0013-7227/01/$03.00/0
Endocrinology

Vol. 142, No. 7
Printed in U.S.A.

Sucrose Ingestion Normalizes Central Expression of Corticotropin-Releasing-Factor Messenger Ribonucleic Acid and Energy Balance in Adrenalectomized Rats: A Glucocorticoid-Metabolic-Brain Axis?*

K. D. LAUGERO†, M. E. BELL, S. BHATNAGAR, L. SORIANO, AND M. F. DALLMAN

Department of Physiology (K.D.L., M.E.B., M.F.D.), University of California San Francisco, San Francisco, California 94143-0444; Department of Psychology (S.B.), University of Michigan, Ann Arbor, Michigan 48109-1109; and AGY Therapeutics (L.S.), South San Francisco, California 94080

ABSTRACT

Both CRF and norepinephrine (NE) inhibit food intake and stimulate ACTH secretion and sympathetic outflow. CRF also increases anxiety; NE increases attention and cortical arousal. Adrenalectomy (ADX) changes CRF and NE activity in brain, increases ACTH secretion and sympathetic outflow and reduces food intake and weight gain; all of these effects are corrected by administration of adrenal steroids. Unexpectedly, we recently found that ADX rats drinking sucrose, but not saccharin, also have normal caloric intake, metabolism, and ACTH. Here, we show that ADX (but not sham-ADX) rats prefer to consume significantly more sucrose than saccharin. Voluntary ingestion of sucrose restores CRF and dopamine-β-hydroxylase messenger RNA expression in brain, food intake, and caloric efficiency and fat deposition, circulating triglyceride, leptin, and insulin to normal. Our results suggest that the brains of ADX rats, cued by sucrose energy (but not by nonnutritive saccharin) maintain normal activity in systems that regulate neuroendocrine (hypothalamic-pituitary-adrenal), behavioral (feeding), and metabolic functions (fat deposition). We conclude that because sucrose ingestion, like glucocorticoid replacement, normalizes energetic and neuromodulatory effects of ADX, many of the actions of the steroids on the central nervous system under basal conditions may be indirect and mediated by signals that result from the metabolic effects of adrenal steroids. (*Endocrinology* **142:** 2796–2804, 2001)

ADRENALECTOMY (ADX) alters CRF, ACTH secretion, sympathetic outflow, and energy balance (1–3). These effects result from the loss of glucocorticoids because replacement with the adrenal steroid corrects these deficiencies (1–4). However, and unexpectedly, we have found that, like corticosteroid replacement, voluntary ingestion of sucrose also corrects increased ACTH secretion, sympathetic outflow, and energy balance in ADX rats (4). Furthermore, whereas intact rats normally like equally sweet but nonnutritive saccharin to drink, ADX rats consume very little saccharin, and these rats exhibit the normal imbalances that accompany ADX (1). These results suggest that ADX rats develop a preference for calorically rich sucrose compared with nonnutritive saccharin, and that this behavioral expression is critical to the restoration of normal metabolic, neuroendocrine, and autonomic function. The results also suggest that the postingestive (energetic) effects of sucrose alter systems in the central nervous system (CNS) that control neuroendocrine, autonomic, and behavioral expression in the absence of adrenal steroids.

As with stress, many of the behavioral, neuroendocrine, and autonomic effects of ADX may be mediated by changes in central CRF. CRF administered to the brain mimics (5–7) and mediates (8–11) stress-induced changes in behavior, caloric intake and storage, autonomic outflow, and hypothalamic-pituitary-adrenal (HPA) activity. Glucocorticoids modulate central CRF under both stressed and basal conditions, and the expression of this neuropeptide in the paraventricular nuclei (PVN) of the hypothalamus is inhibited in strict relationship to the systemically provided dose of corticosterone (6, 12, 13). CRF messenger RNA (mRNA) and content in the central nucleus of the amygdala (CeA) is also responsive to ADX and glucocorticoids (3, 14–16), and the PVN and CeA are integral to an anatomical and functional circuit that mediates neuroendocrine, autonomic, and metabolic function (17). Furthermore, CRF in both sites is responsive to the state of feeding (18, 19), and it is well known that the HPA and autonomic responses to stress depend on the nutritional state of the animal (20, 21). Therefore, sucrose ingestion in the ADX rat may alter these central CRF systems.

Finally, brain stem catecholaminergic systems project to these central CRF cell groups (22, 23), and ADX alters norepinephrine turnover and concentration in various brain sites (*e.g.* (2)]. Norepinephrine also stimulates activity in the hypothalamic PVN after both stress and ADX (24, 25). Moreover, glucocorticoids and ADX specifically influence catecholaminergic activity in the brain stem medulla and pons (26–28). Catecholaminergic cell groups in A2/C2 and locus coeruleus (LC) respond to metabolic cues from the periphery and innervate CRF-expressing neurons in the PVN (23) and the forebrain (29), where norepinephrine is important for learning, arousal, and attention (29–31).

In these studies, we asked whether ADX induces a pref-

Received January 25, 2001.
Address all correspondence and requests for reprints to: Mary F. Dallman, Department of Physiology, Box 0444, University of California San Francisco, San Francisco, California 94143-0444. E-mail: dallman@itsa.ucsf.edu.
* This work was supported, in part, by NIH Grant DK-28172.
† Supported by Grant DK-07418

erence for sucrose, a metabolically useful substrate. Although our previous results (1, 4) suggested an ADX-induced preference for sucrose, rats in those studies were not given a choice between the two sweet drinks. Here we examine consummatory behavior in ADX and control rats allowed choice between the two sweet drinks and saline, in a three-bottle test. To determine the effects of drinking sucrose on the central nervous system, we measured the expression of CRF mRNA in hypothalamus and amygdala, and dopamine-β-hydroxylase (DBH) in A2/C2 and locus coeruleus in ADX and control (sham-ADX) rats drinking saline ± saccharin or sucrose. We also examined the effects of sucrose consumption on circulating metabolites (*e.g.* glucose) and metabolic hormones (*e.g.* leptin). These metabolic variables are known to convey information of energy use and storage to the CNS, and thus might mediate the metabolic effects of sucrose on brain.

Materials and Methods

In all experiments, male rats (Sprague Dawley from Bantin and Kingman, Gilroy, CA) weighing 190 ± 10 g were individually caged in a temperature-controlled (21–23 C) and light-controlled (lights on 0700–1900 h) room. Rats were allowed to adapt to their new environment for at least 2 days before studies were begun. All studies were approved by the University of California San Francisco Institution Animal Care and Use Committee.

Bilateral ADX or sham-ADX was performed under isoflurane anesthesia by the dorsal approach. Skin incisions were clipped. All rats were provided with access to rat chow to eat (Purina no. 5008) and 0.5% NaCl to drink *ad libitum*. Body weights as well as food, saline, sucrose and saccharin intakes were measured daily. For experiments that tested the effects of sucrose ingestion on neuropeptide mRNA expression, energy balance, and hormones, rats were decapitated under basal conditions, brains were removed, and trunk blood was collected between 0800–0830 h. Brains and plasma were stored at −80 C and −20 C, respectively. Plasma corticosterone, leptin, and insulin concentrations were analyzed by RIA (ICN Biomedicals, Inc., Orangeburg, NY, and Linco Research, Inc., St. Charles MO, respectively). Enzyme (colorimetric) assays were used to determine plasma concentrations of glucose, triglyceride (both Sigma Diagnostics, St. Louis, MO) and FFA (Wako Chemicals USA Inc., Richmond, VA), and selected fat pads were also collected and weighed (18). Brain sections (14 μm), from a 1 in 8 series of fixed and acetylated coronal slices encompassing the rostral-caudal extent of each nucleus, were hybridized, *in situ*, with an antisense riboprobe to CRF mRNA (Dr. Kelly Mayo, Northwestern University, Evanston, IL) or DBH mRNA (Dr. Dona Wong, Stanford University, Stanford, CA). Hybridizations for each experiment were carried out in a single lot followed by analysis of the signal on x-ray films (14-day experiment) or emulsion (5-day experiment), guided by cresyl violet staining of adjacent sections (n = 4–7 rats/group). *In situ* hybridization and semiquantitative analyses were carried out as previously described by Viau *et al.* (16).

Experiments

1) We tested whether ADX induces a preference for sucrose compared with saccharin. ADX and sham-ADX rats were provided with an *ad libitum* three-bottle choice among 1 M sucrose, 2 mM saccharin, and 0.5% saline immediately following surgery and for the next 5 days. Daily intakes were measured, and bottles were rotated each day to minimize any positional effects. 2) Previously, in a 14-day experiment, we had tested in ADX rats the effects of corticosterone and/or 9 days of drinking either sucrose or saccharin on energy balance and hormones (1, 4). Here, we measured in the brains of those rats the effects of sucrose or saccharin ingestion on CRF and DBH mRNA expression. 3) In a second experiment of 5 days duration, we measured the brain neuropeptide mRNA expression, energetic, and hormonal effects of continuous access to sucrose or saccharin in ADX or sham-ADX rats. Five groups were studied: sham-ADX, sham-ADX + sucrose, ADX + saline, ADX + saline and sucrose, and ADX + saline and saccharin. All drinks were provided immediately following the adrenal surgery, which initiated the experiment.

Statistical analyses

Data were analyzed by one-way ANOVA. Comparison of the results from ADX rats drinking only saline, and those allowed saccharin as well, showed that saccharin had no effect on any variable; therefore, the results from the two groups were pooled (saline ± saccharin) for further analysis and presentation of all data. A significant ($P \leq 0.05$) global effect of ANOVA was followed by posthoc tests of individual group differences (Fisher's PLSD). Simple linear regression analyses were performed to determine the possible relationship between some variables. ANOVA was used to test the null hypothesis that slopes did not significantly differ from 0 ($P \leq 0.05$).

Results

Sucrose preference in ADX rats

In all experiments to date, our results have shown that ADX consistently and substantially curbs saccharin drinking compared with that of sucrose. While those results suggested that ADX rats prefer sucrose to the equally sweet saccharin, we tested this hypothesis directly by providing rats with a three-bottle choice (saline, saccharin, and sucrose). The results definitively showed that ADX rats prefer sucrose to saccharin, whereas sham-ADX rats ingested equal amounts of both solutions (Fig. 1, $P \leq 0.01$). Sham-ADX rats drank a total volume of 129 ± 23 ml sucrose, 123 ± 18 ml saccharin and 118 ± 42 ml saline in 5 days; ADX rats drank a total volume of 56 ± 11 ml sucrose, 13 ± 1 ml saccharin and 209 ± 22 ml saline in 5 days. Thus, despite the distinct preference shown by the ADX rats for sucrose, they drank only 43% as much sucrose solution as sham-ADX rats ($P < 0.001$), as shown previously (4).

Changes in metabolism provided by sucrose

Sucrose consumption by ADX rats maintained normal energy balance during the 5-day study (Fig. 2). Body weight gain (2A), caloric intake (2B), caloric efficiency (2C), and fat

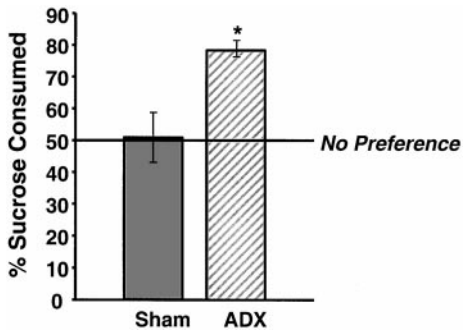

FIG. 1. Adrenalectomized rats prefer sucrose (1 M) to saccharin (2 mM), when given a choice between these two equally sweet drinks. Shams like the sweet drinks equally. Rats were given a three-bottle choice between sucrose, saccharin, and 0.5% saline. Drinks were provided *ad libitum* and bottles were rotated daily to avoid positional effects. The percentage of sucrose consumed = the cumulative amount of sucrose drunk over the 5-day study divided by the cumulative consumptions of sucrose + saccharin, with the result multiplied by 100. See *Results* for absolute intake of sucrose and saccharin. *, Groups are significantly different ($P \leq 0.05$). *Bars*, Group means ± SEM (n = 7 ADX, 5 sham-ADX rats).

stores (2D) were normal compared with sham-ADX controls, and all variables were significantly greater in ADX rats drinking sucrose than those in ADX rats drinking saline ± saccharin ($P \leq 0.05$). Sucrose ingestion also significantly ($P \leq 0.05$) prevented the ADX-induced decrease in circulating leptin and triglycerides (TG), but not insulin (Table 1). Insulin was, however, 40% higher ($P \leq 0.10$) in sucrose-drinking ADX rats compared with ADX rats drinking saline ± saccharin and did not differ significantly from sham-ADX controls. There were no differences in basal circulating glucose but FFA concentrations were increased in sham-ADX rats drinking sucrose. We cannot explain the significant increase in free-fatty acids in the sham-ADX group drinking sucrose compared with all other groups; however, we do know that this finding is not consistent across several sets of subsequent experiments.

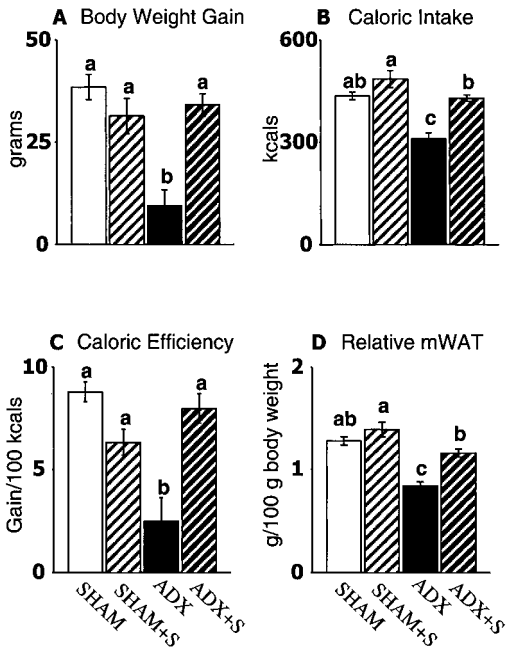

FIG. 2. Sucrose (ADX-S) consumption by ADX rats restored: A, total body weight gain (g); B, caloric intake (kcal); C, caloric efficiency (mg body weight gain/kcal intake); and, D, relative mesenteric white adipose tissue (mWAT) weight to normal. All variables represent the net quantity over 5 days. All data were analyzed by one-way ANOVA and, when significant, posthoc analyses were performed (Fisher's PLSD). Groups with *different letters* differ significantly ($P \leq 0.05$). *Bars*, Group means ± SEM (n = 5–11/group). In this, and subsequent figures, SHAM, sham ADX; SHAM + S, sham ADX rats drinking sucrose; ADX, ADX rats drinking saline ± saccharin; ADX + S, ADX rats drinking sucrose.

CRF mRNA in the PVN

In the 14-day experiment, CRF mRNA expression in the paraventricular nuclei PVN was elevated, as expected in the ADX rats drinking saline ± saccharin (Fig. 3). By contrast, in the rats which had been ADX for 14 days and drinking sucrose, CRF mRNA expression in PVN was not different from the levels in sham-ADX rats that drank saline ± sucrose (Fig. 3; ANOVA: $F_{3,17} = 32.9$, $P < 0.0001$).

In the 5-day experiment, CRF mRNA levels in the PVN were not significantly reduced by allowing ADX rats sucrose to drink (Fig. 4A; ANOVA:$F_{3,24} = 4.4$, $P = 0.014$); however, the variance in this group was unusually high. Linear regression of the relationship of CRF on sucrose (Fig. 4, B and C) explains why the overall mean CRF mRNA (Fig. 4A) was not significantly reduced in sucrose-drinking adrenalectomized rats. Essentially the entire variance (97%) of CRF in the PVN of the ADX rats drinking sucrose was explained by the quantity of sucrose ingested during the last day of the experiment. In the 14-day study, the level of CRF mRNA in the PVN did not correlate with sucrose ingested during the last day (not shown).

CRF mRNA in the CeA

In both the 14-day and 5-day studies, CRF mRNA expression in the amygdala (CeA) of ADX rats drinking saline ± saccharin was significantly reduced, compared with sham-ADX (Fig. 5; 5-day ANOVA: $F_{3,22} = 3.2$, $P = 0.045$, 14-day ANOVA: $F_{3,17} = 5.4$, $P = 0.009$). In contrast to ADX rats drinking saline ± saccharin, CRF mRNA expression in the CeA of ADX rats drinking saline and sucrose was not different from values observed in sham-ADX rats in either study. Only on day 5, sham-ADX rats drinking saline and sucrose had significantly decreased CRF mRNA expression in the CeA compared with sham-ADX rats drinking saline (Fig. 5B). These changes in CRF expression in both PVN and CeA were specific, because there were neither ADX- nor sucrose-induced changes in CRF expression in the bed nuclei of the stria terminalis (not shown).

DBH in A2/C2 and in LC

At 5 days (n = 3–6/group), DBH expression in the locus coeruleus was increased by ADX rats drinking saline ± saccharin, compared with ADX rats drinking sucrose (5-day ANOVA: $F_{2,11} = 4.7$, $P = 0.034$). By 14 days, the overall effect of sucrose was not significant ($P = 0.22$), although there was still a significant difference between ADX rats drinking sucrose compared with those drinking saline ± saccharin. DBH expression in ADX rats drinking sucrose was not different

TABLE 1. Effect of sucrose ingestion on circulating metabolic hormones and metabolites

Variable	Treatment Values			
	Sham Saline	Sham Sucrose	ADX Saline	ADX Sucrose
Leptin (ng/ml)	2.7 ± 0.1^a	3.1 ± 0.4^a	0.7 ± 0.1^b	2.0 ± 0.2^a
Insulin (ng/ml)	2.8 ± 0.2^a	2.2 ± 0.3^a	1.5 ± 0.3^b	$2.1 \pm 0.2^{a,b}$
Triglyceride (mg/dl)	72.4 ± 5.1^a	117.7 ± 8.1^b	43.0 ± 3.0^c	95.6 ± 18.0^b
Free Fatty Acid (mEq/liter)	0.2 ± 0.1^a	0.9 ± 0.1^b	0.2 ± 0.1^a	0.2 ± 0.1^a
Glucose (mg/dl)	131.8 ± 3.4^a	134.6 ± 5.2^a	124.4 ± 7.0^a	139.8 ± 5.3^a

Values = means ± SEM, n = 5–11.
Within a row, means assigned *different letters* are statistically different ($P \leq 0.05$).

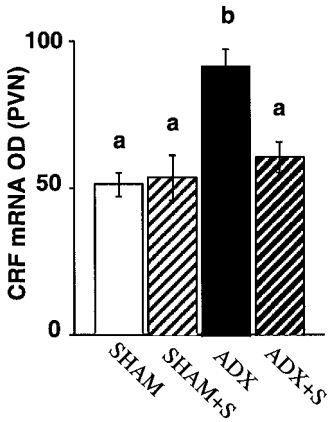

FIG. 3. CRF mRNA expression in the hypothalamic PVN was increased by ADX, but sucrose ingestion completely prevented this ADX effect in the 14-day study (ADX+ S). Groups with *different letters* differ significantly ($P \leq 0.05$). *Bars*, Group means ± SEM (n = 5–6/group).

from sham-ADX rats (Fig. 6, A and B). In the medulla (Fig. 6, C and D) at 5 days there was a clear effect of ADX on DBH mRNA content in A2/C2 with and without sucrose (Fig. 6C; ANOVA: $F_{3,15} = 3.5$, $P = 0.042$). However, by 14 days after ADX, there were no differences in DBH mRNA content in the A2/C2 cell groups in any group (Fig. 6D). Consistent with the increased metabolic water load of animals drinking sucrose, DBH expression in the basolateral medullary cell groups (A1/C1), which preferentially innervate magnocellular AVP-expressing neurons in the PVN (23), was markedly reduced in both groups drinking sucrose compared with groups given only saline ($P \leq 0.01$; not shown).

Discussion

Glucocorticoids have profound effects on the brain that alter the physiology and behavior of the organism. These adrenal steroids influence neural systems that control learning and memory (31–33), feeding (1, 34), drinking sweet solutions (1, 4), autonomic output (1, 4, 35) and activity of the hypothalamic-pituitary-adrenal axis (36). Evidence of the importance of glucocorticoids on these variables is demonstrated by the classic studies of glucocorticoid removal through ADX and steroid replacement. ADX changes CRF and NE activity in brain (2, 3), increases ACTH secretion, sympathetic outflow (1, 4), and disrupts behavior [*e.g.* feeding (1, 34), wheel running (37, 38) and drinking sweet solutions (1, 4)]; all are corrected by treatment with adrenal steroids. Our results demonstrate that, like the effects of glucocorticoid replacement, when adrenalectomized rats drink sufficient sucrose energy balance, and expression of CRF in hypothalamus and amygdala are normal. Furthermore, sucrose ingestion may prevent enhanced catecholaminergic activity in medullary and LC cell groups, as suggested by the normal levels of expression of DBH mRNA. Finally, the loss of glucocorticoids alters ingestion of sweet solutions so that a preference for sucrose is developed, and access to this metabolically useful substrate, but not saccharin, induces normal caloric intake. Together, our results suggest that: 1) the metabolic effects of sucrose ingestion induce signals (metabolic and/or hormonal) in the periphery that alter expression of central CRF; 2) the preference for sucrose to the equally palatable saccharin drink in ADX rats develops as a consequence of the postingestive energy that follows sucrose ingestion; 3) the signals that arise from restored metabolism may be transmitted, at least in part, via catecholaminergic pathways that are known to innervate central CRF neurons; and finally, 4) the normal action of glucocorticoids on CRF, under basal conditions, is mediated by effects of the steroid on metabolism rather than a direct effect on brain (summarized in Fig. 7).

CRF in the PVN

The results of studies in ADX rats have suggested that corticosterone acts directly on brain to inhibit expression of CRF in the PVN, and consequently, the downstream activity in the HPA axis. Replacement of ADX rats with corticosterone maintains normal CRF expression (3, 12, 13, 39), and CRF expression in the PVN is inhibited in strict relationship to the systemically provided dose of corticosterone. However, whereas the brain mediates the feedback effects of the adrenal steroid, the site(s) of glucocorticoid action under basal conditions have yet to be identified. Various brain lesions have been shown to alter activity in the HPA axis (40–43), and implants of corticosterone in amygdala, the preoptic area and in the prefrontal cortex have been shown to inhibit stress-induced ACTH secretion [*e.g.* (44–46)]. However, attempts to determine a specific site of action in brain of corticosterone feedback on basal HPA activity have been generally unsuccessful (47, 48) with one exception.

Corticosterone implants were shown to inhibit basal ACTH concentrations in ADX rats after implantation into the hippocampus, amygdala and lateral septum, but not PVN (49). Those results were obtained in rats with chronic implants, and the feedback efficacy of the steroid may have been mediated by implant-induced changes in metabolism. Here, we show that sucrose ingestion, independently of glucocorticoids, restores CRF mRNA expression in the parvocellular region of the PVN of ADX rats. The decrease in CRF mRNA suggests that sucrose ingestion inhibits drive to the CRF neuron. CRF cell bodies in the PVN send axons to the median eminence and secrete CRF into the pituitary portal vasculature to stimulate ACTH. The sucrose-induced reduction in CRF mRNA in the 14-day ADX rats probably was reflected by reduced CRF peptide secretion, because, unlike the ADX group drinking saccharin (1) circulating ACTH concentrations were not significantly elevated in the ADX group drinking sucrose compared with sham-ADX (4). Thus, sucrose ingestion is capable of supplanting the negative feedback function of glucocorticoids in the hypothalamic-pituitary-adrenal axis.

CRF in the CeA

As in the PVN, our results demonstrate a glucocorticoid-independent effect of sucrose ingestion on CRF mRNA expression in the CeA of ADX rats. Several lines of evidence suggest that high concentrations of glucocorticoids also increase CRF expression in the CeA. Corticosterone pellets implanted over the dorsal margin of the CeA increases CRF mRNA expression in intact rats (15). Systemic administration

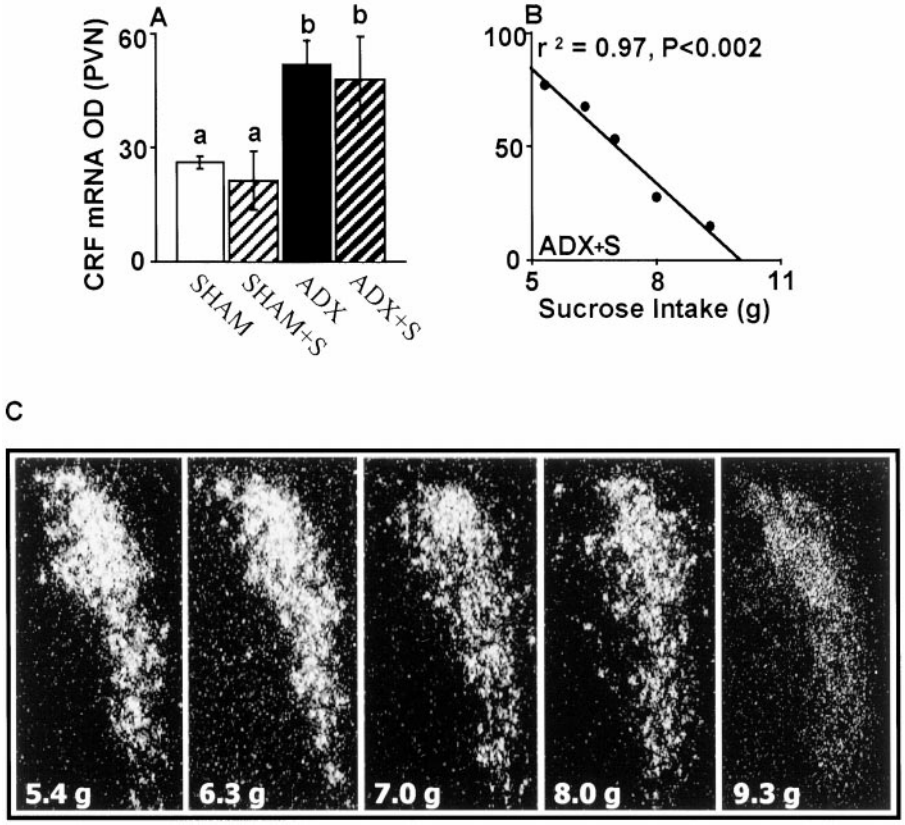

FIG. 4. As expected, ADX increased CRF mRNA expression in the PVN relative to shams (SHAM). Although the mean CRF mRNA expression in ADX, sucrose-drinking rats (ADX + S) was not different from that of ADX rats drinking saline ± saccharin in the 5-day study (A), the variance was essentially explained by the amount of sucrose drunk over the last 24 h in ADX rats (B, C). Groups with *different letters* differ significantly ($P \leq 0.05$). *Bars*, Group means ± SEM (n = 5–11/group).

of high concentrations of corticosterone also increases CRF mRNA expression in the CeA and parallel elevations in median eminence CRF peptide have been shown to occur following treatment with the steroid (3, 14). However, these studies, which exposed the CeA to high (stress) concentrations of corticosterone, do not prove that low to average glucocorticoid concentrations act directly on brain to maintain basal CRF expression in ADX rats. After adrenalectomy, CRF mRNA expression in the CeA decreases; this change, and its reversal by corticosterone, is blocked by lesions of the PVN (39). These results suggest strongly that the ADX-induced decrease in CRF expression in CeA is not directly glucocorticoid-dependent. Although high glucocorticoid concentrations clearly increase CRF expression in the CeA in intact rats, they also increase CRF mRNA in the bed nuclei of the stria terminalis (3, 14), although CRF in the BNST does not change after ADX (this study and Refs. 3, 50). There is no evidence, to our knowledge, that demonstrates a direct restorative effect of centrally administered glucocortcoids on CRF expression in the CeA of ADX rats. Therefore, it seems unlikely that glucocorticoids normally act directly on brain to sustain basal levels of CRF expression in the amygdala.

Sucrose on energy and brain pathways

In addition to the effects of sucrose ingestion on brain CRF, we have shown that, like glucocorticoid replacement, sucrose consumption also normalizes energy balance in ADX rats (4). These effects of sucrose on energy balance in ADX rats (1, 4) were confirmed here in the 5-day study. Thus, glucocorticoid replacement and sucrose ingestion in ADX rats appears to induce a common effect (*e.g.* on metabolism) that consequently alters activity in the same, or a closely parallel pathway. The effect of corticosterone on CRF mRNA expression in the PVN and/or the amygdala of ADX rats appears to be a consequence of the steroid's effects on metabolism rather than a direct effect on brain (see Fig. 7). In addition to our results showing parallel effects of sucrose ingestion and corticosterone on central CRF and energy balance, the neuroanatomical and functional substrates for a glucocorticoid-metabolic-brain axis are well described (51).

The amygdala and PVN receive information about metabolic function (22), control autonomic outflow (17), and affect energy balance (51, 52). Bilateral lesions of the amygdala result in fat rats, and it has been suggested that CeA-regulated sympathetic outflow is important to the normal maintenance of fat stores (53–55). Feeding also increases CRF release in the CeA of rats (19). ADX reduces food intake, enhances sympathetic outflow and depresses lipogenesis, whereas sucrose ingestion restores these metabolic variables to normal, as demonstrated by normal uncoupling protein content in thermogenic brown adipose tissue, fat stores and triglyceride production in the ADX, sucrose-drinking rats (4). Like the amygdala, the hypothalamic PVN is also implicated as an important integrator and regulator of food intake and energy balance. Lesions of the PVN result in a food intake-dependent obesity (56), and glucocorticoids are required for expression of this obesity (57) as well as the obese phenotype in *fa/fa* rats (58). Furthermore, the hypothalamic-pituitary-

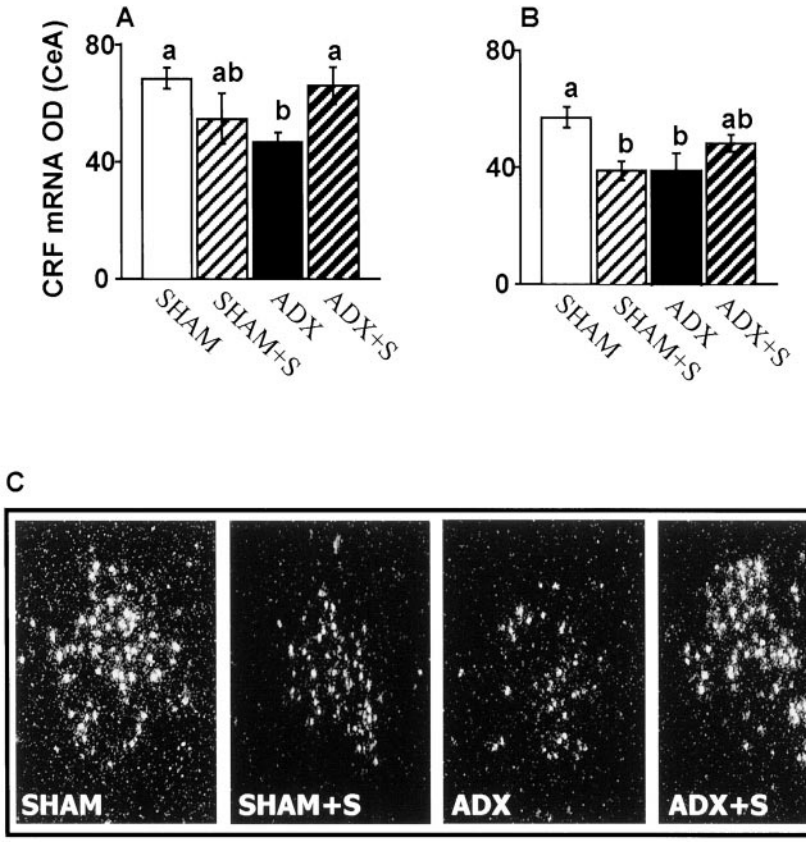

FIG. 5. As expected, ADX decreased CRF mRNA expression in the amygdala (CeA) compared with shams (SHAM), and this did not occur when the ADX rats drank sucrose. A = 14 day (n = 5–6/group); B = 5 day (n = 5–11/group); C = an example from each group in the 5-day experiment. Groups with *different letters* differ significantly ($P \leq 0.05$). *Bars*, Group means ± SEM.

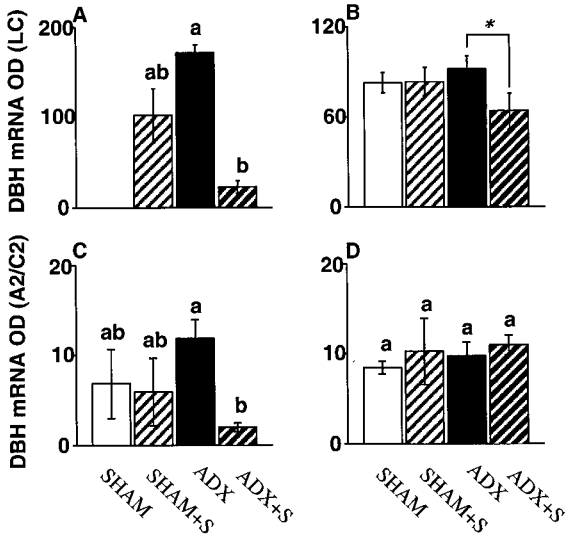

FIG. 6. Compared with ADX rats drinking only saline, DBH mRNA expression in both locus coeruleus (LC; A, B, n = 3–7/group) and medullary (A2/C2; C, D, n = 3–8/group) cell groups was decreased in ADX rats drinking sucrose (A and C). Sham-saline rats are missing in panel A because sections were lost. Although there was no overall treatment effect (ANOVA, $P \geq 0.20$) on DBH expression in the 14-day study, when only ADX rats were compared, sucrose significantly lowered DBH expression in the LC (B; * = significanty different by *t* test). Groups with *different letters* differ significantly ($P \leq 0.05$). *Bars*, Group means ± SEM.

adrenal response to stress depends on the caloric status of the rat (20). Clearly, both of these CRF-rich nuclei are affected by signals related to energy balance.

Brain stem catecholaminergic pathways

Brain stem catecholaminergic systems project to central CRF cell groups (22) and are influenced by glucocorticoids (26, 28, 59). Furthermore, there are studies suggesting that an intact catecholaminergic system is required for glucocorticoid feedback on the CRF neuron (reviewed in Ref. 60). The medullary catecholaminergic systems are major viscerosensory pathways in the brain (22) and relay to hypothalamic and extrahypothalamic nuclei signals related to cardiovascular, respiratory, and metabolic (*e.g.* glucose, lipogenesis) states.

Sucrose ingestion affected DBH expression in the A2/C2 and locus coeruleus (LC) cell groups of ADX rats in the 5-day study, suggesting altered catecholaminergic activity in these neurons. Catecholaminergic fibers from A2/C2 and LC project to PVN and amygdala, and are believed to transmit to these CRF-rich nuclei signals of metabolic origin. Norepinephrine stimulates CRF secretion in the PVN (61), and ipsilateral transection of ascending medullary catecholaminergic fibers reduces basal CRF immunostaining in the PVN (62, 63), suggesting that this pathway tonically stimulates CRF expression. Therefore, it is possible that sucrose ingestion induces metabolic signals that are transmitted from body to brain via catecholaminergic brain stem nuclei. In line with this metabolic effect of sucrose on catecholaminergic cells, it has been shown that the alterations in lower brain stem nuclei (*e.g.* in medullary neurons throughout the rostral-caudal extent of the NTS) resulting from sucrose ingestion are predominantly due to postingestive effects. A significant pro-

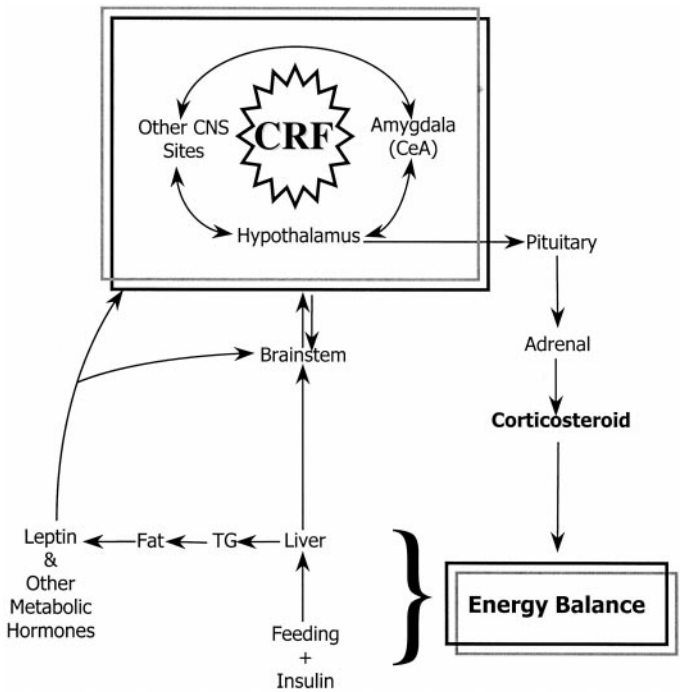

FIG. 7. Schematic view of the proposed glucocorticoid-metabolic-brain feedback axis. Under basal conditions, the metabolic effects of glucocorticoids may mediate the steroid's effects on central CRF expression. Signals resulting from restored feeding (*e.g.* insulin secretion) and metabolism (possibly fat metabolism) may act directly on brain and/or through brain stem mechanisms. Signals to the brain may be transmitted neurally (through the vagus), through metabolites or humorally. It is easy to imagine how carbohydrate ingestion might modulate the effects of stress on CRF and catecholamines in the brain under conditions of chronic stress.

portion of these sucrose-induced effects is likely to be mediated by afferent vagal fibers from liver to brain stem (64, 65).

As well as innervating the amygdala, the locus coeruleus, is innervated and activated by CRF fibers from the CeA and PVN (66, 67); the altered DBH mRNA expression in LC induced by sucrose drinking in ADX rats could be mediated by the restored CRF mRNA, and presumably CRF peptide in the CeA. These effects of sucrose and adrenalectomy on DBH mRNA are transient in both the LC and in the medullary A2/C2; clearly, further exploration of enzyme expression and activity will be required to interpret accurately the effects of drinking sucrose on tonic changes in catecholaminergic activity in brain stem. Others have also shown (27) that ADX-induced changes in the activity of medullary catecholaminergic neurons are temporally related to the time of ADX, and are transient. The largest effect of ADX on metabolism occurs within the first week; thereafter, rats appear to reach a new energetic steady-state, perhaps explaining the transient response of the medullary catecholamine group. The LC is believed to play a role in heightened awareness of and focus on emotional or affective states (68). Signals of importance to the survival of the individual may be integrated and stored for determining future responses by both LC and CeA; the altered catecholamine and CRF expression persists for weeks when the effect of sucrose is compared in ADX rats. These signals need not be aversive; feeding, like restraint stress, provokes CRF secretion in the CeA. It has been suggested that the CRF and NE systems serve to draw attention to significant biological cues or events, such as those of food availability (19), or, as in the case of the ADX rat, the metabolic benefits of ingesting calories as sucrose.

In addition to transmitting visceral information, brain stem nuclei also relay gustatory information to the CeA (69, 70). Thus, the effects of sucrose on the CNS in the present study probably result from either the metabolic actions of sucrose alone or the combined gustatory and metabolic actions of this sweet drink. It appears that, regardless of the gustatory effects of sucrose, there is importance placed on the nutritive value of the sweet drink in the ADX rat.

How are the metabolic effects of sucrose transmitted to the brain?

Several possibilities exist, and the answer probably will be through a combination of many signals and pathways. However, we favor a lipogenic signal in our ADX rat model. ADX did not appear to affect circulating glucose, but it did significantly depress circulating triglycerides and fat stores. Because ADX rats have reduced caloric intake and reduced fat stores due to the loss of corticosterone (34), we believe that sucrose induces restoration of this metabolic pathway in the major lipogenic tissues (liver and fat); indices of lipogenesis or lipolysis then would provide either neural (vagal) or humoral signals that indicate the favorable nutritional effects of sucrose ingestion.

It is also possible that metabolic hormones, such as leptin and insulin, act as mediators of the sucrose-induced changes in metabolic state. These hormones are especially regarded as signals of adiposity, which probably act at the CNS (71). We showed that circulating leptin and insulin are restored to normal in ADX rats drinking sucrose. In addition, there is a strong negative correlation between circulating leptin and mesenteric fat mass and CRF mRNA in the PVN of sucrose-drinking ADX rats (not shown). Leptin may also act at the brain stem (*e.g.* NTS), or at additional hypothalamic cell groups that are known to influence feeding behavior, autonomic output, and overall energy balance. While we are aware of a possible direct fat depot to brain neural pathway (72), sympathetic innervation of adipose tissue is believed to primarily function as an efferent regulator of lipid mobilization. Its afferent, sensory function is uncertain, and leptin (and probably insulin) appears to be the means by which adiposity is signaled to the brain because leptin deficiency or leptin resistance alone causes hyperphagia and obesity in the *ob/ob*, *db/db*, and *fa/fa* rodent models and in humans (71).

General implications

Although these studies deal with the restorative effects of sucrose on deficits induced by ADX under basal conditions, the results also have major implications for understanding the panoply of responses to chronic stress and depression. Under conditions of chronic stress, glucocorticoids are elevated and food intake, insulin and body weights are reduced in rats (73). However, if increased feeding also occurs, more insulin is secreted and, in conjunction with elevated glucocorticoids, calories are stored as fat (74). Carbohydrate feeding is important to behavioral responses to stressors

because rats drinking glucose after inescapable shock exhibit neither the body weight loss nor learned helplessness behavior normally induced by such shock (75, 76). Similarly, rats allowed a high caloric diet and/or carbohydrate to drink during a period of sustained stress have reduced adrenocortical responses to stress (77, 78). Furthermore, sucrose, but not saccharin reduces the quantity of morphine rats self-administer in a pain/tolerance paradigm (79) and this effect is not a consequence of shifts in protein or micronutrient intake (80). In man, provision of a high carbohydrate diet before experimental stress also inhibits cortisol and feelings of depression after the stressor (81). Thus, stress responses can be modulated by experimental provision of carbohydrate in both rats and man. Therefore, in addition to the major effects of sucrose ingestion in the absence of corticosterone, it appears that there may be equally, or more, marked effects in the presence of stress and high glucocorticoid concentrations.

Self-treatment, by increasing carbohydrate and overall intake, may occur in stressed or depressed individuals. Patients who have night-eating syndrome are anorexic in the morning and then exhibit evening hyperphagia and insomnia; the syndrome occurs during periods of life stress and is alleviated when stress is reduced (82). Night eaters ingest more than 50% of their daily calories after dinner, 70% as carbohydrate. They also hypersecrete cortisol and have significantly lower mood scores that decrease further as the evening progresses after dinner time (83). Some patients with unipolar depression overeat, sleep more and are apathetic, rather than being anorexic, insomnic and anxious. From our results, increased carbohydrate intake would be expected to reduce the drive to CRF and norepinephrine in both night eaters and depressed people. Thus, it is plausible to speculate that some stressed and depressed individuals find that it feels better (84) to ingest carbohydrate, to reduce the degree of insomnia, mental anxiety and agitation imposed by chronic stress and melancholic depression.

Brain CRF, particularly in the amygdala, and noradrenergic (NE) systems, particularly in the locus coeruleus, are extensively interconnected (10) and are implicated in chronic stress responses and the etiology of major depression (10, 85). We demonstrate here that sucrose ingestion in ADX rats dramatically alters these glucocorticoid-sensitive neurotransmitters, and that these effects are probably caused by the metabolic changes that result from the consumption of sucrose. Thus, fluctuations in body energy status modulate brain neurotransmitters that cause anxiety, autonomic activity and arousal, and these findings suggest a neural basis for reports that manifestations of chronic stress (76–78, 81) [and possibly depression (83, 86)] are regulated by metabolic signals from body to brain.

References

1. **Bhatnagar S, Bell ME, Liang J, Soriano L, Nagy TR, Dallman MF** 2000 Corticosterone facilitates saccharin intake in adrenalectomized rats. Does corticosterone increase stimulus salience? J Neuroendocrinol 12:453–460
2. **Fuxe K, Hökfelt T, Jonnson G, Levine S, Lofstrom A** 1973 Brain and pituitary-adrenal interaction studies on central monoamine neurons. In: Brodish A, Redgate E (eds) Brain-Pituitary-Adrenal Interrelationships. Karger, Basel, pp 239–269
3. **Watts AG, Sanchez-Watts G** 1995 Region-specific regulation of neuropeptide mRNAs in rat limbic forebrain neurones by aldosterone and corticosterone. J Physiol 484:721–736
4. **Bell ME, Bhatnagar S, Liang J, Soriano L, Nagy TR, Dallman MF** 2000 Voluntary sucrose ingestion, like corticosterone replacement, prevents the metabolic deficits of adrenalectomy. J Neuroendocrinol 12:461–470
5. **Dube MG, Sahu A, Kalra PS, Kalra SP** 1992 Neuropeptide Y release is elevated from the microdissected paraventricular nucleus of food-deprived rats: an *in vitro* study. Endocrinology 131:684–688
6. **Dunn AJ, Berridge CW** 1990 Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses? Brain Res Rev 15:71–100
7. **Owens MJ, Nemeroff CB** 1991 Physiology and pharmacology of corticotropin-releasing factor. Pharmacol Rev 43:425–473
8. **Cole BJ, Cador M, Stinus L, Rivier J, Vale W, Koob GF, Le Moal M** 1990 Central administration of a CRF antagonist blocks the development of stress-induced behavioral sensitization. Brain Res 512:343–346
9. **Kainu T, Honkaniemi J, Gustafsson J-A, Rechardt L, Pelto-Huikko M** 1993 Co-localization of peptide-like immunoreactivities with glucocorticoid receptor- and Fos-like immunoreactivities in the rat parabrachial nucleus. Brain Res 615:245–251
10. **Koob GF** 1999 Corticotropin-releasing factor, norepinephrine and stress. Biol Psychiatry 46:1167–1180
11. **Swiergiel AH, Takahashi LK, Kalin NH** 1993 Attenuation of stress-induced behavior by antagonism of corticotropin-releasing factor receptors in the central amygdala of the rat. Brain Res 623:229–234
12. **Akana SF, Dallman MF** 1997 Chronic cold in adrenalectomized, corticosterone (B)-treated rats: facilitated corticotropin responses to acute stress emerge as B increases. Endocrinology 138:3249–3258
13. **Sawchenko PE** 1987 Adrenalectomy-induced enhancement of CRF and vasopressin immunoreactivity in parvocellular neurosecretory neurons: anatomic, peptide and steroid specificity. J Neurosci 7:1093–1106
14. **Makino S, Gold PW, Schulkin J** 1994 Effects of corticosterone on CRH mRNA and content in bed nucleus of the stria terminalis; comparison with the effects in the bed nucleus of the amygdala and the paraventricular nucleus of the hypothalamus. Brain Res 657:141–149
15. **Shepard JD, Barron KW, Myers DA** 2000 Corticosterone delivery to the amygdala increases corticotropin-releasing factor mRNA in the central amygdaloid nucleus and anxiety-like behavior. Brain Res 861:288–295
16. **Viau V, Chu A, Soriano L, Dallman MF** 1999 Independent and overlapping effects of corticosterone and testosterone on corticotropin-releasing hormone and arginine vasopressin mRNA expression in the paraventricular nucleus of the hypothalamus and stress-induced adrenocorticotropic hormone release. J Neurosci 19:6684–6693
17. **Herman JP, Cullinan WE** 1997 Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. Trends Neurosci 20:78–83
18. **Dallman MF, Akana SF, Bell ME, Bhatnagar S, Choi S, Chu A, Horsley C, Levin N, Meijer OC, Strack AM, Soriano L, Viau V** 1999 Starvation: early signals, sensors and sequelae. Endocrinology 140:4015–4023
19. **Merali Z, McIntosh J, Kent P, Michaud D, Anisman H** 1998 Aversive and appetitive events evoke the release of corticotropin-releasing hormone and bombesin-like peptides at the central nucleus of the amygdala. J Neurosci 18:4758–4766
20. **Akana SF, Strack AM, Hanson ES, Dallman MF** 1994 Regulation of activity in the hypothalamo-pituitary-adrenal axis is integral to a larger hypothalamic system that determines caloric flow. Endocrinology 135:1125–1134
21. **Davidovic V, Vasilev I, Stojanovic-Susulic V** 1992 Dependence of the sympatho-adrenal activity on the nutritional status in corticosterone treated rats. Comp Biochem Physiol 101A:309–312
22. **Loewy AD** 1990 Central autonomic pathways. In: Loewy AD, Speyer KM (eds) Central Regulation of Autonomic Functions. Oxford University Press, New York, pp 88–103
23. **Sawchenko PE, Swanson LW** 1982 The organization of noradrenergic pathways from the brainstem to the paraventricular and supraoptic nuclei in the rat. Brain Res Rev 4:275–325
24. **Pacak K, Kvetnansky R, Palkovits M, Fukuhara G, Yadid G, Kopin IJ, Goldstein DS** 1993 Adrenalectomy augments in vivo release of norepinephrine in the paraventricular nucleus during immobilization stress. Endocrinology 133:1404–1410
25. **Pacak K, Palkovits M, Kopin IJ, Goldstein DS** 1995 Stress-induced norepinephrine release in hypothalamic paraventricular nucleus and pituitary-adrenocortical and sympathoadrenal activity: in vivo microdialysis studies. Front Neuroendocrinol 16:89–150
26. **De Villiers AS, Russell VA, Taljaard JJF** 1992 Effect of corticosterone on noradrenergic nuclei in the pons medulla and [3H] NA release from terminals in hippocampal slices. Neurochem Res 17:273–280
27. **Lachuer J, Buda M, Tappaz M** 1992 Differential time course activation of the brain stem catecholaminergic groups following chronic adrenalectomy. Neuroendocrinology 56:125–132
28. **Joseph V, Dalmaz Y, Cottet-Emard JM, Pequignot JM** 1998 Dexamethasone's influence on tyrosine hydroxylase activity in the chemoreflex pathway and on the hypoxic ventilatory response. Pflugers Arch Eur J Physiol 435:834–839
29. **Moore RY, Bloom FE** 1979 Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. Annu Rev Neurosci 2:113–168

30. **Curtis AL, Lechner SM, Pavcovich LA, Valentino RJ** 1997 Activation of the locus coeruleus noradrenergic system by intracoerulear microinfusion of corticotropin-releasing factor: effects on discharge rate, cortical norepinephrine levels and cortical electroencephalographic activity. J Pharmacol Exp Ther 281:163–172
31. **McGaugh JL, Cahill L, Roozendaal B** 1996 Involvement of the amygdala in memory storage: interaction with other brain systems. Proc Natl Acad Sci USA 93:13508–13514
32. **Roozendaal B, McGaugh JL** 1997 Basolateral amygdala lesions block the memory-enhancing effect of glucocorticiod administration in the rat. Eur J Neurosci 9:76–83
33. **Vaher PR, Luine VN, Gould E, McEwen B** 1994 Effects of adrenalectomy on spatial memory performance and dentate gyrus morphology. Brain Res 656:71–78
34. **Dallman MF, Strack AM, Akana SF, Bradbury MJ, Hanson ES, Scribner KA, Smith M** 1993 Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. Front Neuroendocrinol 14:303–347
35. **Brown MR, Fisher LA** 1986 Glucocorticoid suppression of the sympathetic nervous system and adrenal medulla. Life Sci 39:1003–1012
36. **Dallman MF, Akana SF, Jacobson L, Levin N, Cascio CS, Shinsako J** 1987 Characterization of corticosterone feedback regulation of ACTH secretion. Ann NY Acad Sci 512:402–414
37. **Moberg GP, Clark CR** 1976 Effect of adrenalectomy and dexamethasone treatment on circadian running in the rat. Physiol Behav 4:617–619
38. **Richter CP** 1936 The spontaneous activity of adrenalectomized rats treated with replacement and other therapy. Endocrinology 20:657–666
39. **Palkovits M, Young III WS, Kovacs K, Toth T, Makara GB** 1998 Alterations in corticotropin-releasing hormone gene expression of central amygdaloid neurons following long-term paraventricular lesions and adrenalectomy. Neuroscience 85:135–147
40. **Allen JP, Allen CF** 1975 Amygdalar participation in tonic ACTH secretion in the rat. Neuroendocrinology 19:115–125
41. **Fichette CT, Komisaruk BR, Edinger HM, Feder HH, Siegel A** 1980 Differential fornix ablations and the circadian rhythmicity of adrenal corticosterone secretion. Brain Res 195:373–383
42. **Herman JP, Cullinan WE, Watson SJ** 1994 Involvement of the bed nucleus of the stria terminalis in tonic regulation of paraventricular hypothalamic CRH and AVP mRNA expression. J Neuroendocrinol 6:433–442
43. **Herman JP, Schafer MK-H, Young EA, Thompson RH, Douglass J, Akil H, Watson SJ** 1989 Evidence for hippocampal regulation of neuroendocrine neurons of the hypothalamo-pituitary-adrenocortical axis. J Neurosci 9:3072–3083
44. **Diorio D, Viau V, Meaney MJ** 1993 The role of the frontal cortex in the regulation of hypothalamic-pituitary-adrenal activity. J Neurosci 13:3839–3847
45. **Van de Kar LD, Piechowski RA, Rittenhouse PA, Gray TS** 1991 Amygdaloid lesions: differential effect on conditioned stress and immobilization-induced increases in corticosterone and renin secretion. Neuroendocrinology 54:89–95
46. **Viau V, Meany M** 1996 The inhibitory effect of testosterone on hypothalamic-pituitary-adrenal responses to stress is mediated by the medial preoptic area. J Neurosci 16:1866–1876
47. **Kendall JW** 1971 Feedback Control of Adrenocorticotrophic Hormone Secretion. Oxford University Press, New York
48. **Kovacs K, Kiss JZ, Makara GB** 1986 Glucocorticoid implants around the hypothalamic paraventricular nucleus prevent the increase of corticotropin-releasing factor and arginine vasopressin caused by adrenalectomy. Neuroendocrinology 44:229–234
49. **Kovacs K, Makara GB** 1988 Corticosterone and dexamethasone act at different brain sites to inhibit adrenalectomy-induced adrenocorticotropin secretion. Brain Res 474:205–210
50. **Viau V, Soriano L, Dallman MF,** Androgens alter corticotropin-releasing hormone and vasopressin mRNA within forebrain sites known to regulate activity in the hypothalamic-pituitary-adrenal axis. J Neuroendocrinol, in press
51. **Luiten PGM, ter Horst GJ, Steffens AB** 1987 The hypothalamus, intrinsic connections and outflow pathways to the endocrine system in relation to the control of feeding and metabolism. Prog Neurobiol 28:1–54
52. **Scharrer E** 1999 Control of food intake by fatty acid oxidation and ketogenesis. Nutrition 15:704–714
53. **Bamshad M, Aoki VT, Adkison MG, Warren WS, Bartness TJ** 1998 Central nervous system origins of the sympathetic nervous system outflow to white adipose tissue. Am J Physiol 275:R291–R299
54. **Bartness TJ** 1998 Innervation of mammalian white adipose tissue: implications for the regulation of total body fat. Am J Physiol 275:R1399–R1411
55. **King BM, Cook JT, Dallman MF** 1996 Hyperinsulinemia in rats with obesity-inducing amygdaloid lesions. Am J Physiol 271:R1156–R1159
56. **Tokunaga K, Fukushima M, Kemnitz JW, Bray GA** 1986 Comparison of ventromedial and paraventricular lesions in rats that become obese. Am J Physiol 251:R1221–R1227
57. **King BM, Banta AR, Tharel GN, Bruce BK, Frohman LA** 1983 Hypothalamic hyperinsulinemia and obesity: role of adrenal glucocorticoids. Am J Physiol 245:E194–E199
58. **Freedman MR, Stern JS, Reaven GM, Mondon CE** 1986 Effects of adrenalectomy on in vivo glucose metabolism in insulin resistant Zucker obese rats. Horm Metab Res 18:296–298
59. **Lachuer J, Gaillet S, Barbagli B, Buda M, Tappez M** 1991 Differential early time course activation of the brain stem catecholaminergic groups in response to various stresses. Neuroendocrinology 53:589–596
60. **Sawchenko PE, Swanson LW** 1989 Organization of CRF immunoreactive cells and fibers in the rat brain: immunohistochemical studies. In: DeSouza EB, Nemeroff CB (eds) CRC Critical Reviews in Corticotropin-Releasing Factor: Basic and Clinical Studies of a Neuropeptide. CRC Press, Boca Raton, FL, pp 29–51
61. **Plotsky PM, Cunningham Jr ET, Widmaier EP** 1989 Catecholaminergic modulation of corticotropin-releasing factor and adrenocorticotropin secretion. Endocr Rev 10:437–458
62. **Pacak K, Palkovits M, Kopin IJ, Goldstein DS** 1995 Stress-induced norepinephrine release in the hypothalamic paraventricular nucleus and pituitary-adrenocortical and sympathoadrenal activity: *in vivo* microdialysis studies. Front Neuroendocrinol 16:89–150
63. **Sawchenko P** 1988 Effects of catecholamine-depleting medullary knife cuts on corticotropin-releasing factor and vasopressin immunoreactivity in the hypothalamus of normal and steroid-manipulated rats. Neruoendocrinology 48:459–470
64. **Yamamoto T, Sawa K** 2000 Comparison of c-Fos-like immunoreactivity in the brainstem following intraoral and intragastric infusions of chemical solutions in rats. Brain Res 866:144–151
65. **Yamamoto T, Sawa K** 2000 c-Fos-like immunoreactivity in the brainstem following gastric loads of various chemical solutions in rats. Brain Res 866:135–143
66. **Van Bocksdale EJ, Peoples J, Valentino RJ** 1999 Anatomic basis for differential regulation of the rostrolateral peri-locus coeruleus region by limbic afferents. Biol Psychiatry 46:1352–1363
67. **Pavcovich LA, Valentino RJ** 1997 Regulation of a putative neurotransmitter effect of corticotropin-releasing factor: effects of adrenalectomy. J Neurosci 17:401–408
68. **Gold PW, Chrousos G** 1998 The endocrinology of melancholic and atypical depression: relation to neurocircuitry and somatic consequences. Proceedings of the Association of American Physicians 111:22–34
69. **Yamamoto T, Sako N, Nobuyuki S, Iwafune A** 1997 Gustatory and visceral inputs to the amygdala of the rat: conditioned taste aversion and induction of c-fos-like immunoreactivity. Neuroscience Lett 226:127–130
70. **Scott TR** 1992 Taste: The Neural Basis for Body Wisdom. Karger, Basel
71. **Baskin DG, Figlewicz Latteman DP, Seeley RJ, Woods SC, Porte Jr D, Schwartz MW** 1999 Insulin and leptin: dual adiposity signals to the brain for the regulation of food intake and body weight. Brain Res 848:114–123
72. **Fishman RB, Dark J** 1987 Sensory innervation of white adipose tissue. Am J Physiol 253:R942–R944
73. **Dallman MF, Bhatnagar S** 2001 Chronic Stress and Energy Balance: Role of the Hypothalamo-Pituitary-Adrenal Axis. Oxford University Press, New York
74. **Strack AM, Sebastian RJ, Schwartz MW, Dallman MF** 1995 Glucocorticoids and insulin: reciprocal signals for energy balance. Am J Physiol 268:R142–R149
75. **Dess NK, Choe S, Minor TR** 1998 The interaction of diet and stress in rats: high-energy food and sucrose treatment. J Exp Psychol Anim Behav Processes 24:60–71
76. **Minor TR, Saade S** 1997 Postsstress glucose mitigates behavioral impairment in rats in the "learned helplessness" model of psychopathology. Biol Psychiatry 42:324–334
77. **Levin BE, Richard D, Michel C, Servatius R** 2000 Differential stress responsivity in diet-induced obese and resistant rats. Am J Physiol 279:R1357–R1364
78. **Strack AM, Akana SF, Horsley CJ, Dallman MF** 1997 A hypercaloric load activates thermogenesis but decreases stress responses in both the sympathetic and adrenocortical systems. Am J Physiol 272(3 pt. 2):R840–R848
79. **D'Anci KE** 1999 Tolerance to morphine-induced antinociception is decreased by chronic sucrose or polycose intake. Pharmacol Biochem Behav 63:1–11
80. **Kanarek RB, D'Anci KE, Przypek J, Mathes MW** 1999 Altering dietary levels of protein or vitamins and minerals does not modify morphine-induced analgesia in male rats. Pharmacol Biochem Behav 62:203–208
81. **Markus R, Panhuysen G, Truiten A, Koppeschaar H** 2000 Effects of food on cortisol and mood in vulnerable subjects under controllable and uncontrollable stress. Physiol Behav 70:333–342
82. **Strunkard AJ, Grace WJ, Wolff HG** 1955 The night eating syndrome: a pattern of food intake among certain obese patients. Am J Med 19:78–86
83. **Birketvedt GS, Florholmen J, Sundsfjord J, Osterud B, Dinges D, Bilker W, Stunkard A** 1999 Behavioral and neuroendocrine characteristics of the night-eating syndrome. JAMA 282:657–663
84. **Damasio AR** 1999 The Feeling of What Happens: Body and Emotion in the Making of Consciousness. Harcourt Brace, New York
85. **Wong ML, Kling MA, Munson AJ, Listwak S, Licinio J, Prolo P, Karp B, McCutcheon IE, Geracioti Jr TD, DeBellis MD, Rice KC, Goldstein DS, Veldhuis JD, Chrousos G, Oldfield EH, McCann SM, Gold PW** 2000 Pronounced and sustained central hypernoradrenergic function in major depression with melancholic features: relation to hypercortisolism and corticotropin-releasing hormone. Proc Natl Acad Sci USA 97:325–330
86. **Strunkard AJ, Fernstrom MH, Price A, Frank E, Kupfer D** 1990 Direction of weight change in recurrent depression. Consistency across episodes. Arch Gen Psychiatry 47:857–860