, suggesting that the reduction in plasma corticosterone was not due to diminished pituitary ACTH release.

These data are similar to those of Jezova et al. (1999) who found that elevated plasma epinephrine and corticosterone (but not ACTH) following immobilization could be significantly attenuated by icv administration of a non-specific CRH receptor antagonist. This suggests the potential hypothesis that a small portion of the effect of SEA on plasma corticosterone may derive from CRH-responsive central autonomic pathways (e.g., the locus coeruleus) modulating neural input to the adrenal medulla, which in turn may influence adrenocorticoid activity. However, further research is required to confirm this. In addition, the role of CRH released into the hypophyseal portal system needs to be tested, since increased ACTH release induced by LPS and the bacterial superantigen, SEB, was attenuated by intravenous CRH receptor antagonists or anti-CRH antibodies (Kusnecov et al., 1999; Rivier et al., 2003). Similar confirmation is required for SEA, although the present results involving central CRH receptor antagonism reveal that significant attenuation of the hypophagic effect of SEA is not associated with a loss of the pituitary–adrenal response.

In conclusion, the present results support the view that immunogenic substances that induce proinflammatory cytokines can recruit the central CRH system. Anorexic or hypophagic effects are among the most overt behavioral changes induced by immunogenic challenge, and it is notable that this is also a prominent characteristic of central CRH infusion (Dunn and Berridge, 1990). However, what is not clear is the degree to which immunologically induced suppression of food intake is a function of pure anorexia (i.e., appetite suppression) independent of changes in arousal. In a recent review of the literature, it was suggested that in light of the extensive cortical distribution of CRH-R1 receptors in the brain, CRH mediated effects via this receptor may contribute to arousal (Steckler and Holsboer, 1999). Therefore, it is possible that SEA activation of central CRH neurons contributes to a CRH-R1 mediated augmentation of arousal that is sufficient to suppress intake of a novel food substance. Whether this is the case, needs to be tested using a selective CRH-R1 antagonist. Nonetheless, SEA appears to be more effective in altering behavior under conditions of contextual novelty (Kawashima et al., 2002). This suggests that changes in attention and orientation as a natural consequence of increased arousal are a prominent and adaptive influence of systemically derived immune signals. It is possible that the CRH system represents one key neurochemical mechanism promoting this function.

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