Psychopharmacology (1992) 109:177–184

Psychopharmacology

Corticotropin-releasing factor modulates dietary preference in nutritionally and physically stressed rats

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Received August 26, 1991 / Final version February 18, 1992

Abstract. In order to evaluate the action of central nervous system Corticotropin-Releasing Factor (CRF) in the control of feeding behavior the present studies employed a dietary self-selection task sensitive both to overall appetite as well as preferential intake of familiar versus unfamiliar foods. Prior to the diet selection test, one group of nutritionally stressed animals was fed a protein deficient diet in order to increase the preference for unfamiliar foods relative to nutritionally replete subjects. Both CRF (0.05 and 0.5 μg ICV) and physical restraint (30 min) attenuated selectively the consumption of a novel food choice by deficient animals without affecting concurrent intake of familiar food. Further, CRF administration did not alter water intake or consumption of either diet by the replete control group suggesting that the peptide produced a stress dependent, enhanced response to novelty without a general effect on appetite. The CRF antagonist, α-helical CRF_{9-41} (1, 5 and 25 μg ICV), increased familiar diet consumption in nutritionally deficient subjects without affecting the self-selection pattern or replete controls. Chlordiazepoxide (5 mg/kg) also increased selectively the intake of familiar food suggesting that this action is the anxiolytic complement of the effect of stress in this paradigm. The CRF antagonist (5 and 25 μg) reversed the anorexia produced by CRF (0.5 μg) as well as that induced by restraint stress. These results favor a direct role for endogenous CRF systems in coordinating the behavioral responses to dietary stress.

Key words: Dietary self-selection – Corticotropin-releasing factor – α-Helical CRF_{9-41} – Chlordiazepoxide – Neophilia – Protein – Rat – Restraint stress

The consumption of novel foods in a dietary-preference test has been proposed as an animal model of anxiety since benzodiazepine anxiolytics reduce the latency to eat and increase the total consumption of unfamiliar foods in animals that normally prefer familiar foods (Cooper 1980; Shephard and Broadhurst 1982). This increased appetite for novelty, or neophilia, also arises from dietary stress induced in self-selecting rats by feeding subjects a nutritionally inadequate diet (Deutsch et al. 1989). By contrast, foot shock stress suppresses selectively the consumption of novel foods without affecting intake of familiar chow and this enhanced neophobia is counteracted by benzodiazepines (Cooper 1980). These results suggest that stress-inducing and stress-protective treatments are able to modulate appetite for novel foods in a choice situation.

The present studies were designed to evaluate the role of Corticotropin-Releasing Factor (CRF) in mediating stress-induced changes in preference for novel diets since the behavioral responses to such stressors as environmental novelty, electric shock, physical confinement and acoustic startle stimuli are likely to involve the activation of central nervous system CRF substrates (Baldwin et al. 1990; Dunn and Berridge 1990). Exogenous CRF pretreatment reduces the duration of contact with novel objects in a multiple compartment chamber (Berridge and Dunn 1987, 1989b) and reduces the frequency of approach to a food pellet supported by a pedestal in a novel open field environment (Britton et al. 1982). When an animal is habituated to novel cues by repeated exposure, the pattern of locomotor and exploratory activity induced by CRF in a familiar testing environment is often distinct from the pattern of CRF-induced behavior produced during the animal's first exposure to the environment (Takahashi and Kalin 1989; Baldwin et al. 1990). Hence, intake of both familiar and unfamiliar food alternatives was examined in the present studies after application of dietary and restraint stress and administration of CRF and a CRF antagonist in an ethologically derived choice paradigm known as dietary self-selection.

Materials and methods

Subjects. Male, Wistar rats (Charles River) were housed as groups of three in a colony room lighted from 05:00 to 17:00 hours with continuous water access. Rodent chow (Teklad) was available

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continuously prior to feeding of experimental diet. Subjects were handled on arrival and acclimated to the animal quarters for 1 week prior to any experimental procedure.

Surgery. For intracerebroventricular injections (ICV), rats were equipped with a cannula aimed above the lateral ventricle. This surgery requires anesthetized (Nembutal, 50 mg/kg) subjects to be secured in a Kopf stereotaxic instrument where a 7 mm long, 23 gauge stainless steel guide cannula is lowered to within 1 mm of the ventricle and anchored to the skull with screws and dental cement. With the tooth bar 5.0 mm above interaural zero, implantation coordinates were 0.6 mm posterior to bregma, 3.2 mm below the skull surface at point of entry and ± 2.0 mm lateral.

ICV injection procedure. After a 7 day post-surgical recovery period, cannula patency was confirmed by gravity flow through an 8 mm, 30 gauge injector inserted through the guide to 1 mm beyond its tip. To perform an injection, 5 μl of solution was infused over 30 s from a 10 μl Hamilton syringe connected to the injector by 70 cm of tubing. The injector was then left in place for 30 s to prevent backflow leakage and the cannula was closed subsequently by a 7 mm stylet.

Diet. The protein deficient diet was compounded by weight using 70% corn starch, 20% sucrose, and 10% protein-free diet (ICN Biochemicals). The protein-free diet was composed of the following non-protein macronutrients, vitamins, and minerals formulated to surpass the minimum requirements for rodent growth and maintenance: 67% sucrose, 15% corn starch, 8% non-nutritive bulk, 5% corn oil, 4% minerals and 1% vitamins. The mineral mixture included calcium phosphate dibasic, sodium chloride, potassium citrate monohydrate, potassium sulfate, magnesium oxide, manganous carbonate, ferric citrate, zinc carbonate, cupric carbonate, potassium iodate, sodium selenite, chromium potassium sulfate and sucrose. The vitamin mixture included vitamin A acetate, vitamin D_2, DL-alpha-tocopherol acetate, ascorbic acid, inositol, choline chloride, menadione, p-aminobenzoic acid, niacin, riboflavin, pyridoxine hydrochloride, thiamine hydrochloride, calcium pantothenate, biotin, folic acid and vitamin B_{12}. The protein replete diet was supplemented with 20% milk-derived protein (purified high nitrogen edible, New Zealand casein; ICN Biochemicals), which displaced the same amount of corn starch from the recipe. These powdered solids were mixed, hydrated and heated to achieve a semi-solid cake of diet with a moist consistency. Experimental diets wsere fed in washed stainless steel bowls placed inside of hanging wire mesh cages so that spillage could be measured.

The 20 g per rat daily portion of diet fed during the three day maintenance period (Fig. 1) exceeded the meal size of most subjects with a daily average consumption of 12.7 ± 0.9 g of 0% protein diet ($N = 188$) and 14.4 ± 0.6 g of 20% protein diet ($N = 121$), Thus, the level of pre-test food intake by protein deprived and replete groups was equivalent although the 96 h period of limited access to the experimental diets resulted in a 10–15% loss of body weight. For instance, rats weighing 263 ± 3 g at the beginning of the experiment lost 26.3 ± 1.1 g ($N = 12$) while consuming the protein replete diet and a corresponding protein deficient diet group weighing 266 ± 3 g lost 33.4 ± 1.6 g ($N = 12$). Heavier rats weighing 320–340 g at the beginning of the experiment also failed to display significant group differences in premeal body weight on the Choice Test day [Protein Replete: 293 ± 4 g ($N = 12$); Protein Deficient: 290 ± 5 g ($N = 13$)].

Procedure. At 24 h following removal of home cage laboratory chow at 17:00 hours, each subject was placed singly in a testing cage separate from the home cage and given access for 1 h on 3 consecutive days to a 20 g portion of one of two diets (Fig. 1). One group remained protein replete by consuming a 20% protein-containing diet that exceeded the daily nutritional requirement for adult, male rats of 7–8% protein. The 20% level of dietary protein reflects the amount recommended for adult rats under 1 year of age by the American Institute of Nutrition and approximates the quantity of protein formulated in commercially available laboratory rodent chow. A second group fed a diet lacking entirely in protein gradually developed protein deficiency. There are three results which suggest that adult rats fed a protein-free diet develop incipient protein deficiency. First, rats fed a protein deficient diet over 4 days exhibit an increase in preference for any of several protein-containing foods, but not novel non-protein foods, relative to protein replete subjects (Deutsch et al. 1989). Second, equivalent protein seeking behavior is manifested in pregnant female rats whose mid-gestational nutritional requirements for food protein are elevated (Deutsch et al. 1989). Finally, rats fed the protein deficient versus replete diets differ in plasma levels of unbound tryptophan, an amino acid product of dietary protein metabolism, after a four day limited access to the experimental diets (Heinrichs and Deutsch, unpublished observations). Taken together, this evidence suggests that behavioral and physiological differences arise in groups fed protein deficient vs. replete diets and that a specific appetite for protein arises from an internal state of nutritional deficiency.

On the fourth day at 17:00, hours each subject was placed singly in a testing cage separate from the home cage and given a two-choice preference test between a 20 g portion of the familiar food consumed over the previous three meals versus a 20 g portion of an unfamiliar alternative formulated with 20% of one of the following proteins (ICN Biochemicals): spray dried egg white (ovalbumin), peanut meal, or yeast torula (Ex: Canadida Utilis). A variety of such protein-containing diets are preferred over non-protein diets by protein deficient rats relative to replete rats (Deutsch et al. 1989) and the three proteins used in the present studies were tested in separate replications of each experiment and the data were then pooled to strengthen the generality of the results. The weight of leftover food as well as the volume of water consumed from graduated Richter tubes were measured after the 1 h meal.

Peptides/drug. CRF and α-helical CRF_{9-41} were synthesized by Dr. Jean Rivier of the Salk Institute's Clayton Foundation

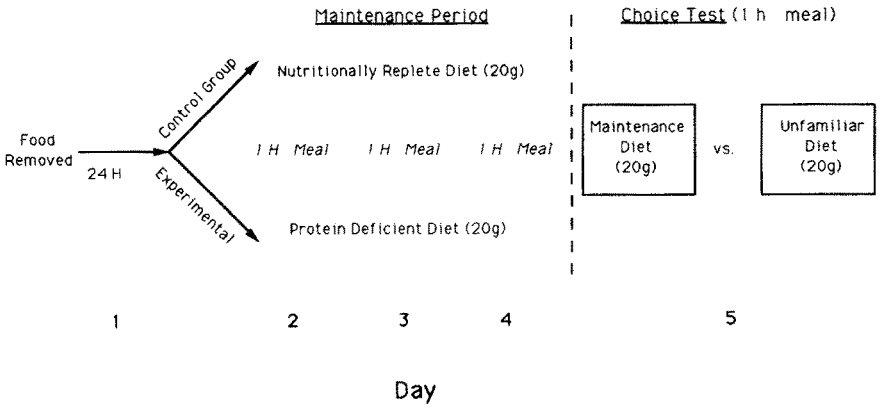

Fig. 1. Over a 96 h period, subjects deprived of laboratory chow received three daily, 1 h meals of a specially formulated diet. Control animals were fed a *Nutritionally Replete Diet* containing corn starch, sugar, protein and a vitamin/mineral/fat supplement. The experimental group was fed a *Protein Deficient Diet* composed of corn starch, sugar and a vitamin/mineral/fat supplement. A 1 h choice test measured preference for the familiar maintenance period diet versus an alternative diet which contained an unfamiliar protein

Laboratory for Peptide Biology. CRF was dissolved in distilled water, α-hel CRF in pH adjusted distilled water (pH 6.7) and chlordiazepoxide (Sigma) in 0.9% physiological saline. All solutions were prepared fresh as needed each experimental day.

Statistics. Mean intake values ± SEM were compared using ANOVA or with Dunnett's *t*-test for multiple, a posteriori comparison of treatment means with a control.

Experiment 1

Several reports link administration of CRF during nocturnal feeding (Morley and Levine 1982) and in food deprived rats (Gosnell et al. 1983a; Arase et al. 1988; Shibasaki et al. 1988a) with suppressed intake of a familiar chow diet. However, suppression by CRF of palatable sucrose solution and plain water intake (Morley and Levine 1982) as well as evidence for conditioned taste aversion to saccharin (Gosnell et al. 1983a) indicate that CRF may affect ingestive behavior non-specifically. In particular, appetitive responses may be incompatible with the expression of other behaviors produced by CRF such as grooming and locomotor arousal which are characteristic of the heightened emotionality produced by CRF (Britton et al. 1982). Similarly, feeding may be disrupted by aversive properties of the peptide. In order to distinguish overall appetite inhibition from selective changes in dietary preference, the first experiment was designed to measure the intake of both familiar and unfamiliar diets in a two-choice test. Further, half the subjects were fed nutrient deficient diets prior to the choice test so that the attractiveness of novel foods was increased relative to nutrient replete controls. In this way flavor/taste cues as well as nutritional status are manipulated to produce a dynamic pattern of intake which can reveal the selectivity of CRF's effects on ingestive behavior.

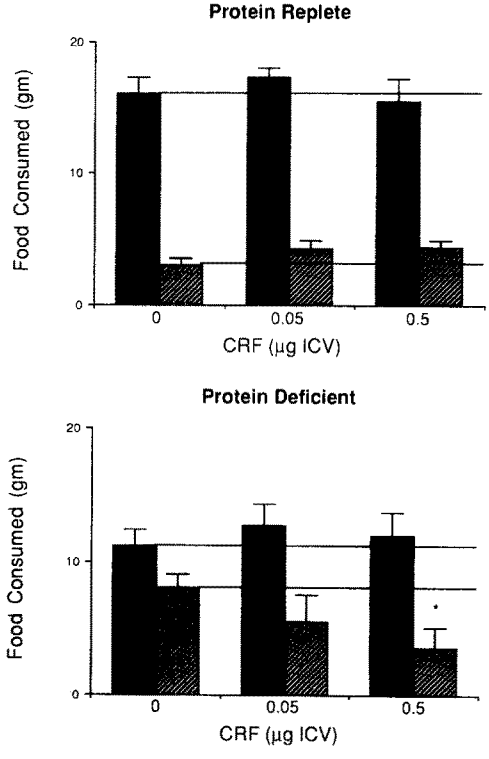

Fig. 2. Protein replete control (*upper panel*) and protein deficient (*lower panel*) subjects were administered 0, 0.05 or 0.5 μg doses of CRF intracerebroventricularly 30 min prior to a choice test of familiar versus unfamiliar foods. Mean control level consumption is shown by the upper horizontal line for the familiar food (*black bars*) and by the lower horizontal line for the unfamiliar food (*hatched bars*). $^*P < 0.05$

Method

Forty-one male rats weighing 300–350 g were separated into two groups and fed either protein replete or deficient diets over four days (Fig. 1). Subjects were then infused ICV with 0 (Replete, $N = 7$; Deficient, $N = 7$), 0.05 (Replete, $N = 6$; Deficient, $N = 6$), or 0.5 (Replete, $N = 8$; Deficient, $N = 7$) μg CRF 30 minutes prior to the 1 h choice test and returned to their home cages.

Results

CRF pretreatment (0.5 μg) suppressed intake of unfamiliar food in protein deficient subjects [$t(17) = 2.13$, $P < 0.05$, one-tailed] without altering either the consumption of familiar food by this group or the selection pattern or replete control rats (Fig. 2). Neither dose of CRF affected water intake during the meal [Vehicle—7.9 ± 1.9 ml, 0.05 μg—8.1 ± 1.3 ml, 0.5 μg—6.5 ± 0.8 ml; $F(2,31) < 1$, n.s.].

Discussion

The well documented intake suppressive effect of CRF is reflected in the present diet self-selection paradigm by reduced consumption of an unfamiliar food. However, as this effect appears only in nutrient deprived subjects which continue to eat a familiar alternative diet and not at all in protein replete controls, the effect of CRF is not on appetite per se but depends on a particular interaction of nutrient hunger and dietary novelty. Further, this intake reduction is specific for food as concurrent drinking was unaffected. Finally, no evidence for peptide-induced malaise was obtained with single exposures to 0.05 and 0.5 μg ICV doses of CRF in a Conditioned Taste Aversion paradigm (Heinrichs et al. 1991), making it unlikely that the peptide suppressed feeding by inducing malaise. Administration of CRF to self-selecting rats seems to suppress dietary neophilia, an increase in preference for unfamiliar, protein containing foods brought about by mild protein nutrient malnutrition (Deutsch et al. 1989). Such inhibition of feeding produced by novel foods or environments has long served as a measure of emotionality in animal models of anxiety (Shephard and Broadhurst 1982; Eysenck and Broadhurst 1964; Bodnoff et al. 1989). In order to determine if this disruption of dietary self-selection reflects one component of the stress-like behavioral profile of exogenous CRF, the next experiment applied physical restraint stress immediately prior to the two-choice test.

Experiment 2

Physical immobilization is a stressor which reduces intake of familiar chow in food deprived rats (Krahn et al. 1986; Shibasaki et al. 1988b). The present study examines the effect of restraint stress on self-selection performance in nutrient replete and deficient subjects.

Method

Fifty eight rats weighing 300–350 g were separated into two groups and fed either protein replete or deficient diets over four days (Fig. 1). Subjects either remained in the home cage (Replete, $N = 11$; Deficient, $N = 12$) or were confined in hemi-cylindrical Plexiglas tubes (Replete, $N = 17$; Deficient, $N = 18$) for 30 min prior to the one hour preference test.

Results

Confinement stress reduced significantly the intake of unfamiliar food by protein deprived rats [$F(1.33) = 13.11$, $P < 0.001$] without altering either the consumption of familiar food by this group or the selection pattern of protein replete controls (Fig. 3). Restraint did not affect water intake over the one hour test [Unrestrained—8.6 ± 0.7 ml, Restrained—6.9 ± 0.6 ml; $F(1,41) = 3.53$, n.s.].

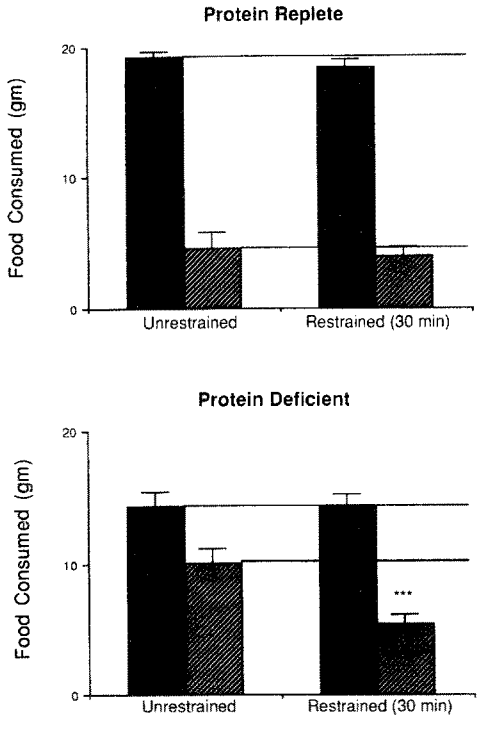

Fig. 3. Protein replete control (*upper panel*) and protein deficient (*lower panel*) subjects were either taken directly from the home cage (Unrestrained) or physically restrained in immobilization tubes for 30 min prior to a choice test of familiar versus unfamiliar foods. Mean control level consumption is shown by the upper horizontal line for the familiar food (*black bars*) and by the lower horizontal line for the unfamiliar food (*hatched bars*). ***$P < 0.001$

Discussion

These results demonstrate that the selective disruption of dietary neophilia by central administration of CRF can be mimicked by external application of a bodily stressor. Restraint stress altered neither the selection pattern of replete controls nor the intake of a familiar diet by deficient rats and hence probably reflects the effect of stress on a dietary preference driven by nutrient hunger rather than on general appetite. The specificity of this effect is further supported by the failure of restraint stress to alter fluid intake in the present experiment or to induce a Conditioned Taste Aversion when applied immediately following a meal of saccharin solution (Unpublished observations). If this reduced preference for novelty is mediated by CRF neurons in the central nervous system, then pretreatment with a CRF antagonist, α-helical CRF_{9-41}, may protect subjects from restraint stress-induced changes in intake.

Experiment 3

Centrally administered CRF anti-serum and α-helical CRF_{9-41} blunt or reverse changes in feeding induced by CRF and immobilization stress while neither treatment alone affects intake of familiar chow in 24 h food deprived rats (Krahn et al. 1986; Shibasaki et al. 1988b). Hence, the present experiment tested the ability of α-hel CRF to modify changes in dietary self-selection induced by restraint stress.

Method

One hundred and thirteen rats weighing 300–350 g were separated into two groups and fed either protein replete or deficient diets over four days (Fig. 1). Subjects were then infused ICV with 0 (Replete, $N = 10$; Deprived/Unrestrained, $N = 13$; Deprived/Restrained, $N = 16$), 1 (Replete, $N = 9$; Deprived/Unrestrained, $N = 7$; Deprived/Restrained, $N = 8$), 5 (Replete, $N = 7$; Deprived/Unrestrained, $N = 8$; Deprived/Restrained, $N = 14$) or 25 (Replete, $N = 6$; Deprived/Unrestrained, $N = 7$; Deprived/Restrained, $N = 8$) μg α-hel CRF and returned to their home cages or restraint tubes for 30 min prior to the 1 h preference test. Protein replete groups were administered peptide but not restrained.

Results

As in experiment 2, restraint stress reduced intake of unfamiliar food in protein deficient subjects [$F(1,73) = 6.29$, $P < 0.02$] without affecting intake of familiar food [$F(1,73) = 1.87$, n.s.]. Administration of CRF antagonist increased significantly the overall intake of familiar food [$F(3,73) = 5.76, P < 0.002$] and produced a complementary decrease in consumption of unfamiliar food [$F(3,73) = 3.91, P < 0.02$]. Comparing individual means (Fig. 4), the 25 μg dose of α-hel CRF increased consumption of familiar food by protein deprived and unrestrained subjects relative to unrestrained controls [$t(18) = 2.35$, $P < 0.05$]. Similarly, 5 and 25 μg doses of α-hel CRF increased consumption of familiar food by

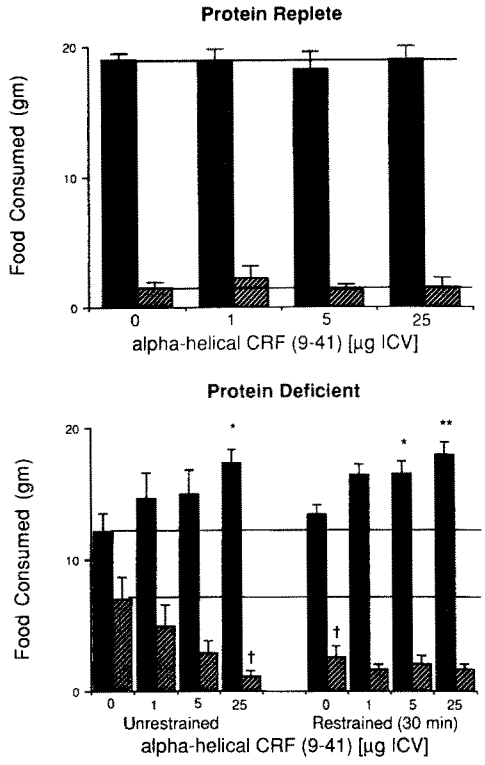

Fig. 4. Protein replete control (*upper panel*) and protein deficient (*lower panel*) subjects were administered 0, 1, 5 or 25 μg doses of α-hel CRF (9–41) intracerebroventricularly and returned to their home cages or restrained physically for 30 min prior to a choice test of familiar versus unfamiliar foods. Mean control level consumption is shown by the upper horizontal line for the familiar food (*black bars*) and by the lower horizontal line for the unfamiliar food (*hatched bars*). $*P < 0.05$, $**P < 0.01$ relative to familiar food intake of respective vehicle-treated controls; $\dagger P < 0.05$ relative to unfamiliar food intake of vehicle-treated, unrestrained control group

protein deprived and restrained subjects relative to restrained controls [$t(44) = 2.7$, $P < 0.05$; $t(44) = 3.29$, $P < 0.01$]. Significant decreases in unfamiliar food intake in unrestrained subjects treated with the 25 μg dose of α-hel CRF [$t(33) = 2.81$), $p < 0.05$] accompanied the enhanced appetite for familiar food. Restraint stress suppressed total food intake only in vehicle treated subjects [$t(27) = 1.90$, $P < 0.05$; one-tailed] and this effect was reversed by the 25 μg dose of α-hel CRF [$t(22) = 2.41$, $P < 0.05$].

Discussion

The CRF antagonist increased intake of familiar food only under conditions of dietary stress. This preference for familiar diet probably does not arise from a complementary decrease in preference for unfamiliar foods since the effect persisted when unfamiliar diet intake was suppressed independently by restraint stress. Further, selective suppression of neophilia by CRF and restraint stress in experiments 1 and 2 did not result in compensatory increases in familiar diet intake. Indeed, the CRF antagonist dose dependently transformed the selection pattern of protein-deprived subjects to resemble closely the dietary preferences of the protein replete control group (see Fig. 4).

In addition, α-hel CRF attenuated the anorexic effect of physical confinement stress on total intake. Both the reversal of restraint stress-induced anorexia and the increased familiar diet intake in experiment 3 occurred at doses of CRF antagonist which are 10–100 times lower than those reported to be effective in antagonizing the suppression of feeding in response to stress (Krahn et al. 1986; Shibasaki et al. 1988b). The present data suggest that α-hel CRF modifies the self-selection pattern of nutritionally and physically stressed animals to resemble that of nutritionally replete and unrestrained controls.

Experiment 4

As the CRF antagonist attenuated changes in diet selection induced by immobilization stress, restraint may alter food intake by a mechanism which is mediated by endogenous CRF systems. Indeed, two reports indicate that α-hel CRF attenuates restraint stress-induced anorexia (Krahn et al. 1986; Shibasaki et al. 1988b). In order to confirm that α-hel CRF is acting on CRF substrates in the present studies, a direct competition of the effects of exogenous CRF and CRF antagonist on dietary self-selection was conducted.

Method

Twenty-four rats weighing 300–350 g were all maintained on the protein-free diet over 4 days (Fig. 1). Subjects were then administered two sequential ICV injections 30 min prior to the 1 h choice test and returned to their home cages. The control group received the α-hel CRF vehicle followed by the CRF vehicle ($N = 7$) while three separate groups received either 0 ($N = 6$), 5 ($N = 6$) or 25 ($N = 6$) μg α-hel CRF followed by 0.5 μg CRF. Each peptide was administered in a 2 μl volume by gravity flow from a 70 cm length of tubing and injectors were left in place for 30 seconds following each injection (Swerdlow et al. 1989).

Results

As in experiment 1, the 0.5 μg ICV dose of CRF reduced choice test intake of an unfamiliar diet [$t(11) = 2.54$, $P < 0.05$] which contributed to an overall suppression of total intake [$t(11) = 3.21$, $P < 0.01$] (Fig. 5). In contrast with experiment 1, CRF administration also suppressed familiar diet intake [$t(11) = 2.40$, $P < 0.05$] perhaps by potentiating the stressful effects of prolonged handling required to perform back to back ICV injections. Although the anorexia induced by this combination of procedural and treatment factors was more intense than any intake reduction produced in experiments 1, 2 or 3, pretreatment with both the 5 and 25 μg doses of α-hel CRF antagonized the effects of CRF so that familiar diet [5 μg—$t(20) = 1.34$, n.s.; 25 μg—$t(20) = 1.23$, n.s.], unfamiliar diet [$t(20) = 0.69$, n.s.; $t(20) = 2.14$, n.s.] and total intake [$t(20) = 1.45$, n.s.; $t(20) = 0.03$, n.s.] of α-hel CRF-treated groups did not differ significantly from controls. The 25 μg dose of α-hel CRF reversed significantly the reduction in total intake produced by CRF [$t(15) = 3.11$, $P < 0.05$].

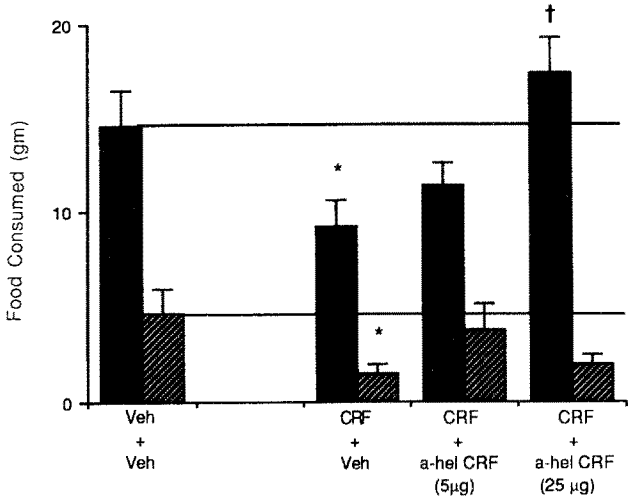

Fig. 5. Protein deficient subjects received intracerebroventricular injections of 0, 5 or 25 μg α-hel CRF followed immediately by either 0 or 0.5 μg doses of CRF ICV. After a 30 min home cage delay, subjects selected for one hour between familiar and unfamiliar diets. Mean control level consumption is shown by the upper horizontal line for the familiar food (*black bars*) and by the lower horizontal line for the unfamiliar food (*hatched bars*).*$P < 0.05$ relative to respective food intake of vehicle-treated controls; †$P < 0.05$ relative to the familiar food intake of the group treated with CRF alone

Discussion

As α-helical CRF_{9-41} blocked the effects of CRF in the present experiment, the CRF antagonist probably alters the selection behavior of nutrient deprived rats by acting on central nervous system CRF receptors. Other investigators also report direct antagonism by α-hel CRF of various behavioral effects of CRF (Britton et al. 1986b; Swerdlow et al. 1989). In order to characterize the nature of changes induced by α-hel CRF in experiment 3, the final experiment examined the effect of an anxiolytic benzodiazepine in the diet-selection paradigm.

Experiment 5

Chlordiazepoxide shortens the latency to begin a meal and enhances feeding at low doses (5 and 10 mg/kg) and also increases preference for novel foods at higher doses (15 mg/kg) (Cooper 1980; Cooper et al. 1981). The latter effect of chlordiazepoxide in attenuating the reduction in intake produced by unfamiliar flavor or consistency of foods suggests that dietary preference tests are sensitive to levels of emotionality in which the inhibition of appetitive behavior by novelty is lessened by anxiolytic doses of benzodiazepines (Shephard and Broadhurst 1982). Here chlordiazepoxide is used as a reference for α-hel CRF's disinhibitory effect on familiar diet consumption and to contrast stressor-induced changes in self-selection pattern.

Method

Twenty-four rats weighing 250–300 g were separated into two groups and fed either protein replete or deficient diets on 4 days (Fig. 1). Subjects were then injected intraperitoneally with 0 (Replete, $N = 12$; Deficient, $N = 12$) or 5 (Replete, $N = 12$; Deficient, $N = 12$) mg/kg chlordiazepoxide in a 1 ml/kg vehicle and returned to their home cages for 30 min prior to the 1 h choice test.

Results

In Fig. 6, chlordiazepoxide (5 mg/kg) pretreatment increased significantly the consumption of familiar diet in protein deficient subjects [$F(1,22) = 8.83$, $P < 0.01$] but did not alter significantly the intake of unfamiliar diet [$F(1,22) = 3.85$, n.s.] or the total amount eaten [$F(1,22) = 1.52$, n.s.]. The chlordiazepoxide-induced increase in preference for familiar diet was specific to protein deprived rats as the selection pattern of replete controls was not affected. Intake of unfamiliar food by both replete and deprived subjects may have been reduced from the level set in the previous four experiments by premeal infusions into the peritoneal cavity or by the lack of prior habituation to this injection procedure. Water intake during the choice test was not altered by the benzodiazepine (Vehicle—7.2 ± 0.6 ml, 5 mg/kg—6.3 ± 0.5 ml; $F(1,46) = 1.16$, n.s.).

Discussion

Chlordiazepoxide modified selectively the choice pattern of nutritionally stressed rats without affecting fluid intake

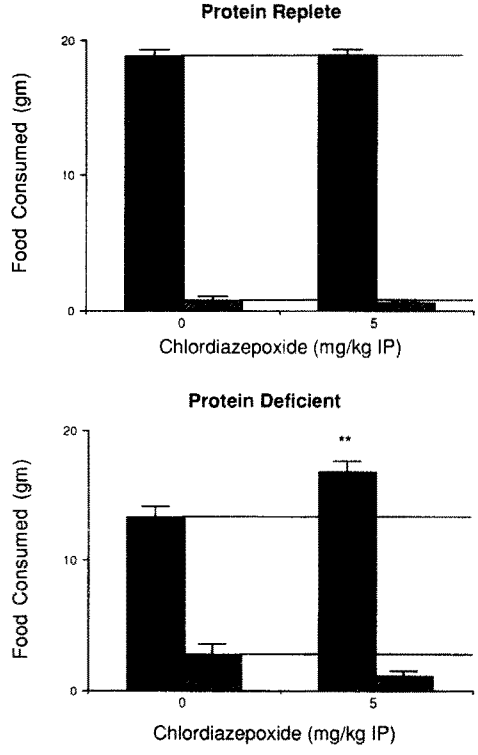

Fig 6. Protein replete control (*upper panel*) and protein deficient (*lower panel*) subjects were administered 0 and 5 mg/kg chlordiazepoxide intraperitoneally 30 min prior to a one hour choice test of familiar versus unfamiliar foods. Mean control level consumption is shown by the upper horizontal line for the familiar food (*black bars*) and by the lower horizontal line for the unfamiliar food (*hatched bars*). **$P < 0.01$

or the overall appetite of protein replete subjects. Copper (1989) also observed an increase in preference for familiar foods in a dietary choice test using a 5 mg/kg dose of chlordiazepoxide. As the selection pattern observed with the CRF antagonist in experiment 3 is analogous to the present results, the actions of α-hel CRF on dietary self-selection may be an anxiolytic complement to the actions of CRF. Similarly, the effects of α-hel CRF and chlordiazepoxide on CRF-induced changes in conflict behavior, social interaction and acoustic startle amplitude are opposite in direction to the profile of CRF actions in these paradigms (Britton et al. 1985, 1986b; Dunn and File 1987; Swerdlow et al. 1989; Baldwin et al. 1990).

General discussion

These experiments suggest that CRF disrupts dietary neophilia, an enhanced preference for novel foods, while α-hel CRF antagonizes this effect as well as the neophilia-producing effect of nutritional stress in self-selecting rats. Britton et al. (1982) suggest that opposing actions of exogenous CRF versus anxiolytic treatments in a novel open field arena indicate that release of CRF within the brain could mediate behavioral responses to novelty. While the link between enhanced response to novelty and CRF activation can be challenged on the basis of physical differences in familiar versus unfamiliar testing environments (Sherman and Kalin 1987), novel cues in the present studies were contained within specially formulated foods presented in a single feeding cage to which each subject had been habituated during three, 1 h pretest meals. Hence, changes in intake induced by CRF peptides reflect a modified preference for familiar and unfamiliar diets rather than non-specific stress provoked by environmental novelty (Sherman and Kalin 1987; Bodnoff et al. 1989).

The actions of CRF and CRF antagonist on dietary self-selection arise only under nutritional stress. The lack of effect of α-hel CRF on intake of food deprived but nutritionally replete rats has been reported previously (Krahn et al. 1986). Shibasaki et al. (1988b) observe that α-hel CRF and anti-CRF γ-globulin administered intracerebroventricularly are effective in antagonizing restraint stress-induced anorexia but do not modify food intake in 24 h fasted rats. These authors argue that endogenous CRF is involved in the control of ingestive behavior only when subjects are exposed to stressful conditions. The present results support this hypothesis both in the case of stress induced by physical confinement and in the event of nutritional imbalance resulting in specific hunger for protein. In contrast, dietary restriction, a significant stressor in and of itself (Suemaru et al. 1986), resulted in comparable weight loss among both protein replete and deficient subjects so that group differences are not explained easily by caloric deprivation alone.

While CRF is reported to suppress ingestive behavior non-specifically in both home and novel cages (Britton et al. 1982; Levine et al. 1983), during nocturnal feeding and in fasted subjects (Gosnell et al. 1983a) and when offered food, drinking water or attractively sweetened solutions (Morley and Levine 1982), the present data argue for a selective effect of CRF in decreasing the attractiveness of unfamiliar foods in nutrient hungry subjects. It is not likely that low levels of unfamiliar diet intake by some experimental control groups prevented detection of CRF treatment effects since several investigators report anorectic actions of CRF relative to a basal intake level of only 1–2 g of food (Britton et al. 1982; Gosnell et al. 1983a; Shibasaki et al. 1988a). The selective effect of CRF on unfamiliar food may be expressed only at the doses used in the present studies which are lower than those which produce non-specific ingestive inhibition (Morley and Levine 1982) and yet are large enough to mimic changes in self-selection pattern produced by immobilization stress. Indeed, at least one report documents a dose dependent, bi-phasic effect of CRF on food intake (Gosnell et al. 1983a). Evidence for a selective effect on appetite may also depend on the use of a two-choice procedure which, unlike protocols employing only a single food, is well suited to the detection and discrimination of shifts in appetite and preference (Cooper 1980).

While the paraventricular nucleus of the hypothalamus has been identified as a specific brain site known to be involved in the control of food intake at which CRF exerts anorexic effects (Krahn et al. 1988), the present changes in appetite probably reflect actions on central nervous system CRF neurons other than those which mediate the secretion of adrenocorticotropic hormone (ACTH). The α-hel CRF peptide has a relatively low affinity for hypothalamic receptors at which CRF activates the neuroendocrine axis (Rivier et al. 1984) and the anorexic, pro-conflict, locomotor activating and sympathetic arousal effects of CRF persist in hypophysectomized and dexamethasone treated animals (Brown et al. 1982; Gosnell et al. 1983b; Eaves et al. 1985; Britton et al. 1986a; Berridge and Dunn 1989a).

Taken together, the present studies support the hypothesis that CRF systems exert specialized control over feeding behaviors occurring within the context of a stressor such as protein nutrient deficiency. In contrast to certain experimental stressors which include noxious or painful components, protein-calorie malnutrition produces an endocrine stress profile (Smith et al. 1975) by placing a simple and gradually felt physiological demand on the body. Further, the neophilic response to nutritional stress is adaptive since it restores nutrient balance and may be unlearned in the rat (Deutsch et al. 1989). Along with the model of Defensive Withdrawal developed by Takahashi et al. (1989) in which exploratory activity is constrained by an innate fear of predation, instances in which CRF modulates appropriate behavioral responses to naturalistic stressors serve to promote the integral role of CRF in brain mechanisms which allow an organism to cope with stress.

Abnormal levels of CRF are described in the CSF of patients suffering from appetite disorders (Kaye et al. 1989; Krahn and Gosnell 1989). The present data regarding subtle changes in the microstructure of food preference therefore may suggest an instrument for early diagnosis of pathological feeding behavior. For instance, bulimia and anorexia nervosa are characterized by a shift in hedonic preference for the sensory qualities of food resulting in a craving for or rejection of sweet tastes (Drewnowski

et al. 1987a; Kaye et al. 1989) and fat-containing foods (Drewnowski et al. 1987b). As such aberrant food choices stand in striking contrast to these patient's usual diets, some feeding mechanism involved in diet selection may be disabled in these individuals by overexpression of CRF in the same manner that the peptide disrupts adaptive food preference in the present studies.

Acknowledgements. We thank Ed Fox for critical reading of the manuscript and student apprentices John Dunlop and Brent Heffron for experimental assistance. This research was supported by grants NIADDK 26741 to G.F.K. and NIAAA 07456/06420 to the Alcohol Research Center of the Scripps Research Institute. This is publication 6989-NP of the Scripps Research Institute.

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