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Short communication

Influence of peptide CRF receptor antagonists upon the behavioural effects of human/rat CRF

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Abstract

The effects of the corticotropin-releasing factor (CRF) receptor antagonists, α-helical CRF-(9–41), [D-Phe^{12},Nle^{21,38}, Cα MeLeu^{37}]humanCRF-(12–41) (D-PheCRF-(12–41)) and astressin ([cyclo(30–33){D-Phe^{12},Nle^{21,38},Glu^{30},Lys^{33}}]humanCRF-(12–41) upon hypophagic and motor activation response to human/ratCRF (h/rCRF) were investigated. All three antagonists (100 μg intracerebroventricular (i.c.v.)) blocked the effects of h/rCRF (1 μg i.c.v.) upon food intake and body weight change in food-deprived rats. In contrast, α-helical CRF-(9–41) and astressin (both at 100 μg i.c.v., but not lower doses), but not D-PheCRF-(12–41) (up to 100 μg i.c.v.), blocked h/rCRF (0.3 μg i.c.v.)-induced motor activation in rats in a familiar environment. The ability of D-PheCRF-(12–41) to block CRF-induced hypophagia, but not motor activation, suggests a selective action of this antagonist upon the behavioural effects of centrally administered h/rCRF.

Keywords: CRF-(9–41), α-helical; [D-Phe^{12},Nle^{21,38},Cα MeLeu^{37}]humanCRF-(12–41); Astressin; Motor activation; Hypophagia; CRF (corticotropin-releasing factor)

1. Introduction

Corticotropin releasing factor (CRF), a 41-residue peptide, plays a crucial role in the integration of the body's response to stress and is the primary regulator of the hypothalamic–pituitary–adrenal axis (Chappell et al., 1986; Fisher, 1991). In addition, there is widespread extrahypothalamic distribution of CRF and its receptors, including subtypes CRF_1, CRF_2 and $CRF_{2\beta}$ (Behan et al., 1996), in the CNS consistent with actions beyond the hypothalamic–pituitary–adrenal axis (see Dunn and Berridge, 1990). Indeed, centrally administered CRF causes a wide range of behavioural effects in rats, which are independent of the hypothalamic–pituitary–adrenal axis (see Dunn and Berridge, 1990; De Souza et al., 1995; Jones et al., 1998). Further, animal and clinical studies suggest that elevated CRF may be involved in psychiatric diseases, including depression, anxiety and anorexia (Chalmers et al., 1996), leading to the suggestion that CRF receptor antagonists may be useful in the treatment of these diseases (De Souza et al., 1995).

Until recently, there have been few pharmacological tools available to investigate the biology of the CRF system. However, recent reports have described novel peptide and non-peptide CRF receptor antagonists (Menzaghi et al., 1994; Gulyas et al., 1995; see Smagin et al., 1998). The present study compared the effects of three peptide CRF receptor antagonists, α-helical CRF-(9–41) (Rivier et al., 1984), [D-Phe^{12},Nle^{21,38},Cα MeLeu^{37}]human-CRF-(12–41) (D-PheCRF-(12–41), Curtis et al., 1994) and astressin ([cyclo(30–33){D-Phe^{12},Nle^{21,38}, Glu^{30},Lys^{33}}]humanCRF-(12–41), Gulyas et al., 1995) upon the motor activating and hypophagic effects of centrally administered human/rat CRF (h/rCRF, see Dunn and Berridge, 1990; Jones et al., 1998).

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2. Materials and methods

2.1. Subjects

Sprague–Dawley rats (Charles River, 250–300 g at surgery) were implanted under anaesthesia (Domitor® (medetomidine HCl, 0.4 mg/kg s.c., Pfizer, Sandwich, UK), Sublimaze® (fentanyl, 0.45 mg/kg i.p., Janssen-Cilag, High Wycombe, UK)) with a cannula directed towards either the left or right lateral ventricle (co-ordinates: ±1.6 mm from midline, 0.8 mm caudal from bregma, −4.1 mm from skull surface, incisor bar at −3.2 mm below zero, Paxinos and Watson, 1998). Anaesthesia was reversed by Antisedan® (atipamezole HCl, 2.5 mg/kg s.c., Pfizer, Kent, UK) and post-operative analgesia provided by Nubain® (nalbuphine HCl, 2 mg/kg s.c., Du Pont Pharmaceuticals, Letchworth Garden City, UK). All rats were singly housed after surgery and for the duration of the study to avoid damage to the guide and dummy cannulae. Rats were handled frequently during the studies to prevent the development of hyperactivity and aggression. Following 7 days recovery from surgery, correct cannula placement was verified by an intense drinking response to angiotensin II (100 ng intracerebroventricular (i.c.v.) (Simpson et al., 1978). At least 7 days elapsed before further drug testing. All testing took place between 08:00 and 18:00 h during weekdays. Rats were housed under a 12-h light cycle with lights on at 07:00 h. Animals were checked daily and a formal health check was carried out by a veterinary technician each week.

In order to reduce the number of animals used, each group of rats was tested in several experiments with a minimum of 7–10 days between testing. Rats received no more than four treatments in total. All experiments were carried out in strict accordance with the United Kingdom Animals (Scientific Procedures) Act, 1986 (Home Office Project Licence PL 80/1003) and were subject to ethical review within SmithKline Beecham.

2.2. Influence upon food intake and body weight in food-deprived rats

The effects of CRF receptor antagonists upon CRF-induced anorexia were determined using the methods described by Jones et al. (1998). Briefly, food was removed 22–23 h prior to measuring food intake. All testing took place in the home cage. Rats were habituated to the food deprivation protocol on two separate occasions (3–4 days apart) at least 7 days prior to drug testing. The effects of α-helical CRF-(9–41), astressin and D-PheCRF-(12–41) (all at 100 μg i.c.v.) upon h/rCRF-induced hypophagia were determined in a single experiment. The peptide antagonists were administered 15–20 min prior to h/rCRF administration (1.0 μg i.c.v., Jones et al., 1998). A pre-weighed amount of soaked rat chow (CRMX, Special Diet Services, Witham, Essex) was placed in the cage 15 min after h/rCRF injection. Two hours later, both the rats and the remaining food were weighed.

2.3. Motor activity in a familiar environment

The motor activity chambers consisted of eight clear Perspex cages (42 × 21 × 20 cm) positioned within a frame equipped with a series of infrared beams along its length and width (AM1052 Activity Monitor, Benwick Electronics, Essex, UK). Prior to drug testing, rats were habituated to the motor activity chambers in at least two separate sessions (approximately 60 min/session).

Initial studies determined the effects of the peptide antagonists, α-helical CRF-(9–41) (10, 30 or 100 μg i.c.v.), astressin (3, 10 or 30 μg i.c.v.) and D-PheCRF-(12–41) (1, 3, 10, 30 or 100 μg i.c.v.) against h/rCRF-induced hyperactivity separately. To allow a direct comparison and to confirm the findings of the previous experiments, the effects of all three peptide antagonists (all at 100 μg i.c.v.) were also determined in a single experiment. In each case, the peptide antagonists were administered 15–20 min prior to h/rCRF administration (0.3 μg, Jones et al., 1998). Motor activity was measured for 70 min following h/rCRF administration. Additionally, α-helical CRF-(9–41) (100 μg i.c.v., 15 min prior to saline i.c.v.) was tested upon baseline activity following the suggestion that α-helical CRF-(9–41) may act as a partial agonist in vivo (Menzaghi et al., 1994).

2.4. Drugs

Peptides or vehicle were injected i.c.v. in a volume of 5 μl over 60 s. The injection needle (extending 1 mm from the end of the guide cannula) was left in place for a further 90 s to allow diffusion of the drug. Peptides were obtained from Bachem UK (Saffron Walden, Essex, UK), except astressin, which was kindly supplied by Prof. J. Rivier, The Salk Institute. Initial experiments with D-PheCRF-(12–41) used a sample kindly supplied by Prof. Rivier (batch 1) and was dissolved in sterile saline. In all other experiments, D-PheCRF-(12–41) was obtained from Bachem UK (batch 2) and was dissolved in sterile water following advice from Prof. Rivier (minimum quantity of acetic acid, approximately 1 μl per 250/300 μl). α-Helical CRF-(9–41) was dissolved in sterile water (with minimum NaOH (500 nM), approximately 1 μl per 175/200 μl) for all experiments except for the dose–response curve for motor activity (sterile saline). Astressin was dissolved in sterile water (with minimum acetic acid). h/rCRF was dissolved in sterile saline (with minimum NaOH (500 nM)). In all cases, pH was approximately 6.5–7 measured

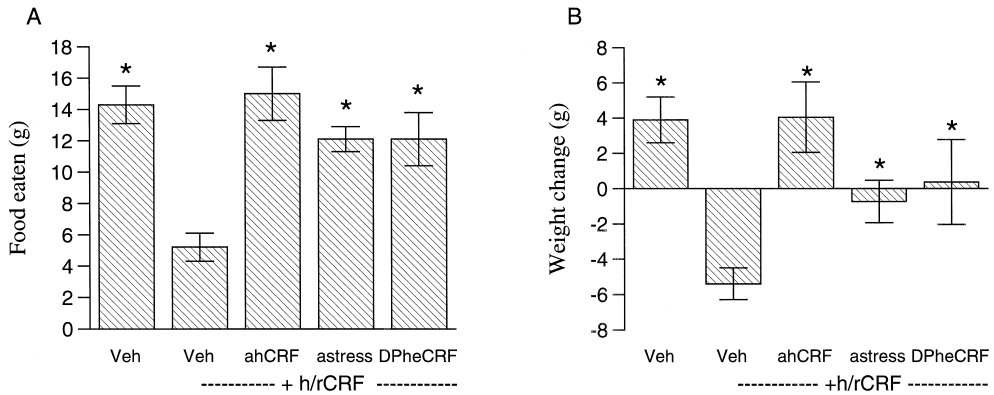

Fig. 1. The effect of α-helical CRF-(9–41) (ahCRF), astressin (astress) and D-PheCRF-(12–41) (all at 100 μg i.c.v.) upon h/rCRF-induced (A) reduction in food intake, and (B) body weight gain (g) in 23 h food-deprived rats. Data are shown as mean (±SEM) food eaten (g) or body weight change (g) following a 2-h access period following h/rCRF (1 μg i.c.v.). Peptide antagonists or vehicle (sterile water) were administered 15–20 min before h/rCRF treatment. Significant differences from h/rCRF-treated rats (Veh/h/rCRF) are shown by $^{*}P < 0.05$, $n = 10–12$.

using pH strips. Aliquots of peptides sufficient for each experiment were frozen on cardice (stored at −20°C) and thawed immediately prior to use to avoid multiple freeze/thawing.

2.5. Data analysis

Data for motor activity and food intake (g) were analysed (SAS-RA©, SAS Institute) by one-way ANOVA followed by Dunnett's *t*-test. The data were $\log_{10}$ transformed to correct for heterogeneity of variance when appropriate. Data for body weight change were analysed by Kruskal–Wallis test for overall significance followed by Mann–Whitney *U*-tests for between-group comparisons (SAS-RA©, SAS Institute).

3. Results

3.1. Influence upon food intake and body weight in food-deprived rats

h/rCRF (1 μg) significantly reduced food intake compared with Veh/Veh treated rats which was reversed by pretreatment with all of the peptide antagonists ($F_{4,48} = 8.49$, $P < 0.0001$, $n = 10–12$, Fig. 1a). h/rCRF also re-

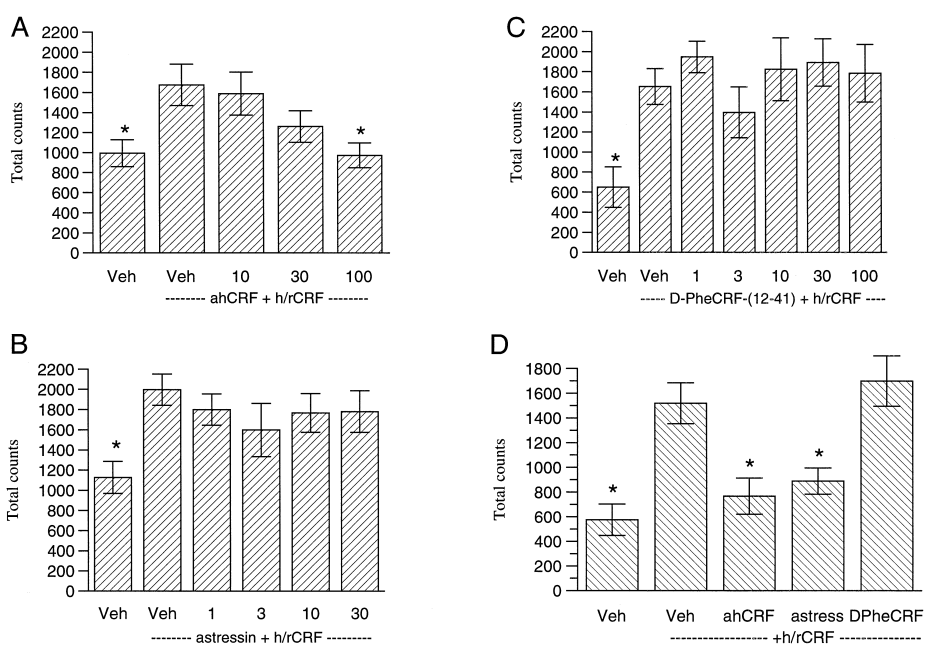

Fig. 2. The effect of (A) α-helical CRF-(9–41) (ahCRF; 10–100 μg i.c.v.); (B) astressin (astress, 1–30 μg i.c.v.) and (C) D-PheCRF-(12–41) (1–100 μg i.c.v.) and (D) all three CRF receptor antagonists (100 μg i.c.v.) upon h/rCRF-induced motor activity (total beam breaks). Activity was measured for a 70-min period following h/rCRF (0.3 μg i.c.v.). Peptide antagonists or vehicle were administered 15–20 min before h/rCRF treatment. Significant difference from h/rCRF-treated rats (Veh/h/rCRF) is shown by $^{*}P < 0.05$.

duced body weight gain compared with Veh/Veh treated rats ($P < 0.001$) which was reversed by all of the antagonists ($P < 0.05$, Fig. 1b).

3.2. Motor activity in a familiar environment

Pretreatment with α-helical CRF-(9–41) dose-dependently antagonised h/rCRF-(0.3 μg) induced motor activation, which reached significance at the top dose (100 μg) ($F_{4,59} = 2.66$, $P < 0.05$, $n = 10–14$, Fig. 2a). α-helical CRF-(9–41) had no effect upon baseline activity (data not shown). In contrast, pretreatment with astressin (1–30 μg, $F_{5,41} = 3.09$, $P < 0.05$, $n = 6–9$, Fig. 2b), D-PheCRF-(12–41) (either 3–30 μg, $F_{4,34} = 5.99$, $P < 0.001$, $n = 6–11$, batch 1, data not shown) or 1–100 μg, $F_{6,47} = 7.32$, $P < 0.00001$, $n = 6–9$, batch 2, Fig. 2c) failed to influence h/rCRF-induced motor activity. In a final experiment, animals received either vehicle (water), α-helical CRF-(9–41), D-PheCRF-(12–41) (batch 2) or astressin (all at 100 μg). α-helical CRF-(9–41) and astressin, but not D-PheCRF-(12–41), significantly attenuated h/rCRF-induced motor activation ($F_{4,49} = 10.6$, $P < 0.0001$, Fig. 2d).

4. Discussion

h/rCRF reduced food consumption in food-deprived rats and increased motor activity as previously described (Dunn and Berridge, 1990; Jones et al., 1998). However, while all three CRF receptor antagonists were able to block the feeding effects of h/rCRF, α-helical CRF-(9–41) and astressin, but not D-PheCRF-(12–41), blocked the motor activating effects of h/rCRF.

In the present study, astressin blocked both the hypophagic and motor activating effects of h/rCRF, extending the literature on this CRF receptor antagonist. A relatively high dose was required to block the motor activating effects of h/rCRF compared with previous studies (Gulyas et al., 1995; Martinez et al., 1997). Gulyas et al. (1995) reported a higher potency of astressin to block adrenocorticotrophin-releasing hormone (ACTH) release in vitro and in vivo compared with both α-helical CRF-(9–41) and D-PheCRF-(12–41). However, this is the first report of a direct comparison of these antagonists following i.c.v. administration and the first describing astressin blockade of CRF-induced activation.

In contrast to the motor activating effects of h/rCRF, all three peptide antagonists blocked the effects of a larger dose of h/rCRF (1 μg) upon both food intake and change in body weight. This suggests a clear difference in the mechanism of the effects of h/rCRF upon motor activity and food intake. Feeding has been more closely linked with the CRF_2 receptor subtype (Smagin et al., 1998). However, the relative selectivity differences between the peptide antagonists for CRF_1 over CRF_2 receptors are unlikely to explain their different actions upon CRF-induced hypophagia and activation (Gulyas et al., 1995). Further, for peptide CRF receptor antagonists, direct comparisons of efficacy are complicated by differences in affinity for CRF receptor subtypes (e.g., CRF_1 receptor, astressin > D-PheCRF-(12–41) > α-helical CRF-(9–41)), the CRF binding protein (α-helical CRF-(9-41) > D-PheCRF-(12–41) ≫ astressin) (Gulyas et al., 1995) and likely differences in relative in vivo stability/distribution.

Under the present test conditions, α-helical CRF-(9–41) and astressin, plus several non-peptide antagonists (Jones et al., 1996) were able to block h/rCRF-induced motor activity. The effects of α-helical CRF-(9–41) upon h/rCRF-induced motor activation is in agreement with previous findings (Britton et al., 1986; Dunn and Berridge, 1990). Further, α-helical CRF-(9–41) failed to influence baseline activity. D-PheCRF-(12–41) was inactive under the same test conditions. This is unlikely to reflect peptide degradation as D-PheCRF-(12–41) was able to block h/rCRF-induced hypophagia and the integrity of D-PheCRF-(12–41) was verified at the end of the experiment using analytical techniques (mass measured by matrix-assisted laser desorption ionisation and time-of-flight (MALDI–TOF) and mass spectrometry and amino acid sequencing). Our findings with D-PheCRF-(12–41) contrast with those of Menzaghi et al. (1994) who reported potent inhibition of h/rCRF-induced motor activity by both α-helical CRF-(9–41) and D-PheCRF-(12–41). The reason for this discrepancy is unclear.

In conclusion, the motor activating effects of h/rCRF in rats was sensitive to α-helical CRF-(9–41) and astressin, but not D-PheCRF-(12–41). In contrast, the effects of h/rCRF upon feeding was blocked by all three peptide antagonists, suggesting a selective action of D-PheCRF-(12–41).

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