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Corticotropin-releasing hormone-binding protein in brain and pituitary of food-deprived obese (*fa/fa*) Zucker rats

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Timofeeva, Elena, Yves Deshaies, Frédéric Picard, and Denis Richard. Corticotropin-releasing hormone-binding protein in brain and pituitary of food-deprived obese (*fa/fa*) Zucker rats. *Am. J. Physiol.* 277 (*Regulatory Integrative Comp. Physiol.* 46): R1749–R1759, 1999.—The present study was conducted to verify whether experimental conditions such as obesity and food deprivation, which promote food intake and reduce thermogenesis, could modify the expression of the corticotropin-releasing hormone (CRH)-binding protein (BP) in the rat brain. In situ hybridization, histochemistry, and immunohistochemistry were used to assess the expression of CRH-BP in lean (*Fa/?*) and obese (*fa/fa*) Zucker rats that were fed ad libitum, food deprived for 24 h, or food deprived for 24 h and refed for 6 h. In both lean and obese rats, food deprivation led to a reduction in body weight that was accompanied by a reversible increase in plasma corticosterone levels. Food deprivation and, to a lesser degree, obesity induced the expression of CRH-BP mRNA in the dorsal part of the medial preoptic area (MPOA). This induction of the CRH-BP gene led to by food deprivation was confirmed by the appearance in the dorsal part of the MPOA of neurons immunoreactive to CRH-BP. Food deprivation (in particular) and obesity also increased the levels of CRH-BP mRNA in the basolateral amygdala (BLA). The enhanced CRH-BP expression in the MPOA and BLA in response to food deprivation was reversed by refeeding. In lean *Fa/?* rats, the CRH-BP mRNA level in the pituitary cells was significantly decreased after food deprivation and restored after refeeding. When food was provided ad libitum, the number of cells expressing CRH-BP in the anterior pituitary was significantly higher in lean rats than in obese animals. Food deprivation for 24 h decreased dramatically the number of pituitary cells expressing CRH-BP in lean rats. Altogether, the present results demonstrate that food deprivation and, to a lesser extent, obesity can selectively affect the expression of CRH-BP. Given both the inactivating effect of CRH-BP on the CRH system and the potential roles played by the MPOA and BLA in the thermogenic and anorectic effects of CRH, it can be argued that the induction of the CRH-BP gene in obesity and after food deprivation occurs as a mechanism to reduce energy expenditure and to stimulate food intake.

amygdala; brain; energy balance; food intake; hypothalamus; thermogenesis

CORTICOTROPIN-RELEASING HORMONE (CRH) is a 41-amino acid peptide whose expression in the brain is wide and abundant. This expression is particularly noticeable in the hypothalamic paraventricular nucleus (PVH), from which an important cluster of CRH neurons projects to the median eminence to control the activity of the pituitary-adrenal axis. The control of this activity represents the best-characterized, though not the sole action of CRH (2). Among the best documented effects of CRH that are unrelated to the pituitary-adrenal axis are its thermogenic and anorectic actions (33, 36). CRH has been reported to be involved in the anorectic effects of treadmill running (34), restraint stress (37), estradiol (12), and caffeine (32) as well as in the thermogenic actions of fenfluramine (22) and the serotonin 2A/2C-receptor agonist, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (7). The site of the anorectic action of CRH has been identified as potentially being the PVH (20). Data obtained in our (13) and other (15) laboratories have provided evidence that the medial preoptic area (MPOA) could be the site of the thermogenic action of CRH. Recently, CRH has been reported to alter hoarding threshold in food-deprived rats, suggesting that CRH can genuinely be involved in the regulation of energy balance (9).

In the rat brain, CRH targets two classes of transmembrane receptors referred to as CRH type 1 (27) and CRH type 2α (24) receptors. CRH also binds a CRH-binding protein (BP) that is, similar to CRH and its receptors, widely distributed throughout the brain. The exact function of this protein has yet to be fully defined, but there is strong evidence that suggests that CRH-BP could inactivate CRH (4); the inhibition of CRH-BP by the CRH-molecule fragment $CRH_{6—33}$ has been reported to raise CRH tone in the brain (5).

Given the anorectic and thermogenic effects of CRH, one can predict that the activity of the CRH system is reduced in obesity or after food deprivation. However, apart from a reduced expression of CRH type 2α-receptor mRNA in the ventromedial hypothalamus of obese (*fa/fa*) or food-deprived rats (41), there has so far been no direct indication that CRH activity is reduced in obesity or after food deprivation. The reduction in CRH mRNA expression led to by food deprivation in the PVH of lean rats (8) cannot be ascribed to a reduction in a CRH activity associated with the regulation of energy balance, the PVH CRH neurons primarily fulfilling a hypophysiotropic role. In that respect, it is noteworthy that CRH mRNA expression is not decreased in unrestrictedly fed obese *fa/fa* (Zucker) rats and *ob/ob* mice, and it is even induced in these rodents after food deprivation (18, 41).

Recently, Heinrichs et al. (17) showed that raising CRH tone by inhibiting CRH-BP with $CRH_{6—33}$ can efficiently reduce weight gain in the obese *fa/fa* Zucker rat. These results raised the possibility that CRH-BP can play a role in the development of obesity, which prompted us to test the hypothesis of an upregulation of

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the CRH-BP protein in obesity and after food deprivation. The present study was conducted to investigate the effects of food deprivation and refeeding on CRH-BP synthesis in the central nervous system of food-deprived lean and obese Zucker rats. Food deprivation has proved very useful to investigate the regulation of energy balance as it induces fat store variations leading to marked adjustments in the control of food intake and energy expenditure (18).

METHODS

Animals and treatments. Lean (*Fa/?*) and obese *fa/fa* male Zucker rats, aged 6 wk, were purchased from the Canadian Breeding Laboratories (St. Constant, Canada). All rats were cared for and handled according to the Canadian Guide for the Care and Use of Laboratory Animals, and our institutional animal care committee approved the present protocol. The animals were housed individually in wire-bottom cages suspended above absorbent paper and, unless otherwise specified, fed ad libitum a stock diet (Agway Prolab, Rat/Mouse/Hamster 1000 Formula). They were subjected to a 14:10-h dark-light cycle (lights on between 1200 and 2200) and kept at an ambient temperature of 23 ± 1°C. Rats were killed after *1*) ad libitum feeding, *2*) 24 h of food deprivation, or *3*) 6 h of ad libitum refeeding subsequent to 24 h of food deprivation. All rats were killed between 1200 and 1300, that is during the first hour of the light phase. Because we intended to keep the time of death constant, the times at which food was removed were varied according to the length of the food deprivation and refeeding periods.

Brain and pituitary preparation. Brains and pituitaries were prepared as previously described (41). Briefly, rats were anesthetized with 1.5 ml of a mixture containing 20 mg/ml of ketamine and 2.5 mg/ml of xylazine. Without delay, they were perfused intracardially with 30 ml of ice-cold isotonic saline followed by 200 ml of a 4% paraformaldehyde solution. The brains and pituitaries were removed at the end of the perfusion and kept in paraformaldehyde for an additional period of 7 days. They were then transferred to a solution containing paraformaldehyde and sucrose (10%) before being cut 24 h later using a sliding microtome (Histoslide 2000, Reichert-Jung). Brain sections were taken from the olfactory bulb to the brain stem. Thirty-micrometer-thick sections were collected and stored at −30°C in a cold sterile cryoprotecting solution containing sodium phosphate buffer (50 mM), ethylene glycol (30%), and glycerol (20%).

In situ hybridization histochemistry. In situ hybridization histochemistry was used to localize CRH-BP mRNA on tissue sections taken from the entire brain and the pituitary. The protocol used was largely adapted from the technique described by Simmons et al. (38). Briefly, one of every five brain sections as well as all the pituitary specimens were mounted onto poly-L-lysine-coated slides and allowed to desiccate overnight under vacuum. The sections were then successively fixed for 20 min in paraformaldehyde (4%), digested for 30 min at 37°C with proteinase K (10 µg/ml in 100 mM Tris·HCl containing 50 mM EDTA, pH 8.0), acetylated with acetic anhydride (0.25% in 0.1 M triethanolamine, pH 8.0), and dehydrated through graded concentrations (50, 70, 95, and 100%) of alcohol. After vacuum drying for at least 2 h, 90 µl of the hybridization mixture, which contained an antisense ^{35}S-labeled cRNA probe (10^7 counts·min^{-1}·ml^{-1}), were spotted on each slide. The slides were sealed under a coverslip and incubated overnight at 60°C in a slide warmer. The next day, the coverslips were removed and the slides were rinsed four times with 4× SSC (0.6 M NaCl, 60 mM trisodium citrate buffer, pH 7.0), digested for 30 min at 37°C with RNAse-A (20 µg/ml in 10 mM Tris-500 mM NaCl mixture containing 1 mM EDTA), washed in descending concentrations of SSC (2×, 10 min; 1×, 5 min; 0.5×, 5 min; 0.1×, 30 min at 60°C), and dehydrated through graded concentrations of alcohol. After a 2 h-period of vacuum drying, the slides were exposed for 48 h on an X-ray film (Eastman Kodak, Rochester, NY). Once removed from the autoradiography cassettes, the slides were defatted in xylene, dipped in NTB2 nuclear emulsion (Eastman Kodak), and exposed for 14 days before being developed in D19 developer (Eastman Kodak) for 3.5 min at 14°C and fixed in rapid fixer (Eastman Kodak) for 5 min. Finally, tissues were rinsed in running distilled water for 1–2 h, counterstained with thionine (0.25%), dehydrated through graded concentrations of alcohol, cleared in xylene, and coverslipped with DPX.

Antisense ^{35}S-labeled cRNA probe. The CRH-BP cRNA probe was generated from the 630-bp *Pst* I fragment of rat CRH-BP cDNA (Dr. W. Vale, Peptide Biology Laboratory, The Salk Institute), subcloned into pBluescript SKII plasmid (Stratagene, La Jolla, CA), and linearized with *Eco*R I and *Xba* I (Pharmacia Biotech, Canada) for antisense and sense probes, respectively. The specificity of the CRH-BP cRNA probe was confirmed by the absence of positive signal in sections hybridized with the sense probe. Radioactive riboprobes were synthesized by incubation of 250 ng linearized plasmid in 10 mM NaCl, 10 mM dithiothreitol, 6 mM MgCl, 40 mM Tris (pH 7.9), 0.2 mM ATP/GTP/CTP, α-^{35}S-UTP, 40 U RNasin (Promega, Madison, WI), and 20 U T3 or T7 RNA polymerase (for antisense or sense probe, respectively) for 60 min at 37°C. The DNA templates were treated with 100 µl of DNAse solution (1 µl DNAse, 5 µl of 5-mg/ml tRNA, and 94 µl of 10 mM Tris and 10 mM $MgCl_2$). The preparation of the riboprobes was completed through a phenol-chloroform extraction and ammonium acetate precipitation.

Immunocytochemical procedure. Immunocytochemical detection of CRH-BP was performed using a rat monoclonal antibody, raised in mouse (generously provided by X.-J. Liu and N. Ling, Neurocrine Biosciences, San Diego, CA). Brain sections were processed using a conventional avidin-biotin-immunoperoxidase method. Briefly, brain slices were washed in sterile 0.05 M potassium phosphate-buffered saline (KPBS). They were then incubated for 48 h at 4°C with a CRH-BP antibody. The CRH-BP antibody was used at a dilution of 1:200 in KPBS with heparin (0.25%), Triton X-100 (0.4%), and bovine serum albumin (2%). Forty-eight hours after incubation at 4°C with the first antibody, the brain slices were rinsed

Table 1. *Effects of food deprivation and refeeding on body weight, plasma glucose, and plasma corticosterone of Fa/? and fa/fa Zucker rats*

	Body Weight, g	Plasma Glucose, mM	Plasma Corticosterone, µM
	Fa/?		
Ad libitum	143.8 ± 8.8	8.80 ± 0.27	0.10 ± 0.06
Food deprived, 24 h	122.0 ± 7.8	7.16 ± 0.32	0.42 ± 0.24
Refed, 6 h	139.5 ± 4.0	7.84 ± 0.47	0.01 ± 0.01
	fa/fa		
Ad libitum	235.7 ± 7.6	8.35 ± 0.60	0.75 ± 0.19
Food deprived, 24 h	205.5 ± 6.6	7.99 ± 0.43	1.09 ± 0.11
Refed, 6 h	238.7 ± 2.8	9.03 ± 0.27	0.39 ± 0.23
Significant effects, $P < 0.05$	Nutrition, phenotype		Nutrition, phenotype

Values represent means ± SE of 4 animals per experimental condition. *Fa/?*, lean Zucker rats; *fa/fa*, obese Zucker rats.

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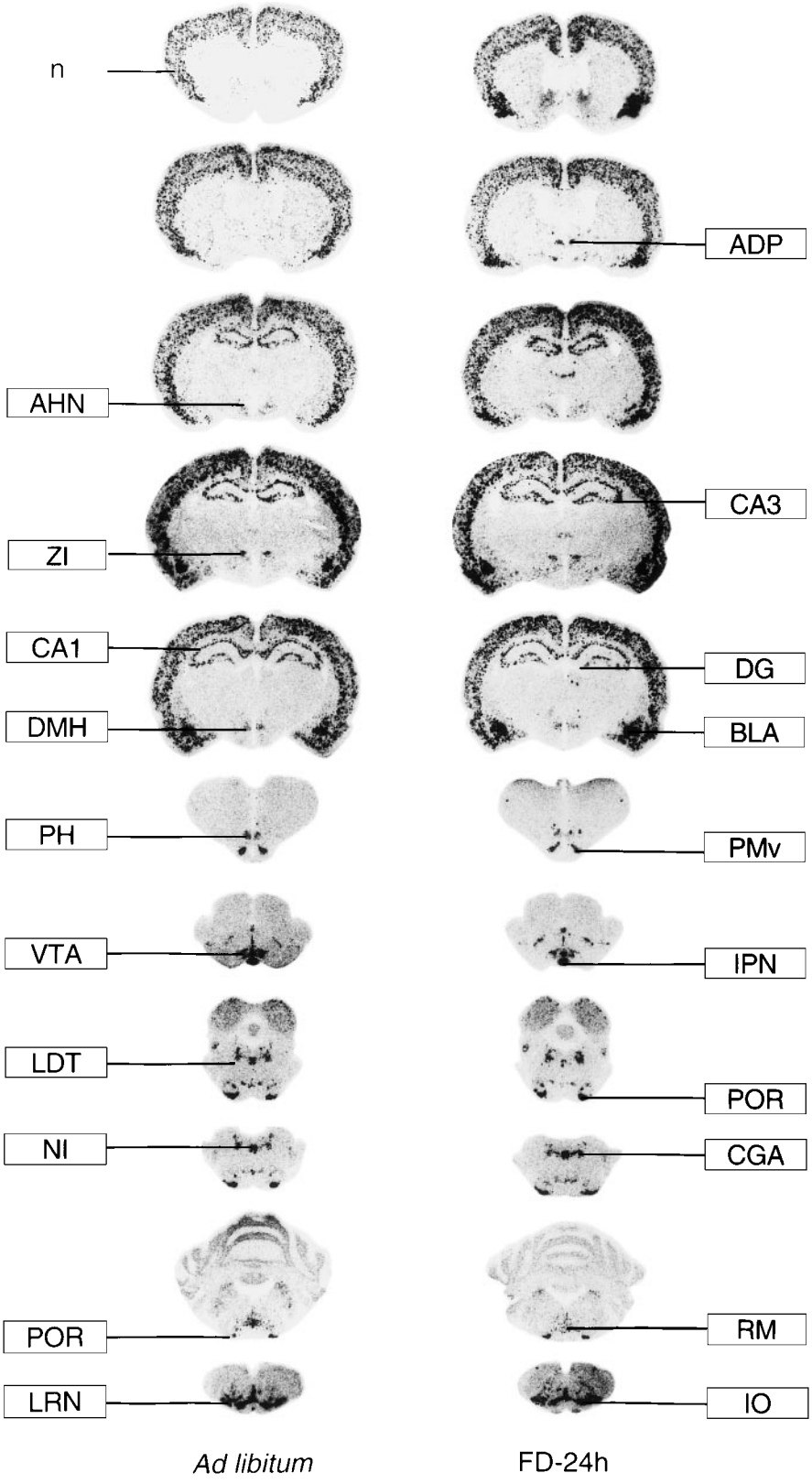

Fig. 1. Rostrocaudal distribution of corticotropin-releasing hormone (CRH)-binding protein (BP) in brain of lean (*Fa/?*) Zucker rats either fed ad libitum or food deprived for 24 h (FD-24 h). Pictures are film autoradiograms of coronal brain sections that were hybridized with an antisense riboprobe complementary to rat CRH-BP mRNA. n, neocortex; ADP, anterodorsal preoptic nucleus; AHN, anterior hypothalamic nucleus; CA1, field CA1 of Ammon's horn; CA3, field CA3 of Ammon's horn; DG, field DG of Ammon's horn; ZI, zona incerta; BLA, basolateral complex of amygdala; DMH, dorsomedial hypothalamic nucleus; PH, posterior hypothalamic nucleus; PMv, ventral premammillary nucleus; VTA, ventral tegmental area; IPN, interpeduncular nucleus; LDT, laterodorsal tegmental nucleus; POR, periolivary nucleus; NI, nucleus incertus; CGA, central gray, alpha; RM, nucleus raphe magnus; LRN, lateral reticular nucleus; IO, inferior olivary complex.

in sterile KPBS and incubated with a mixture of KPBS, Triton X-100, heparin, and biotinylated goat antimouse IgG (1:1,500 dilution; Vector Laboratories) for 90 min. Sections were then rinsed with KPBS and incubated at room temperature for 60 min with an avidin-biotin-peroxidase complex (Vectastain ABC Elite Kit, Vector Laboratories) followed by a second incubation with a mixture of KPBS, Triton X-100, heparin, and biotinylated goat antimouse IgG followed by the ABC Elite solution. After several rinses in sterile KPBS, the brain slices were allowed to react in a mixture containing

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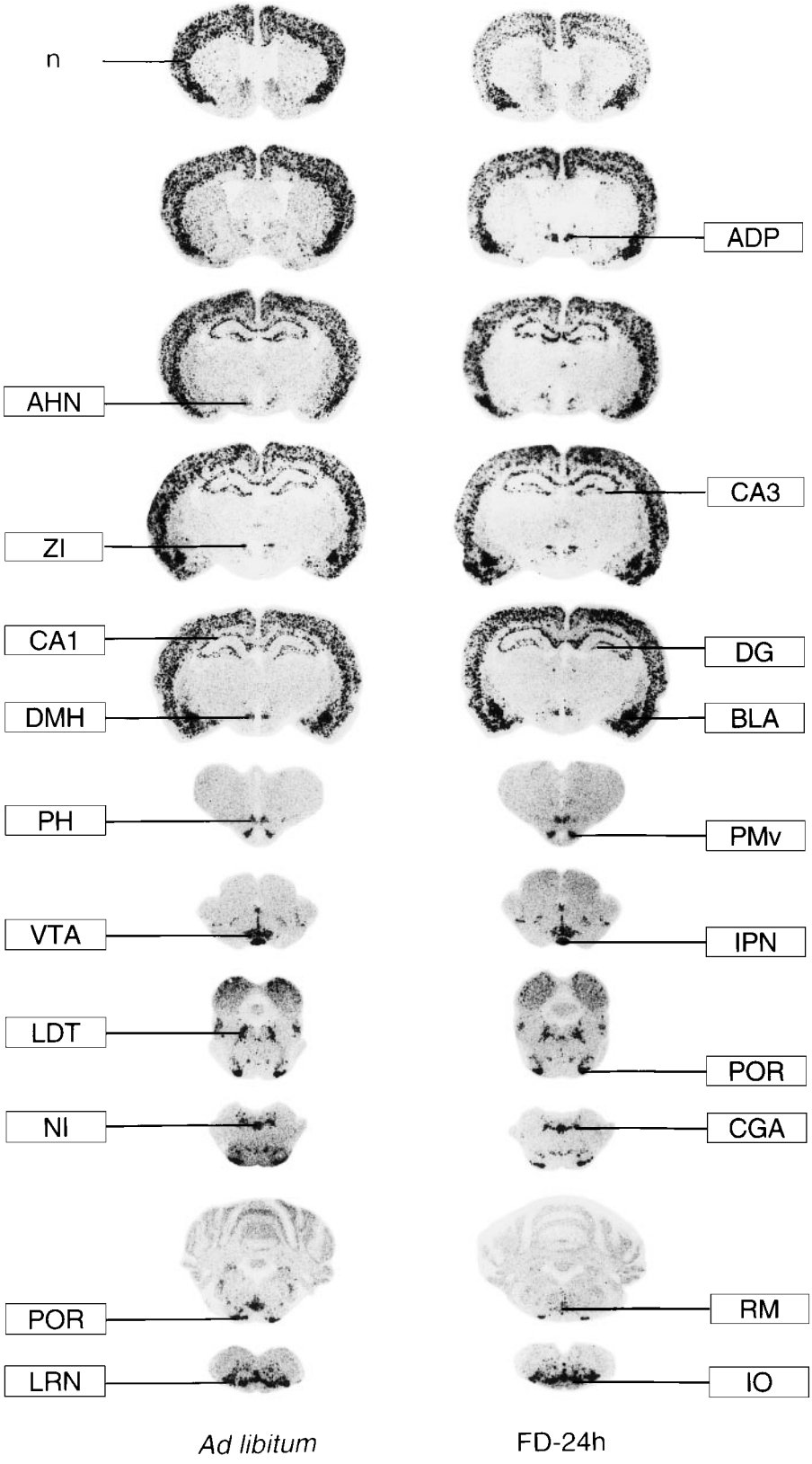

Fig. 2. Rostrocaudal distribution of the CRH-BP protein in brain of obese (*fa/fa*) Zucker rats either fed ad libitum or FD-24 h. Pictures are film autoradiograms of coronal brain sections that were hybridized with an antisense riboprobe complementary to rat CRH-BP mRNA.

sterile KPBS, the chromagen 3,3′-diaminobenzidine tetrahydrochloride (0.05%), and 1% hydrogen peroxide. Thereafter, tissues were rinsed in sterile KPBS, mounted onto poly-L-lysine-coated slides, rapidly dehydrated through graded concentrations of alcohol, cleared in xylene, and coverslipped with DPX.

Quantitative analysis of the hybridization signals. The hybridization signals revealed on NTB2-dipped nuclear emul-

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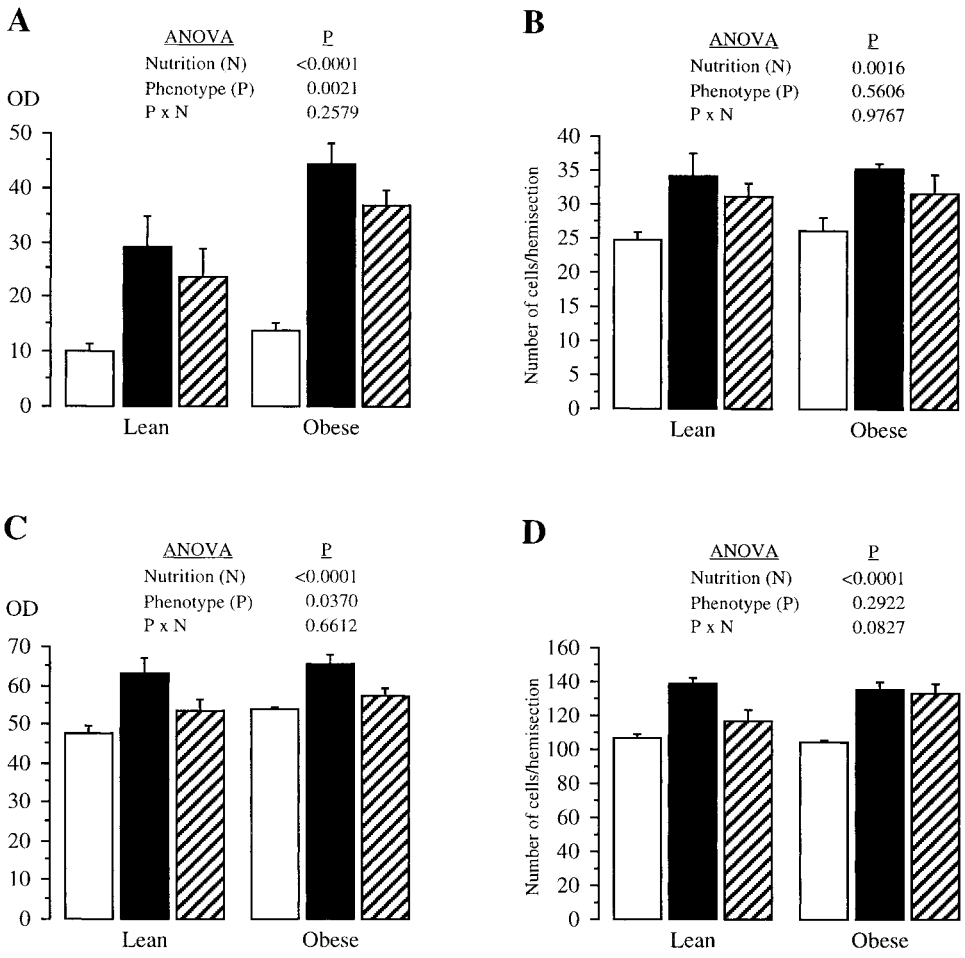

Fig. 3. Effect of 24 h of food deprivation and 6 h of refeeding after 24 h of food deprivation (RF-6h) on expression of CRH-BP mRNA in ADP (*A* and *B*) and BLA (*C* and *D*). Bar graphs illustrate optical density (OD; *A* and *C*) of hybridization signal of CRH-BP mRNA and number of positively labeled cells per hemisection (*B* and *D*) that were determined under microscope on coronal brain sections taken from *Fa/?* and *fa/fa* Zucker rats. A 2 × 3 ANOVA was used to examine the main and interaction effects of phenotype (lean and obese) and nutrition conditions [fed ad libitum (open bars), FD-24h (solid bars), or RF-6h (hatched bars)]. Bars represent means ± SE.

sion slides were analyzed and quantified under the dark-field illumination of a light microscope (Olympus, BX50) equipped with a black and white video camera (Sony, XC-77) using Image software (version 1.57 non-FPU, Wayne Rasband, NIH, Bethesda, MD). Both the number of cells, each identified as a cluster of silver grains overlying a thionine-stained perikaryon, and the optical density (OD) of the mRNA hybridization signal were quantified for each specific region. To measure the number of cells, the signal-to-background ratio was set at four, the background being determined outside the area of interest. The OD for each specific region was corrected for the average background signal, which was determined by sampling unlabeled areas outside of the areas of interest. When no hybridization signal was visible under dark-field illumination, the brain structures of interest were outlined under bright-field illumination and then subjected to densitometric analysis under dark-field illumination. Determination of cell number and OD were performed bilaterally on two or three tissue sections for the dorsal aspect of the MPOA (bregma from −0.15 to −0.50), and on five or six sections for the basolateral amygdala (BLA; bregma from −2.45 to −3.30) for each of the four animals assigned to each treatment. In the case of the pituitary, the number of cells and OD were determined on four to six hemisections of the anterior lobe for each animal. The mean number of cells and OD for individual animals were used to determine the mean ± SE for each group.

It should be mentioned that the analyses of the OD of mRNA levels done in this study are semiquantitative. Such analyses are widely used and have been proven to be valid and reproducible. The data presented herein have been analyzed separately by one of the authors and by an independent person who was blinded to the identification of the samples. For the analyses, care was taken to match brain sections across animals and to avoid reaching saturation of the hybridization signals.

Plasma determinations. An intracardial blood sample was taken in anesthetized rats immediately before the beginning of the intracardial perfusion with paraformaldehyde. Plasma glucose was determined (glucose oxidase method) using a glucose analyzer (Beckman, Palo Alto, CA). Serum corticosterone was determined by a competitive protein-binding assay (sensitivity, 0.058 nmol/l; interassay coefficient of variation, 9.0%) (26).

Statistical analysis. A 2 × 3 ANOVA was used to examine the main and interaction effects of "phenotype" (lean and obese) and "nutrition" [*1*) fed ad libitum, *2*) food deprived for 24 h, or *3*) refed for 6 h after 24 h of food deprivation] on the various dependent variables measured in this study. When a significant phenotype × nutrition interaction occurred, a posteriori comparisons were performed using the Bonferoni/Dunn multiple-comparison procedure. Each of the six groups used included four animals.

RESULTS

Body weight, plasma glucose, and plasma corticosterone. Food deprivation led to a body weight reduction in both lean and obese rats (Table 1). There was no difference between fed, food-deprived, and refed rats in

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plasma glucose levels. In lean and obese rats, food deprivation led to a reversible increase in corticosterone levels. The levels of corticosterone were lower in lean rats than in obese animals.

CRH-BP mRNA distribution in the brain of Zucker rats. The rostrocaudal distribution of CRH-BP mRNA in the brain of lean *Fa/?* and obese *fa/fa* rats is illustrated in Figs. 1 and 2. A positive hybridization signal for CRH-BP mRNA was detected in various regions of the brain including the neocortex, the hippocampal formation (CA1, CA3, and dentate gyrus), the BLA (lateral, basolateral, and basomedial amygdaloid nuclei), the hypothalamus, the midbrain, and the hindbrain. Within the hypothalamus, CRH-BP mRNA was abundantly found in the anterior hypothalamic nucleus, the dorsomedial hypothalamic nucleus, the posterior hypothalamic nucleus, and the ventral premammillary nucleus. Within the midbrain, CRH-BP mRNA was strongly expressed in the ventral tegmental area, the laterodorsal tegmental nucleus, and the interpeduncular nucleus. At the level of the hindbrain, the CRH-BP mRNA transcript was clearly located in the periolivary nucleus, the nucleus incertus, the central gray, the lateral reticular nucleus, and the inferior olivary complex.

Overall, there was no marked significant difference between lean and obese rats and between ad libitum-fed and food-deprived rats in the expression of CRH-BP for most of the regions examined. Careful examination of the brain sections under the microscope, however, revealed significant differences in the levels of expression in two structures, namely the MPOA and the BLA.

Effects of obesity and food deprivation on CRH-BP mRNA in the MPOA and BLA. Food deprivation and refeeding significantly affected the level of CRH-BP mRNA as well as the number of positively labeled neurons in the dorsal part of the MPOA and in the BLA of *Fa/?* and *fa/fa* rats. The increase in the level of CRH-BP transcript and number of positively stained cells was partially blunted by refeeding. The number of labeled cells increased in the anterodorsal preoptic nucleus (ADP) and in the BLA during food deprivation regardless of the phenotype of the animals. However, the levels of CRH-BP mRNA transcript of both the ADP and BLA were significantly higher in obese rats compared with lean animals, under all experimental treatments (Fig. 3). Figures 4 and 5 represent expression of the CRH-BP in the dorsal part of the MPOA and in the BLA.

Effects of obesity and food deprivation on CRH-BP immunoreactivity in the MPOA. The enhanced expression of CRH-BP mRNA levels in the dorsal aspect of the MPOA seen in lean and obese rats in response to food deprivation was paralleled by the appearance of neurons exhibiting CRH-BP immunoreactivity, which was not detected in the preoptic area of the lean and obese rats fed ad libitum (Fig. 6). The average number of the CRH-BP immunoreactive neurons in the dorsal part of

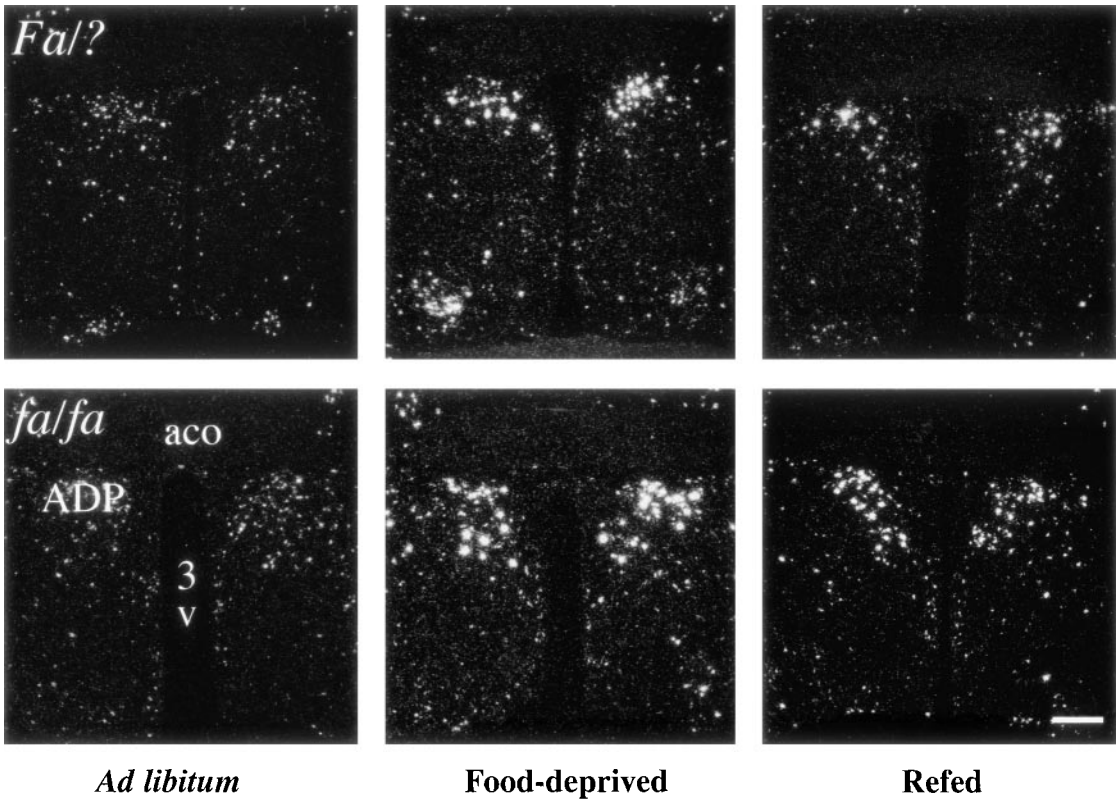

Fig. 4. Dark-field photomicrographs of coronal brain sections taken at level of medial preoptic area (MPOA) and hybridized with an antisense riboprobe complementary to rat CRH-BP mRNA. Brain sections were obtained from *Fa/?* and *fa/fa* Zucker rats fed ad libitum, FD-24h, or RF-6h. ACO, anterior commisure; 3v, third ventricle. Scale bar, 300 μm.

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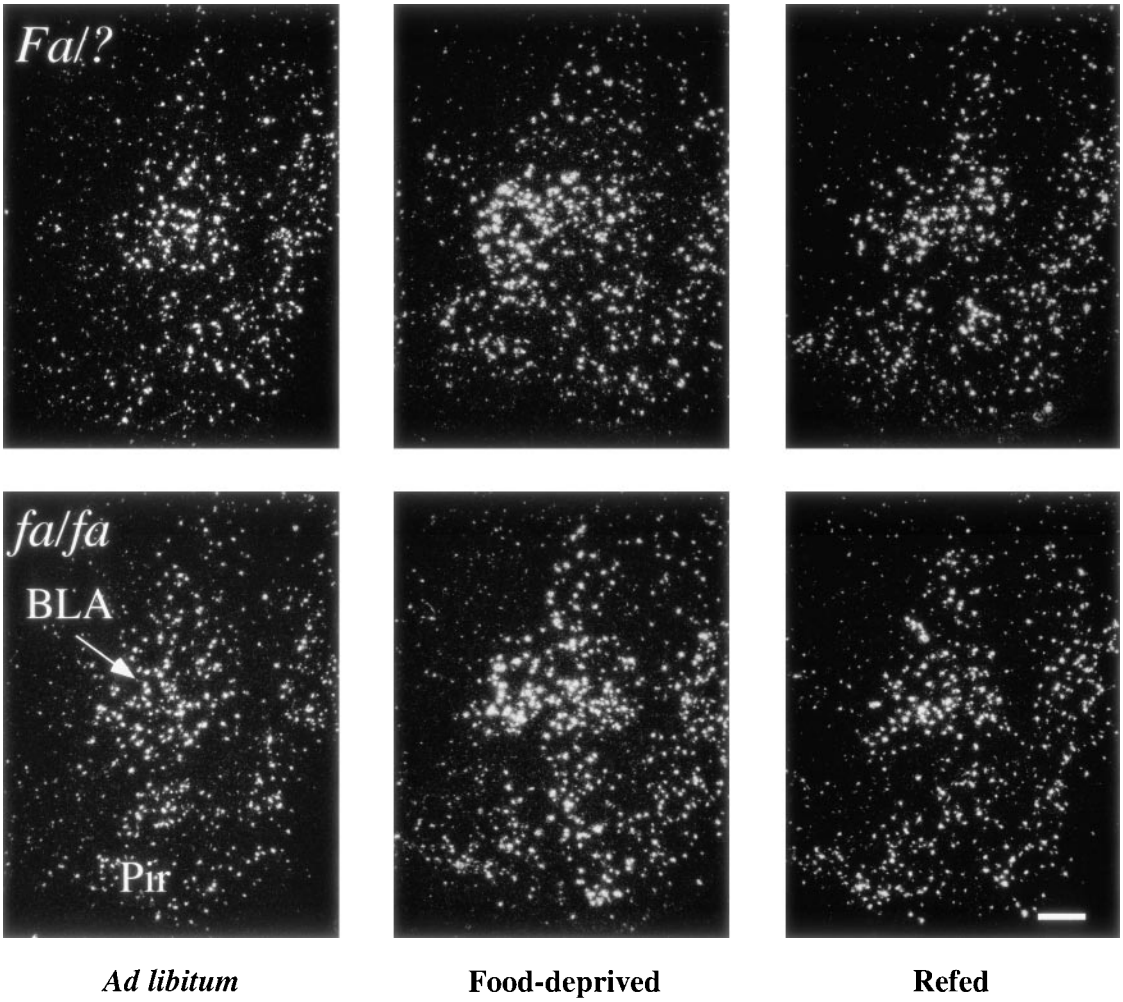

Fig. 5. Dark-field photomicrographs of coronal brain sections taken at level of BLA and hybridized with an antisense riboprobe complementary to rat CRH-BP mRNA. Brain sections were obtained from *Fa/?* and *fa/fa* Zucker rats fed ad libitum, FD-24h, or RF-6h. Pir, piriform area. Scale bar, 300 μm.

MPOA of food-deprived rats was, per each one half of coronal brain section, 7.5 ± 1.6 for lean and 9.7 ± 1.3 for obese rats.

Effects of obesity and food deprivation on CRH-BP mRNA in the pituitary. In the pituitary, the CRH-BP mRNA transcript was detected mostly in the anterior lobe (AL). The levels of CRH-BP mRNA in the AL of food-deprived and refed rats are shown in Fig. 7. In *Fa/?* rats, 24 h of food deprivation significantly decreased CRH-BP mRNA expression in the AL. When food was provided ad libitum, the number of positively labeled cells in the AL was significantly higher in lean rats than in obese animals. The 24 h of food deprivation dramatically decreased the number of cells expressing CRH-BP in the AL of lean rats. The positive hybridization signal for CRH-BP mRNA in the pituitary of the *Fa/?* and *fa/fa* rats is illustrated in Fig. 8.

DISCUSSION

The present study was conducted to verify whether experimental conditions that promote food intake and reduce thermogenesis can influence the expression of CRH-BP. Within the brain, CRH-BP represents a CRH-inactivating protein (4) that can bind up to 60% of all the neuronal CRH (4). Recently, a role for CRH-BP in the regulation of energy balance was suggested by a study of Heinrichs et al. (17), who showed that the CRH-BP inhibitor $CRH_{6—33}$ can efficiently reduce weight gain in obese *fa/fa* rats. An increase in CRH-BP in *fa/fa* rats would occur as a mechanism whereby the CRH tone could be reduced in obesity, as it has been suggested by many investigators (3, 30, 35, 36). CRH is an anorectic/thermogenic molecule whose metabolic effects after brain infusions have been reported to be particularly strong in obese rats (3, 35).

The present results provide evidence that food deprivation and obesity can induce the expression of the CRH-BP gene within the MPOA. The clear increase in the levels of CRH-BP expression in the dorsal part of the MPOA after food deprivation was paralleled by the appearance of neurons exhibiting CRH-BP immunoreactivity in this region. The number of neurons positively labeled for the CRH-BP transcript was, in general, higher than that exhibiting immunoreactivity. The reason for this is not known. The possibility that only a portion of cells that expressed the message

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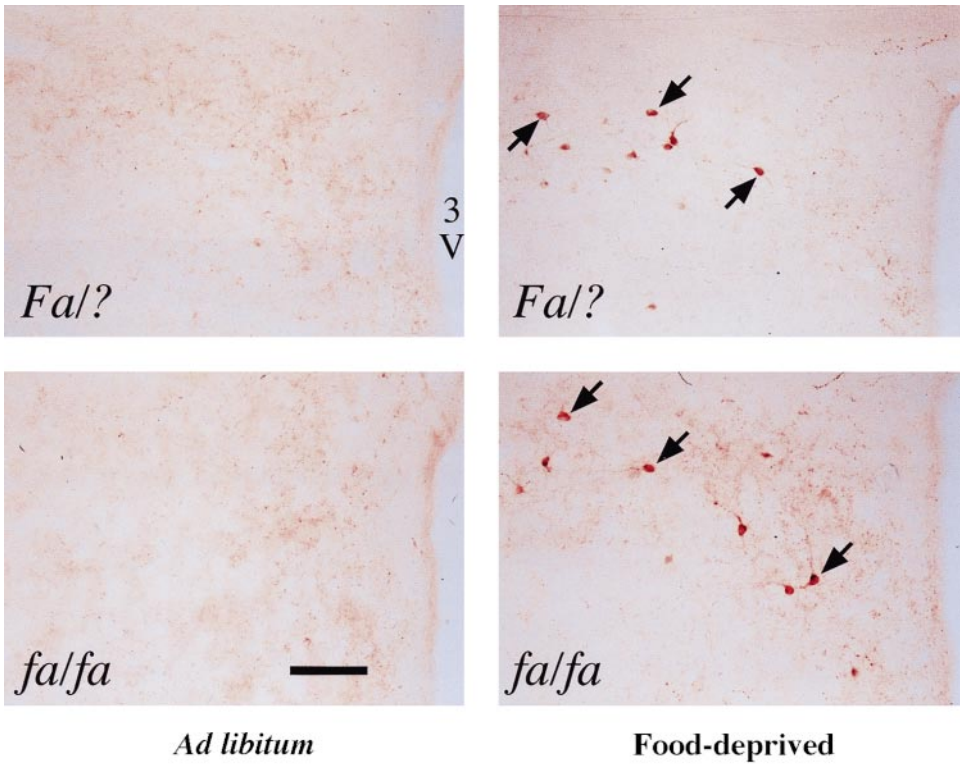

Fig. 6. Bright-field photomicrographs of coronal brain sections taken at level of MPOA and displaying cells immunoreactive to CRH-BP (some cells are indicated with arrows). Brain sections were obtained from *Fa/?* and *fa/fa* Zucker rats killed when fed ad libitum or FD-24h. Scale bar, 100 μm.

expressed the protein cannot be excluded. The difference may also be accounted for by the delay necessary for protein synthesis. The MPOA receives and sends numerous projections to many other areas of the brain, and an enhanced expression of CRH-BP in this part of the hypothalamus could have an impact on many functions. The MPOA is, for instance, involved in thermoregulation (19, 40) and has been suggested to be the site for the sympathetically mediated thermogenic action of CRH (14, 15). If we assume that CRH-BP may exert its quenching action on CRH within the MPOA itself, it may be argued that the enhancement of CRH-BP expression in food-deprived rats occurs as a mechanism to reduce thermogenesis. Whether a reduction in CRH-induced thermogenesis in the MPOA can lead to a substantial reduction in energy expenditure obviously needs to be substantiated. However, it is clear that food deprivation causes, in small laboratory rodents, a drop in energy expenditure by reducing the sympathetic drive to brown adipose tissue (16, 21). The fact that CRH-BP expression in neurons of the MPOA was higher in obese than in lean rats is consistent with the proposition that CRH-BP can play a physiological role in the regulation of energy balance. It is worth pointing out that repeated sessions of treadmill running, which cause an energy deficit, also induce the expression of CRH-BP in the medial preoptic area of the rat (E. Timofeeva and D. Richard, unpublished data).

Food deprivation also enhanced the CRH-BP mRNA expression in the BLA of both lean and obese rats. The BLA is, in terms of its neuroanatomy and neurochemistry, considered as a functional extension of the cortex

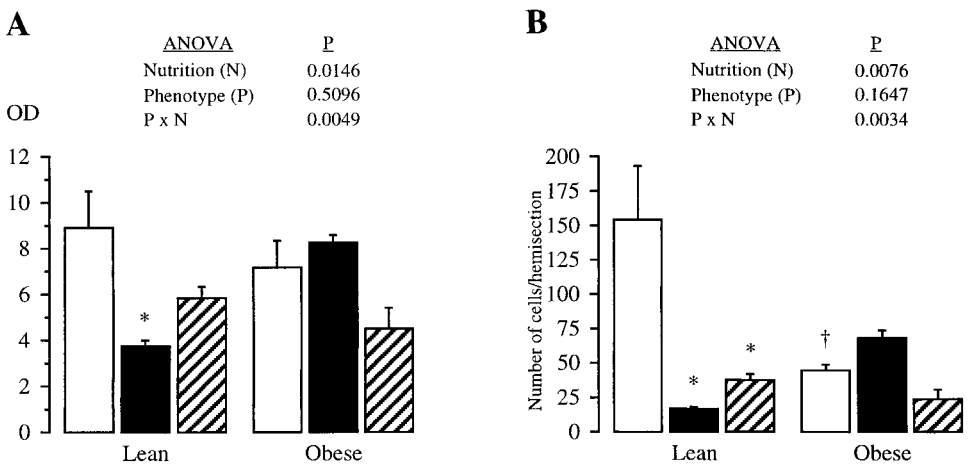

Fig. 7. Effect of FD-24h and RF-6h on expression of CRH-BP mRNA in anterior lobe of pituitary. Bar graphs illustrate OD of hybridization signal of CRH-BP mRNA (*A*) and number of positively labeled cells per hemisection (*B*) that were determined under microscope on pituitary sections taken from *Fa/?* and *fa/fa* Zucker rats. A 2 × 3 ANOVA was used to examine main and interaction effects of phenotype (lean and obese) and nutrition conditions (fed ad libitum, FD-24h, or RF-6h). A posteriori comparisons were performed using the Bonferroni/Dunn multiple-comparison procedure. *Significantly different from ad libitum-fed group within same phenotype; †significantly different from lean group within same nutrition status. Bars represent means ± SE.

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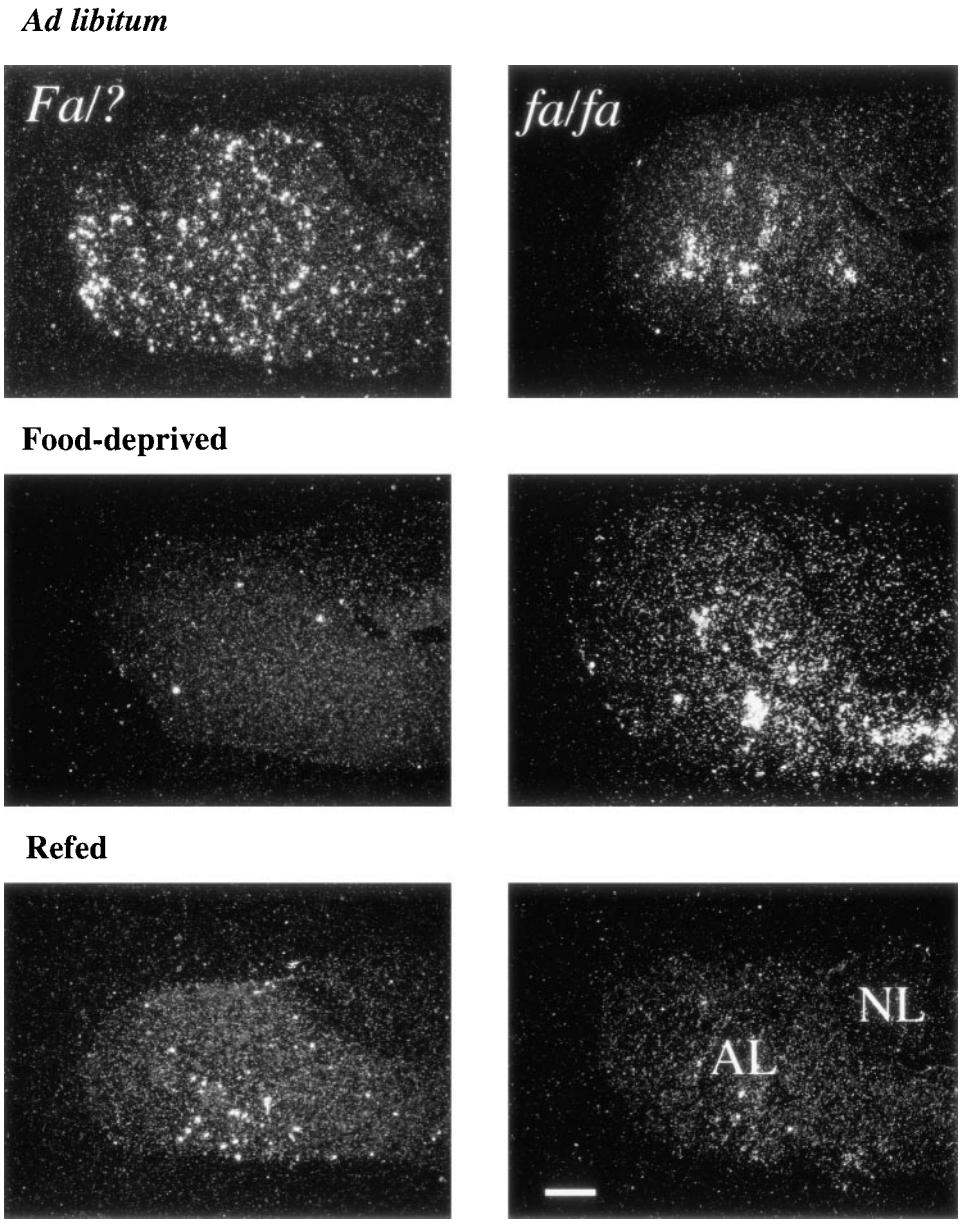

Fig. 8. Dark-field photomicrographs of pituitary sections hybridized with an antisense riboprobe complementary to rat CRH-BP mRNA. Brain sections were obtained from *Fa/?* and *fa/fa* Zucker rats fed ad libitum, FD-24h, or RF-6h. AL, anterior lobe of pituitary; NL, neural lobe of pituitary. Scale bar, 300 μm.

(1, 10), within which CRH-BP is also abundantly expressed (31). The nuclei of the BLA receive sensory information from the frontal and temporoinsular cortices and contain neurons that project to the central nucleus of the amygdala (CeA) (25). A substantial proportion of the cells that emerge from the BLA and project to the CeA are likely to be CRH-BP-containing neurons. Indeed, the present results (not shown) revealed the presence of numerous nerve fibers and terminals immunoreactive to CRH-BP in the CeA. The CeA also comprises CRH terminals and contains neurons abundantly expressing CRH. Within and outside the CeA, CRH could control behavioral responses related to stress, fear, and anxiety (23, 28, 39). In addition, there is evidence that the CeA could be involved in the regulation of energy balance, the lesion of this area promoting fat deposition in the rat (6). The demonstration that CRH levels rise in the CeA during food ingestion (29) is also consistent with the view that the CeA is involved in the feeding behavior. The enhanced CRH-BP expression in the BLA after food deprivation could represent a mechanism to reduce the anorectic action of CRH.

The mechanisms underlying the enhanced expression of CRH-BP in food-deprived and obese rats have yet to be determined. CRH-BP expression is positively stimulated by CRH (11), and there is evidence that food deprivation can stimulate the activity of CRH neurons in the MPOA of lean and obese rats (41), raising the possibility that CRH activity can be the regulator of CRH-BP expression in food-deprived and obese rats. That CRH can be a regulator of the expression of CRH-BP is also supported by the present results that demonstrate a difference between lean and obese rats in the effects of food deprivation on the pituitary expression of CRH-BP. In obese *fa/fa* rats, in which the

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hypophysiotropic CRH neurons are remarkably stimulated after food deprivation (41), there was a tendency for an increase in the expression of CRH-BP mRNA in the AL. In contrast, in lean rats, in which a decrease in the CRH mRNA expression in the PVH has been reported (8) after deprivation, there was a decrease in the expression of CRH-BP in the cells of the AL accompanied by a very large drop in the number of cells positively labeled for the CRH-BP transcript. Some variations in the expression of CRH-BP, therefore, seem to occur as a compensatory mechanism preventing an exaggerated action of CRH.

The reduction in the number of cells expressing CRH-BP at the level of the pituitary in obese rats is noteworthy. Indeed, such a reduction could represent a mechanism accounting for the increased activity of the pituitary-adrenal axis seen in the *fa/fa* rat. A reduction in the levels of CRH-BP in the pituitary could increase the availability of hypophysiotropic CRH. The mechanisms underlying this reduction, as well as those responsible for the abnormal pattern of CRH-BP mRNA distribution in the anterior pituitary, warrant further investigation.

In conclusion, the present study demonstrates that obesity and food deprivation can induce a positive and reversible effect on the expression of CRH-BP in the MPOA and BLA. If we assume the potential roles of these brain structures in the thermogenic and anorectic effects of CRH and the inactivating effect of CRH-BP on the CRH system, it can be argued that the increase in the expression of CRH-BP in food-deprived and obese rats occurs as an adaptation to reduce energy expenditure and to stimulate food intake to limit the effects of food deprivation on energy reserves.

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