Effects of corticotropin-releasing factor on feeding and pancreatic polypeptide response in the dog

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Abstract

We have previously reported that corticotropin-releasing factor (CRF) is a potent stimulator of adrenocorticotrophic hormone and cortisol secretion in the dog. Therefore, in the present study, we investigated the extrahypophysiotropic actions of CRF in this species. When CRF was injected into the third cerebral ventricle, it failed to inhibit food intake significantly at doses of 1·19, 3·57 and 11·9 nmol. This is in sharp contrast with the results in

rodents. At the 3·57 and 11·9 nmol doses, CRF markedly stimulated the secretion of pancreatic polypeptide (PP), a hormone under vagal control, and at the highest dose CRF increased plasma glucose levels. These results suggest species differences in the feeding response to CRF and activation of the parasympathetic nervous system in the dog.

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Introduction

Stress is well known to be accompanied by various physiological changes including activation of the pituitary–adrenal axis. This activation is dependent upon hypothalamic releasing hormones such as corticotropin-releasing factor (CRF) which regulate the secretion of adrenocorticotrophic hormone (ACTH) from the pituitary. CRF is a 41-residue peptide characterized from ovine and rat hypothalami by Vale *et al.* (1981) and Spiess *et al.* (1983). CRF immunoreactivity (Swanson *et al.* 1983) and mRNA (Matthews *et al.* 1991) have been localized in the central nervous system in both the hypothalamus and extra-hypothalamic structures. The CRF-containing cell bodies in the medial parvocellular division of the paraventricular nucleus, the fibers of which abundantly project into the neurohemal zone of the median eminence, are dominantly involved in the control of the pituitary–adrenal axis, which represents the primary role of CRF (Antoni 1986, Harbuz & Lightman 1992). Within and outside the paraventricular nucleus, there are also CRF-containing perikarya, not involved in the pituitary function, which project into neuronal structures that modulate behavioral and autonomic functions (Swanson *et al.* 1983, Matthews *et al.* 1991, Petrusz & Merchenthaler 1992).

In recent years, the effects of CRF on behavioral and autonomic functions have aroused as much interest as the role CRF plays in the secretion of ACTH and other pro-

opiomelanocortin products (Gray 1993, Menzaghi *et al.* 1993, Richard 1993, Tache *et al.* 1993, Vamvakopoulos & Chrousos 1994). However, there are few data on the extrahypophysiotropic actions of CRF in species other than rodents. We and others have previously reported that CRF is a powerful stimulator of ACTH and cortisol secretion in the dog (Inoue *et al.* 1989, Reul *et al.* 1991). Therefore, in the present study, we examined the effects of CRF on food intake and on plasma pancreatic polypeptide (PP) secretion, an index of the activity of the parasympathetic nervous system (Schwartz 1983, Taylor 1989).

Materials and Methods

Animals

Normal adult mongrel dogs ($n=7$), weighing approximately 12 kg, were used in all the studies, which were carried out at least 3 days apart. They were housed in individual cages and kept under standard laboratory conditions (12 h light/12 h darkness cycle; $22 \pm 2^\circ\text{C}$; 200 g dog food (Nissin Pet Food Co., Tokyo, Japan) daily), with tap water available *ad libitum*. After an overnight fast, animals were placed in jackets and allowed to remain in a fully conscious relaxed state during the experiments. All experiments were approved by our university animal care committee.

Surgical procedure

Under sodium thiamylal anesthesia, 20-gauge stainless steel cannulas were permanently implanted toward, but not entering, the third cerebral ventricle of dogs, using dental cement. The methods used have been described previously (Inui *et al.* 1987, 1988, 1989, 1991). Experiments were conducted 2 weeks after implantation of the cannula in the brain ventricle.

On the day of the experiment, an i.v. butterfly needle(s) was placed in the superficial vein of the forelimb for blood sampling. The i.c.v. injection insert (a 24-gauge stainless steel tube) was attached by tubing to a syringe, placed in the cannula, and adjusted so that the tip was in the ventricle. The patency of the ventricular cannula was usually ascertained by observing the reflux of the cerebrospinal fluid. If we could not verify that peptides were injected into the ventricle, we omitted the data from the statistical analysis. The experimental design was a randomized block, and the saline test was run for each of the experiments. Ovine CRF (Peptide Institute, Osaka, Japan) was dissolved in saline vehicle and 100 µl of the solution was injected into the third ventricle (i.c.v.) over 5 min. Experiments were performed under 16 h fasting conditions.

Experimental design

Plasma glucose and PP secretion Seven dogs received i.c.v. injection of CRF (1·19, 3·57 and 11·9 nmol) or saline vehicle as a control. Blood samples were obtained during the basal period and between 0 and 120 min after the injection of CRF. Results were expressed as the mean $\pm$ S.E.M. The incremental integrated responses of PP and glucose were calculated in individual subjects, utilizing the trapezoidal method. The incremental integrated response is the incremental area under the curve after basal subtraction.

Food and water intakes Food was removed 16 h before the experiment (1700–0900 h), while water remained available *ad libitum*. CRF (1·19, 3·57 and 11·9 nmol) or saline vehicle was injected i.c.v. into the seven dogs. The dogs were then given a premeasured quantity of a test meal, a highly palatable dog food (Delicious Cut: Sunrise Co., Osaka, Japan: protein 15%, fat 23%, carbohydrate 62%) and water. Food and water intakes were recorded at 10 min intervals for 2 h after the injection. Results were expressed as g of food and ml of water ingested after i.c.v. CRF or saline administration, and cumulated at 10, 30, 60 and 120 min.

Pretreatment with atropine Previously we found that a 20 µg i.c.v. dose of atropine markedly inhibited PP response to i.c.v. cholecystokinin octapeptide (CCK-8) without producing gross behavioral changes or

inhibition of food intake (Inui *et al.* 1988). Therefore we administered atropine (20 µg/50 µl saline per 2·5 min) or saline vehicle into the third ventricle of seven dogs just before i.c.v. CRF (3·57 nmol/50 µl saline per 2·5 min) and examined its effect on CRF-induced inhibition of food intake and release of PP. To rule out possible leakage of atropine into the peripheral circulation, we measured plasma atropine concentrations before, 60 and 120 min after 100 µg atropine injection into the third ventricle. The sampling points were chosen based on the data that i.c.v. administered substances such as proglumide and PP do not appear acutely in the dog (Inui *et al.* 1987) and that the half-life of atropine is 4·125 h in the peripheral circulation (Adams *et al.* 1982).

Sample determinations Blood samples were collected in chilled tubes containing 400 kallikrein inhibitor units of aprotinin (Trasylo; Bayer AG, Leverkusen, Germany) and 1·2 mg EDTA/ml blood and centrifuged. The plasma was then frozen at -20°C until assayed. PP was measured by RIA, using a modification of the double-antibody technique (Inui *et al.* 1985, 1993), in which first the antibody was incubated with the sample for 48 h before the addition of iodinated PP to increase the sensitivity of the assay. Plasma glucose was determined by the glucose oxidase method. Plasma atropine concentrations were measured by HPLC after ether extraction with the minimum detection limit of 1 ng/ml (coefficient of variation 8·0%).

Statistical methods

Data were evaluated by ANOVA, followed by Dunnett's test. A level of $P < 0\cdot05$ was taken as statistically significant.

Results

Effects of i.c.v. CRF on food intake

Previously we found that i.c.v. CRF at a dose of 1·19 nmol powerfully stimulated ACTH and cortisol secretion in the dog, the latter being nearly equivalent to that produced by insulin-induced hypoglycemia (Inoue *et al.* 1989). At this i.c.v. dose, CRF failed to inhibit food intake significantly (Fig. 1). The actual (cumulative) amount of food eaten at 10, 30, 60 and 120 min was $318\cdot1 \pm 51\cdot8$, $328\cdot0 \pm 46\cdot3$, $335\cdot6 \pm 31\cdot1$ and $380\cdot0 \pm 81\cdot0$ g after control saline injection, $291\cdot4 \pm 51\cdot0$, $304\cdot0 \pm 49\cdot5$, $387\cdot1 \pm 86\cdot0$ and $431\cdot9 \pm 85\cdot4$ g after 1·19 nmol CRF, $280\cdot9 \pm 71\cdot5$, $280\cdot9 \pm 71\cdot5$, $318\cdot6 \pm 77\cdot0$ and $360\cdot6 \pm 89\cdot2$ g after 3·57 nmol CRF, and $249\cdot0 \pm 37\cdot7$, $269\cdot0 \pm 33\cdot1$, $310\cdot3 \pm 38\cdot2$ and $426\cdot5 \pm 50\cdot1$ g after 11·9 nmol CRF injection. Thus dogs had almost finished eating within the first 10 min as reported previously (Inui *et al.* 1987, 1988, 1989, Sakatani *et al.* 1987), and the highest dose of CRF decreased food intake to 80% of control saline injection

Figure 1 Effect of CRF injected into the third cerebral ventricle of seven dogs on food intake and water consumption. Results are expressed as amount of food or water ingested after i.c.v. injections.

although it did not reach statistical significance. This is quite different from the results of other feeding-inhibitory peptides such as CCK, which powerfully inhibited feeding in dogs (Inui *et al.* 1987, 1988, 1989, Sakatani *et al.* 1987). The cumulative food intake after injection of 1.19 nmol CCK-8 was 149.1 ± 39.4 , 194.9 ± 47.6 , 239.1 ± 46.9 and 297.7 ± 62.6 g at 10, 30, 60 and 120 min respectively in this study. CRF did not decrease water consumption (Fig. 1). We found no gross behavioral changes such as vomiting when CRF was injected into the ventricle.

Effects of i.c.v. CRF on autonomic responses

CRF at a dose of 1.19 nmol failed to alter plasma glucose and PP responses significantly (Figs 2 and 3). However, at the dose of 3.57 nmol, CRF significantly stimulated PP secretion, and at the highest dose (11.9 nmol) it markedly increased plasma PP concentrations over the entire 2 h observation period. Plasma glucose was increased only at the highest dose of CRF.

Figure 2 Effect of CRF injected into the third cerebral ventricle of seven dogs on plasma glucose and PP levels. * $P < 0.05$ or less vs basal.

Effects of i.c.v. pretreatment with atropine

Atropine administered i.c.v. at a dose (20 μ g) that did not affect food intake (Inui *et al.* 1988) significantly reduced the PP response to the peptide (integrated PP response: 9.6 ± 6.4 vs 22.2 ± 6.8 ng/min per ml (control); $P < 0.05$). The plasma atropine concentrations remained below the detection limit (1 ng/ml) after injection of 100 μ g atropine, a dose that was five times higher than that used in the study, into the third ventricle ($n=5$).

Discussion

Major advances in the study of CRF on stress responses have been made through almost exclusive use of laboratory rodents. Little information is available for other mammalian species, although Brown and associates performed a variety of studies in dogs examining the actions of i.c.v. CRF on plasma levels of catecholamines and glucose as well as on cardiovascular and hemodynamic function (Brown & Fisher 1983, Lenz *et al.* 1986). In the present study, we examined the effects of CRF on behavioral and

Figure 3 Effect of CRF injected into the third cerebral ventricle of seven dogs on integrated plasma glucose (IGR₁₂₀) and PP (IPPR₁₂₀) responses.

autonomic functions in the dog. We found that CRF injected into the third cerebral ventricle failed to inhibit food intake significantly up to a dose (11.9 nmol) at least ten times higher than that stimulating cortisol secretion (Inoue *et al.* 1989). Thus the inhibitory effect of CRF on food intake was much less potent than other feeding-inhibitory peptides, such as CCK, which powerfully inhibited the initiation of feeding under the same fasting conditions (Inui *et al.* 1987, 1988, 1989). Since CRF is a

major feeding-inhibitory peptide in the hypothalamus of rodents (Britton *et al.* 1982, Morley & Levine 1982, Morley 1987, Richard 1993), the present findings indicate species differences in the feeding response to exogenous CRF.

Although we used ovine CRF instead of canine CRF which is not yet characterized, the possible species difference in CRF structure rather than CRF action is a less likely explanation in view of the potent actions of ovine CRF on various physiological functions of dogs (Brown & Fisher 1983, Lenz *et al.* 1986, Inoue *et al.* 1989, Reul *et al.* 1991, the present study). Urocortin, a CRF-related peptide, was recently discovered in mammalian brain and found to have potent appetite-suppressing effects (Vaughan *et al.* 1995, Spina *et al.* 1996). It is thus to be determined whether urocortin is an endogenous CRF-like peptide in this species that is responsible for the inhibitory effects on appetite, as was demonstrated for rodents (Spina *et al.* 1996).

We also found in this study that CRF potently stimulated the secretion of PP, a hormone under vagal control (Schwartz 1983, Taylor 1989), in a dose-dependent manner in the dog. This action of CRF was observed at lower doses than those that caused an increase in plasma glucose. This immediate elevation of plasma glucose levels suggests that there is early activation of the sympathetic nervous system leading to glucagon secretion and/or glycogenolysis (but not inhibition of insulin secretion (M Okita, A Inui, T Inoue, H Mizuuchi, K Banno, S Baba & M Kasuga, unpublished observations)) and therefore resulting in elevated glucose levels. At the highest dose used, CRF increased plasma PP concentrations to almost the levels observed after two potent stimuli of PP secretion, i.e. ingestion of a meal and insulin-induced hypoglycemia (Inui *et al.* 1993). It was demonstrated that PP, released into the circulation, traverses the blood/brain barrier through leaky regions of this barrier in the area postrema, binds to the specific receptors, and directly regulates vagal input to the stomach and other gastrointestinal organs (Whitcomb *et al.* 1990, McTigue *et al.* 1993). The resulting enhancement of gastric motility and acid secretion (McTigue *et al.* 1993) may contribute in part to the accelerated gastric emptying and acid secretion observed during hypoglycemia, because CRF is well known to be activated by hypoglycemic stress (Petrusz & Merchenthaler 1992, Gray 1993, Menzaghi 1993).

Recent studies suggest that neuropeptides such as thyrotropin-releasing hormone specifically activate vagal efferent neurons in the dorsal motor nucleus (McTigue & Rogers 1992). Thyrotropin-releasing hormone administered to the ventricle also stimulates PP secretion in the dog (M Okita, A Inui, T Inoue, H Mizuuchi, K Banno, S Baba & M Kasuga, unpublished observations). Although the site of action of CRF in the brain is not apparent at this stage, CRF-induced PP secretion was suppressed by i.c.v. pretreatment with atropine which acted without

appearing in the peripheral circulation. Therefore CRF would affect the muscarinic cholinergic system near the ventricular structure to release PP (Ban 1966), as demonstrated for CCK in this species (Inui *et al.* 1988). The dose of atropine used in this study is well within the range (1–100 µg) in which atropine administered locally to the brain or i.c.v. affects behavioral functions such as appetite (Ikemoto & Panksepp 1996), locomotion (Brudzynski *et al.* 1991) and grooming (Torre & Celis 1989) in rats, and aggression (Dawra *et al.* 1988) in cats. These results suggest physiological relevance of the dose of atropine and central cholinergic systems in PP secretion. However, the efferent mechanism of CRF-induced PP release is not yet established. The latency between CRF injection and statistically significant changes in plasma PP levels may suggest that it is a consequence of peripheral changes secondarily produced by central CRF injection. Although this possibility could not be ruled out, peripherally administered CRF was also reported to produce slow PP response in contrast with immediate stimulation of cortisol secretion (Lytras *et al.* 1984). Therefore a more likely explanation may be that activation of sympathetic outflow results in alleviation of PP secretion simultaneously evoked by vagal stimulation.

In conclusion, we found that CRF is a weak inhibitor of feeding but a potent stimulator of PP secretion in the dog. This suggests species differences in the feeding response to CRF, as demonstrated for other peptides such as CCK (Della-Fera & Baile 1979, Morley *et al.* 1985, Inui *et al.* 1993) and neuropeptide Y (Inui *et al.* 1991, Sipols *et al.* 1996), and activation of the parasympathetic nervous system in this species.

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