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Research Article

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Lactation as a Model of Naturally Reversible Hypercorticalism Plasticity in the Mechanisms Governing Hypothalamo-Pituitary-Adrenocortical Activity in Rats

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Abstract

Steady state levels of hypothalamic expression of the genes encoding corticotropin-releasing hormone (CRH), proopiomelanocortin (POMC), arginine vasopressin (AVP), and oxytocin (OT) were studied in rats to investigate the mechanisms underlying the transitions between hypercorticalism during lactation and normocorticalism upon weaning. During lactation, CRH mRNA levels and blood titers of adrenocorticotropin (ACTH) were found to be significantly reduced, although POMC mRNA levels in the anterior pituitary were not significantly different from those found in cycling virgin (control) rats; during all phases of lactation, an inverse relationship was observed between the blood levels of ACTH and corticosterone (CORT). Plasma prolactin (PRL) concentrations were elevated ~ 30-fold during lactation. Whereas steady state levels of OT mRNA were markedly increased throughout lactation, those of AVP mRNA were only transiently (initially) elevated, and the blood levels of these hormones were not significantly altered in lactating as compared with cycling virgin and postlactating rats. CRH and POMC gene expression and blood levels of ACTH, CORT, and PRL were normalized within 1–3 d of removal of suckling pups. The temporal relationships between the biosynthetic profiles of the various peptide hormones and the patterns of ACTH and CORT secretion during the two physiological states suggest that lactation-associated hypercorticalism does not merely result from increased ACTH secretion; although still not well substantiated at this time, the evidence points to contributory roles of PRL, OT, and AVP in the hypercorticalismic state found during lactation. (*J. Clin. Invest.* 1995. 96:1208–1215.) Key words: corticotropin releasing hormone • oxytocin • proopiomelanocortin • adrenocorticotropin • prolactin

Introduction

A variety of neuropeptides originating in the hypothalamus contribute to the regulation of adrenocortical activity (1, 2). Corticotropin-releasing hormone (CRH_{1-41})[1] and arginine vasopressin (AVP) appear to be the principal regulators of adrenocorticotropin (ACTH) and corticosteroid secretion, with oxytocin (OT) and the opioid peptide β-endorphin (β-END) serving accessory modulatory influences. Indeed, the control of the hypothalamo-pitutary-adrenal (HPA) axis is probably more complex than outlined here since each of these peptidergic systems appear to influence one another through intricate intrahypothalamic microcircuits, e.g., bilateral CRH–opioid interactions have been reported (3), CRH is a secretagogue of OT (4, 5) (but not during lactation [6]), opioids influence the activity of OT neurons (7), and CRH neurons are induced to coexpress AVP when glucocorticoid negative feedback is diminished (8, 9); furthermore, AVP potentiates the ACTH-releasing activity of CRH (10–12). These multiple regulatory mechanisms presumably serve to tailor the organism's adaptive reaction to noxious stimuli, with the response of each peptidergic component playing a decisive role in the ultimate intensity and duration of adrenocortical secretion.

In the last few years, inappropriate shutoff of adrenocortical secretion upon cessation of stress has been causally linked to pathophysiological states such as major depression. The participation of classical neurotransmitters as well as CRH, AVP, OT, and opioids in dysregulated HPA axis activity is being intensively researched at present. A large body of evidence suggests that extended exposure to high glucocorticoid levels leads to a loss of hippocampal and (probably) hypothalamic corticosteroid receptors and, thus, to eventual failure of glucocorticoid negative feedback in rats (13) and supposedly to hypercortisolemia-associated depression in humans (14). However, rather than simply concentrating on why this dysregulation occurs, it may also be important to understand the mechanisms that may, in certain circumstances, buffer the organism against glucocorticoid excess and its ill effects. A potential natural model for examining the last question is provided in the lactating rat. Lactation is characterized by hypercorticalism and a flattening of the diurnal rhythm in glucocorticoid secretion (15–17); in some respects, lactation-associated hypercorticalism thus resembles some hypercortisolemic states in humans (14). In rats, elevated corticosterone (CORT) levels are apparently necessary for lactogenesis (18). This hypercorticalism is associated with marked changes in emotional behavior (reduced fear, increased aggression) (19, 20) and reduced neuroendocrine responses (CRH, OT, and enkephalin) to noxious stimuli (21).

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1. *Abbreviations used in this paper:* β-END, β-endorphin; CORT, corticosterone; CRH, corticotropin-releasing hormone; HPA, hypothalamo-pituitary-adrenal; mPVN, magnocellular division of the paraventricular nucleus; OT, oxytocin; POMC, proopiomelanocortin; pPVN, parvocellular division of the paraventricular nucleus; PRL, prolactin; PVN, paraventricular nucleus.

Previous studies have suggested that the latter are not simply related to increased glucocorticoid negative feedback upon the central mechanisms responsible for mounting the hormonal response to stress (16, 22).

As a first step toward gaining an insight into the mechanisms that allow the lactating rat to alternate between hyper- and normocorticalismic states, this study examined the patterns of neuropeptide gene expression (steady state levels) during lactation and at various intervals of the postlactational (weaning) phase. The work concentrated on gene expression for CRH, OT, and AVP in the hypothalamic paraventicular nucleus (PVN), whose parvicellular division may be considered to be the most relevant subnucleus in eliciting the hormonal response to stress (fibers from the parvicellular region terminate in the external zone of the median eminence where from their neurosecretions have direct access to pituitary corticotropes) (23). Transcription of the proopiomelanocortin (POMC) gene was also assessed since POMC serves as the prohormone from which β-END is derived; POMC-immunoreactive fibers, originating in the hypothalamic arcuate nucleus, extend to many cerebral (including hypothalamic) loci and also to the median eminence where they presumably influence adenohypophyseal function (24). The POMC gene also encodes ACTH (25), the primary pituitary stimulatory regulator of adrenocortical function; we therefore monitored its expression in the anterior pituitary concomitantly. Lastly, blood levels of ACTH and CORT were measured as indices of pituitary-adrenocortical activity in lactating and postlactational rats; in addition, plasma concentrations of OT, AV, and prolactin (PRL) were determined.

Methods

Animals and tissue collection. Female virgin and primiparous Wistar rats (Max Planck Institute of Biochemistry, Martinsried, Germany) were obtained at least 1 wk before killing or expected date of parturition, respectively. Rats were individually caged under standard conditions and in compliance with local animal welfare regulations. Virgins serving as controls were rapidly killed on the day of diestrous (determined by vaginal cytology); nursing dams were killed at various postpartum intervals (up to 3 wk); and mothers whose litters had been weaned were killed at various postlactational time points (up to 3 wk). Rats were killed between 1000 and 1400 (lights on in animal facility from 0600–1800 daily), but were not necessarily actively suckling at the point of killing. Each treatment group consisted of six to eight rats. After killing, hypothalami (excluding the preoptic area) and anterior pituitaries were rapidly dissected and snap-frozen in an isopentane/dry-ice bath. These procedures were carried out in RNase-free conditions. Trunk blood was also collected for the determination of ACTH (in prechilled EDTA-coated tubes containing 140 μg aprotinin) and CORT, OT, AVP, and PRL.

Northern blots. Total cellular RNA was isolated using guanidinium isothiocyanate, followed by ultracentrifugation through a cesium chloride gradient, according to standard procedures (26). Using established protocols, aliquots of total RNA (35 μg from hypothalamus, 5 μg from pituitary) were denatured with glyoxal at 50°C for 1 h and electrophoresed on agarose gels before transfer to Nytran membranes by capillary action. Membranes were presoaked in water and 2× SSC before transferring total RNA extracts. After blotting, RNA was fixed to the membranes by baking for 2 h at 80°C. Sample RNA was electrophoresed alongside an RNA ladder (0.16–1.77 kb) to determine migration of the relevant mRNA sequence (ethidium bromide staining). Hybridizations for POMC, CRH, AVP, and OT were carried out on different membranes. Before each hybridization, membranes were prehybridized (4 h, 58°C for riboprobes, 55°C for oligonucleotide probes) with 5× SSPE buffer (with the addition of 50% formamide when riboprobes were to be used),
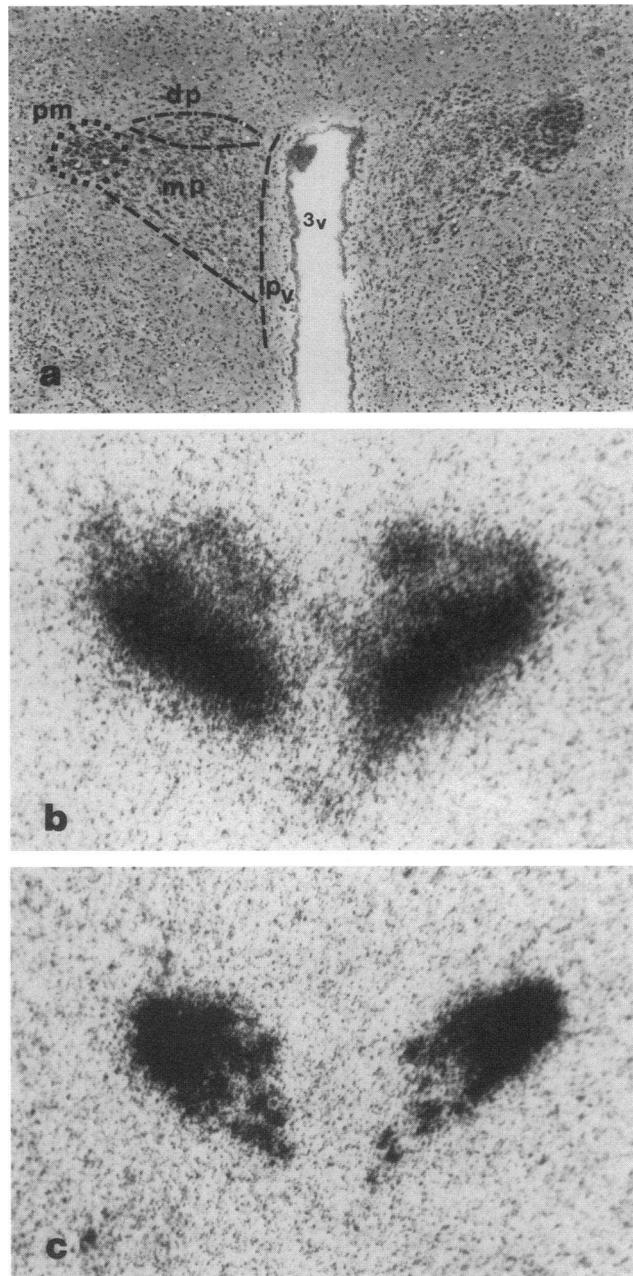

Figure 1. The method for distinguishing the cytoarchitectonic subdivisions of the PVN (mPVN and pPVN, see reference 31) is shown in *a*, the representative low-power photomicrograph obtained from a Nissi-stained coronal section through a rat PVN (corresponding to Bregma: −4.2 mm). Typical hybridization signals for AVP (*b*) and OT (*c*) in noncounterstained, anatomically matched sections from rats in the early phase of lactation are also depicted. *dp*, dorsal parvocellular subdivision; *pm*, posterior magnocellular subdivision; *mp*, medial parvocellular subdivision; p_v, periventricular nucleus; 3_v, third ventricle.

5× Denhardt's solution, 0.5% SDS, and 0.1% denatured herring sperm DNA. To detect POMC mRNA, membranes were hybridized with a single-stranded cRNA probe (mouse POMC plasmid ME-150, containing a 150-bp insert encoding β-END) (27), labeled with [^{32}P]UTP using T3 RNA polymerase (typical specific activity, 10^8–10^9 cpm/μg) for 20 h at 58°C (probe concentration, 2.10^6 cpm/ml). After hybridization, filters were washed under increasingly stringent conditions (final wash, 0.01× SSPE with 0.5% SDS, 60°C, 30 min), dried, and apposed
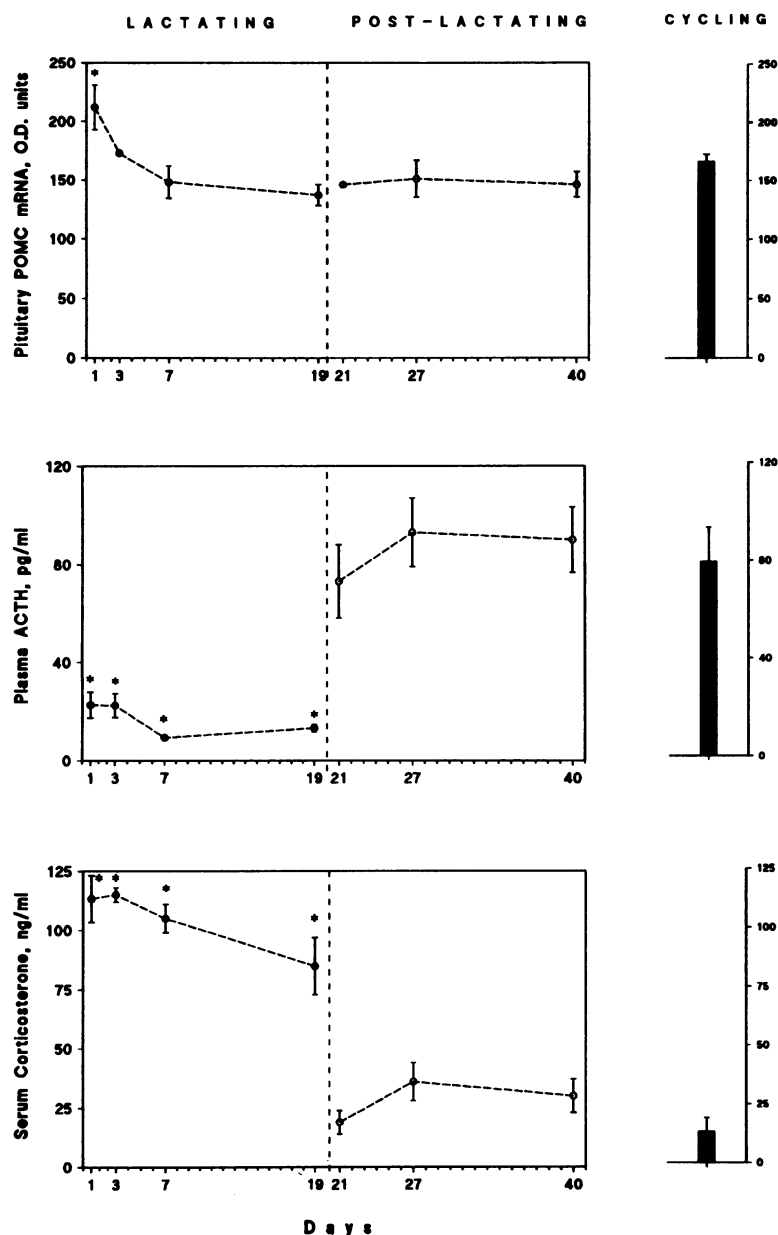
Figure 2. Pituitary-adrenocortical activity in lactating and nonlactating rats. Changes in pituitary POMC mRNA levels (*top*) and blood levels of ACTH (*middle*) and corticosterone (*bottom*) at various intervals during the lactational and postlactational phases are shown, alongside corresponding basal values in virgin, diestrous (control) females (*bar diagrams, far right*). Values represent mean±SEM ($n = 6$–8 rats per time point); asterisks indicate significant differences between lactating and nonlactating groups versus cycling rats ($P \geq 0.05$).

to Hyperfilm MP (Amersham International, Braunschweig, Germany) at $-70°C$ in photographic cassettes outfitted with intensifying screens. CRH mRNA was detected after hybridization with a [^{32}P]dATP-labeled 48-mer oligonucleotide probe (complementary to bases 496–543 of rat pre-pro-CRH) (28); labeling (typical specific activity 10^8 cpm/μg) was achieved using terminal deoxynucleotidyl-transferase. All radiolabeled nucleotides were purchased from Dupont/New England Nuclear (Bad Homburg, Germany). Filters were hybridized with 4.10^6 cpm/ml of labeled probe for 20 h at 55°C and then subjected to washes of increasing stringency (final washes: 0.1× SSC and 0.5% SDS, 42°C, 15 min and, briefly, with 2× SSC at room temperature). Filters were then dried and apposed to autoradiographic film as described above. AVP mRNA and OT mRNA hybridization were carried out under similar conditions to those described above for CRH mRNA, using 48-mer oligonucleotide probes complementary to bases coding for the 16 terminal amino acids of the pro-AVP glycoprotein and bases 247–294 of rat OT, respectively (29).

In situ hybridization histochemistry. To discern parvocellular from magnocellullar PVN cell groups expressing OT and/or AVP, a separate

set of lactating and postlactating rats was examined ($n = 4$–6 rats per group). Sections (14 μm thick) were cut from frozen brains, thaw-mounted on gelatin-subbed slides, and processed for in situ hybridization (30). Hybridizations were carried out using the ^{35}S-labeled oligonucleotides described above for OT and AVP. Similarly, as a means to confirm the Northern assay results, hybridizations for CRH mRNA were carried out. Autoradiographic images of hybridized signal were generated by apposition of the slide-mounted sections to Hyperfilm β-max film (Amersham International) for 5–6 h; radioactive standards were run alongside.

Semiquantitative analysis of autoradiograms. Optical densities (OD, arbitrary units) of the radiographic images (Northern and in situ hybridization assays) were digitized using the Macintosh-based image analysis program IMAGE 1.52 (Wayne Rasband, Research Services Branch, National Institute of Mental Health, Bethesda, MD). For the in situ assays, four measurements in defined areas of interest were made on adjacent brain sections from each animal. Since intense OT and AVP hybridization signals were obtained, reference was made to anatomically matched Nissl-stained coronal sections through the PVN of an indepen-
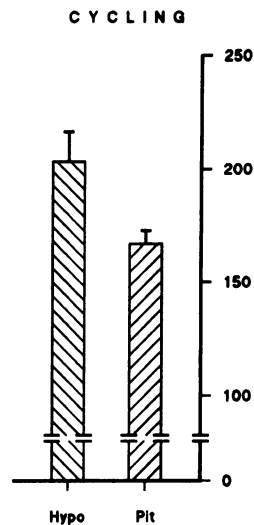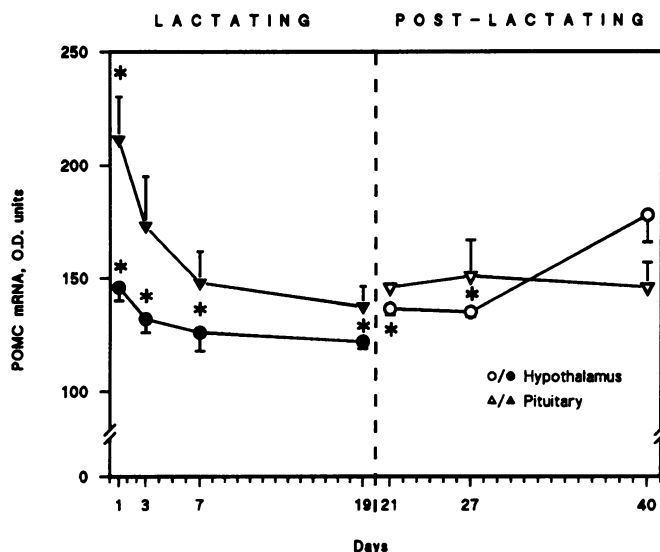
Figure 3. Comparison of POMC gene transcript levels (arbitrary OD units) in hypothalami and anterior pituitaries of lactating, postlactating, and nonparous, cycling rats. Data shown represent mean±SEM ($n = 6-8$ rats per group). The main panel depicts time course of changes at various intervals throughout the lactational and postlactational periods, each lasting 3 wk. Hatched bars in the far right panel show corresponding basal values in diestrous, virgin control rats. Asterisks denote significant differences from values obtained in virgin rats ($P \geq 0.05$). For further details of quantitation of mRNA signals, see the legend to Fig. 4.

dent set of animals to discern between magno- and parvicellular (mPVN and pPVN, respectively) OT- and AVP-expressing cells; Sawchenko and Swanson's delineations of the subnuclei (31) were followed, a representative photomicrograph of which is shown in Fig. 1.

Blood ACTH, CORT, AVP, OT, and PRL levels. ACTH concentrations in plasma samples (stored at $-70°C$ until assay) were measured by an RIA kit (ICN Biomedicals, Costa Mesa, CA), in which rat $ACTH_{1-24}$ (Peninsula Laboratories, Inc., Belmont, CA) was used for preparation of standards; parallelism between serial dilutions of rat plasma and the standard curve pointed to the validity of this assay system. All samples were run in the same assay (intraassay coefficient of variation: $< 7\%$). Sera in which CORT was to be measured were stored at $-20°C$ until the time of assay. All samples were assayed in a single session, using RIA kits from ICN Biomedicals (intraassay coefficients of variation were $< 5\%$). Plasma PRL concentrations were measured using reagents provided by the National Institute of Diabetes and Digestive and Kidney Diseases, with rPRL-RP-3 serving as standard. The intraassay coefficient of variation was $< 5\%$. Blood concentrations of AVP and OT were estimated using antisera obtained from Peninsula Laboratories, Inc. and radioiodinated peptides from Dupont/New England Nuclear.

Statistical analysis. All hormone values and OD data from the various experimental groups were compared using ANOVA and Student's t test, as appropriate (significance level set at $P \geq 0.05$).
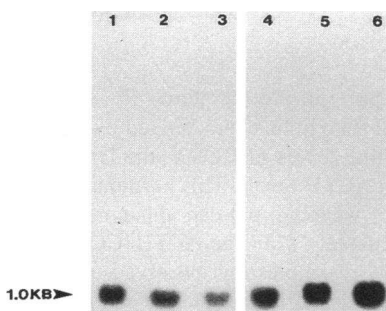

Figure 4. Representative Northern blot for POMC mRNA on lactation days 1 (lane *1*), 7 (lane *2*), and 19 (lane *3*), as well as on postweaning days 1 (lane *4*) and 20 (lane *5*); for comparison, lane *6* shows the hybridization signal obtained for cycling virgin rats. Each lane was loaded with 35 µg of total RNA. Computation of OD values for hybridization signals (for details of replication, see Methods) shown in Fig. 3 included a correction for RNA loading (according to reference 53: ODs of photographic images of ethidium bromide–stained 28S and 18S bands for each blot).

Results

Pituitary-adrenocortical activity. As shown in Fig. 2 (*top*, steady state levels of POMC mRNA were significantly elevated on the first day of suckling as compared with all other lactational and postlactational days and also as compared with the number of POMC mRNA transcripts in virgin (diestrus) controls. Thereafter (day 3 onward), POMC gene expression returned to levels that were not significantly different from those observed in control virgin rats. Despite the relatively unimpressive changes in POMC gene transcription, the lactational and postlactational phases were characterized by major differences in the blood levels of ACTH (Fig. 2, *middle*) and CORT (Fig. 2, *bottom*). Throughout lactation, ACTH concentrations were reduced to about one-third of those found during diestrus; within 1 d of weaning, however, plasma ACTH levels were reinstated to diestrus control values. Serum CORT levels displayed a pattern that was inverse to that seen for ACTH. Compared with diestrus virgins, nursing dams displayed significantly higher (seven- to eightfold) basal levels of CORT during all phases of lactation (experimental days 1–19). Starting from the first day of weaning (experimental day 21), serum CORT titers were similar to those found in virgin controls.

Hypothalamic versus pituitary steady state levels of POMC mRNA. POMC mRNA levels in the hypothalamus were maintained at a significantly reduced level ($\sim 70\%$ compared with control virgin levels) throughout the lactational (20 d) and postlactational (20 d) periods, although there was a tendency (not significant) for levels to increase at the last time point (Fig. 3). The hypothalamic pattern of response differs from that reported above for the pituitary in that, in the latter tissue, POMC gene expression was generally unaltered by lactation and weaning except on day 1 of lactation when they were elevated (Figs. 3 and 4).

CRH mRNA levels in the hypothalamus. Representative results on CRH gene expression (steady state levels) in cycling virgin, lactating, and postlactating rats obtained by in situ hybridization histochemistry are shown in Fig. 5. The results shown in Figs. 6 and 7 show the changes in hypothalamic CRH mRNA levels, as measured by Northern hybridization, during
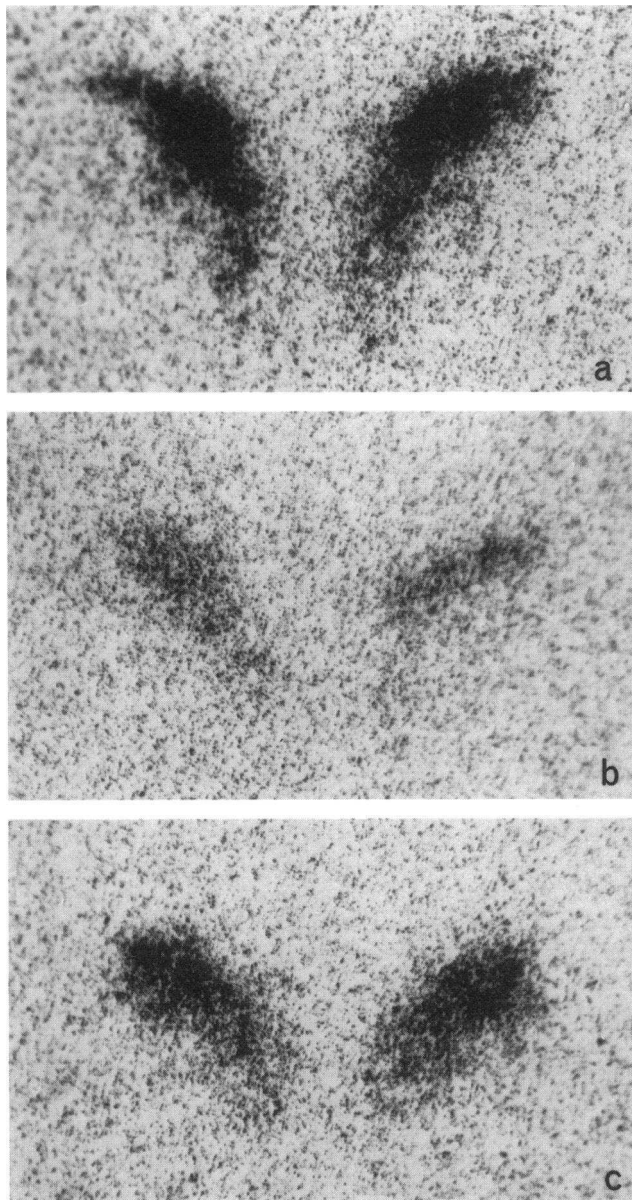
Figure 5. Representative low-power photomicrographs of PVN sections from cycling virgin (*a*), lactating (*b*), and postlactating (*c*) rats hybridized in situ for CRH mRNA.

Figure 6. Representative Northern blot for CRH mRNA on lactation days 1 (lane *1*), 7 (lane *2*), and 19 (lane *3*), as well as on postweaning days 1 (lane *4*) and 20 (lane *5*); for comparison, lane *6* shows the hybridization signal obtained for cycling virgin rats. Each lane was loaded with 35 μg of total RNA. Computation of OD values for hybridization signals (for details of replication, see Methods) shown in Fig. 7 included a correction for RNA loading (according to reference 53: ODs of photographic images of ethidium bromide–stained 28S and 18S bands for each blot).

various lactational phases. Throughout lactation (experimental days 1–19), CRH gene transcripts in the hypothalamus occurred at a level that was significantly lower than that measured in control diestrus rats. Levels of CRH mRNA showed an upward trend upon weaning (experimental day 21 onward), but they still remained lower than those seen in virgin controls. No significant differences were found between CHR mRNA levels in lactating and postlactating rats. Scrutiny of Figs. 3 and 7 indicates a parallelism in the temporal expression of the CRH and POMC genes in the hypothalamus; such a pattern is not easily discernible when hypothalamic CRH and pituitary POMC gene expression profiles (Fig. 3) are compared.

Synthesis and secretion of AVP and OT. Steady state levels of AVP and OT mRNA were determined by two methods: Northern hybridization (data not shown) and in situ hybridiza-
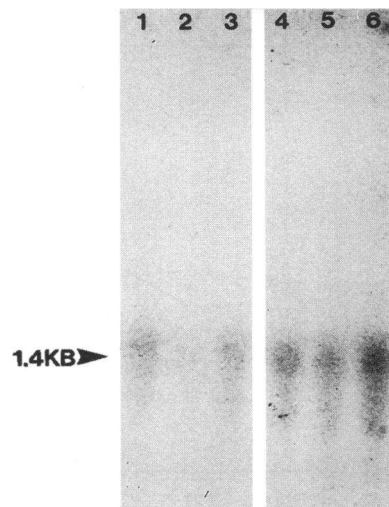
tion histochemistry. The latter was performed on a set of brains that was different from that which provided all the Northern assay and hormonal data reported in this paper. Except for during the first half of lactation (e.g., day 10), when mRNA levels were significantly elevated as compared with diestrus controls, the density of AVP gene transcripts in the pPVN remained unchanged during the second half of lactation and after weaning. In contrast, the levels of AVP mRNA in the mPVN were significantly increased in both lactating and postlactating (up to 20 d after weaning) rats, as compared with cycling virgin controls (Fig. 8 *bottom*). Parallel changes were found in OT mRNA levels in the mPVN and pPVN, with higher levels found in the former (Fig. 8, *top*). In both subdivisions of the PVN, the number of OT mRNA transcripts increased upon the commencement of lactation and declined to virgin control levels only after ~ 20 d after weaning. No significant differences were found between basal blood levels of OT and AVP in virgin (OT: 0.9±0.4 pg/ml; AVP: 5.3±2.4 pg/ml), lactating, and postweaning rats.

Plasma PRL levels. As compared with cycling virgin (1.4±0.6 ng/ml) and 24-h postweaning (2.4±1.3 ng/ml) rats, lactating rats (days 1–21 pooled) had significantly elevated blood concentrations of PRL (33.3±9.1 ng/ml).

Discussion

In keeping with earlier reports in the literature (15–17), this study shows the lactational state to be characterized by a severalfold elevation in circulating levels of CORT; the latter were inversely related to plasma ACTH levels. This hormonal profile was reversed within 1 d of weaning, but can apparently occur within just 3.5 h of pup removal (17). The ACTH/CORT profile during lactation closely resembles that observed in certain affective disorders in humans and in chronically stressed animals (32). However, whereas it has been postulated that CRH hypersecretion may play a casual role in the latter two conditions, measures of CRH mRNA (steady state levels) in this study suggest that adrenal hypersecretion during lactation may ensue from the involvement of other mechanisms, i.e., CRH
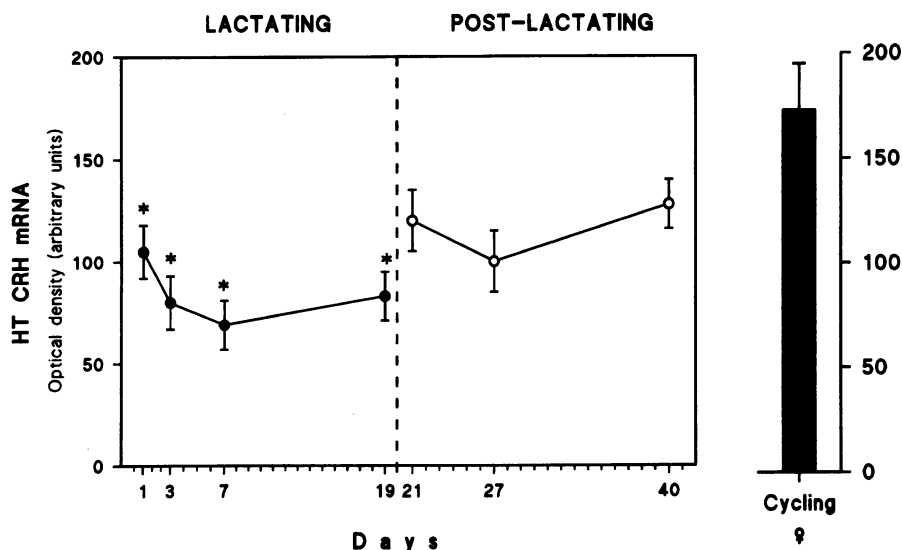
Figure 7. CRH gene expression in hypothalami of lactating, postlactating, and cycling virgin (control) rats. All values (arbitrary OD units) represent mean±SEM ($n = 6$–8 rats per group). The main panel shows changes in CRH gene expression at various time points throughout lactation and weaning. Asterisks indicate significant differences ($P \geq 0.05$) from CRH mRNA levels in nonparous, cycling females (*closed bar, far right*). For further details of quantitation of mRNA signals, see legend to Fig. 4.

gene expression was attenuated, a finding consistent with intact glucocorticoid feedback mechanisms (28, 33). Transcriptional activity of the POMC gene in the hypothalamus also showed feedback regulation by the elevated levels of CORT during lactation, a finding in keeping with observations in nonlactating animals (34); this was not the case with the pituitary POMC gene however (compare with reference 35), which was generally resilient to the hypercorticalismic state. This observation points to possible differential glucocorticoid regulation of POMC gene transcription in the hypothalamus and pituitary. The close parallel between ACTH secretion and the number of hypothalamic CRH gene transcripts probably reflects the ability of CRH to stimulate ACTH secretion directly, independently of ACTH synthesis.

In considering the lack of correlation between ACTH and CORT titers in the blood, it is important to note that although our experimental design may have missed suckling-induced episodes in ACTH secretion that could maintain high levels of adrenocortical secretory activity (compare with reference 17), these ACTH episodes apparently do not adequately account for the CORT hypersecretion during lactation: lactators are reportedly not more sensitive than nonlactators to the stimulatory effects of ACTH upon CORT secretion (15). Thus, ACTH does not seem to be responsible for the persistent drive on adrenocortical activity during lactation, and appraisal of other stimulatory factors is warranted.

As mentioned in the Introduction, two other neuropeptides, OT and AVP, are known to have ACTH-releasing activity (1, 2). Furthermore, there is some evidence that these peptides might directly stimulate adrenocortical secretion (36, 37). Expression of the genes encoding these peptides was therefore also evaluated in this study. Whereas Northern hybridizations of whole hypothalamic extracts (including the supraoptic nucleus) failed to reveal any significant changes in the expression of either gene (compare with references 38–40 reporting equivocal findings), retrospective in situ hybridization histochemical assays permitted an analysis of steady state OT and AVP mRNA levels in the two cellular subdivisions of the PVN mPVN and pPVN; it should be borne in mind that pPVN neurons mainly project to the median eminence (thereby exerting a direct influence upon ACTH secretion), whereas mPVN cells predomi-

nantly terminate in the neurohypophysis from which they reach their peripheral targets (e.g., kidney, mammary glands, uterus) via the general circulation. Both mPVN and pPVN showed elevated levels of AVP and OT gene expression during the first half of lactation; during the latter phase of lactation, the number of AVP gene transcripts returned to control levels while that of OT remained elevated through to the time of weaning. On the other hand, as shown previously (41), basal blood concentrations of OT and AVP were found to be similar in lactating, postlactational, and virgin rats. The latter results therefore tend to question the importance of these neurohypophyseal peptides in lactation-associated hypercorticalism.

It becomes obvious from the above that none of those peptides normally associated with HPA activation appear to play a substantial role in CORT hypersecretion during lactation. However, our confirmation of the well-documented (42) temporal coincidence among serum PRL concentrations, suckling, and pup removal raises the possibility that PRL might be a contributory factor. Early investigations suggesting that PRL might directly stimulate adrenocortical secretion (43–46) support this view, and the facts that (*a*) glucocorticoids potently inhibit PRL synthesis (47) and secretion (48) and (*b*) adrenalectomy results in increased PRL secretion (49) indicate the existence of a classical endocrine feedback loop. In light of these observations, it would appear that glucocorticoid-induced control of PRL secretion may also be altered during lactation.

The blunted responses of the various neuroendocrine parameters reported here and elsewhere (15–17, 21) most probably reflect a unique mechanism whereby the lactating mother is spared from the detrimental effects of persistently elevated glucocorticoid secretion (13). In this context, it should be noted that, at least in the rat, hypercorticalism appears to be necessary for the maintenance of lactogenesis (18) and for a number of maternal behavioral strategies related to survival of the offspring (19, 20). Clearly, these adaptive behaviors and lactation itself present the lactator with a multitude of apparently conflicting demands. The ability to reset tissue glucocorticoid responsiveness may subserve this requirement. Earlier studies have proposed a buffering role for PRL during periods of elevated adrenocortical activity (50, 51); although speculative at this stage, we suggest that analogous protective mechanisms
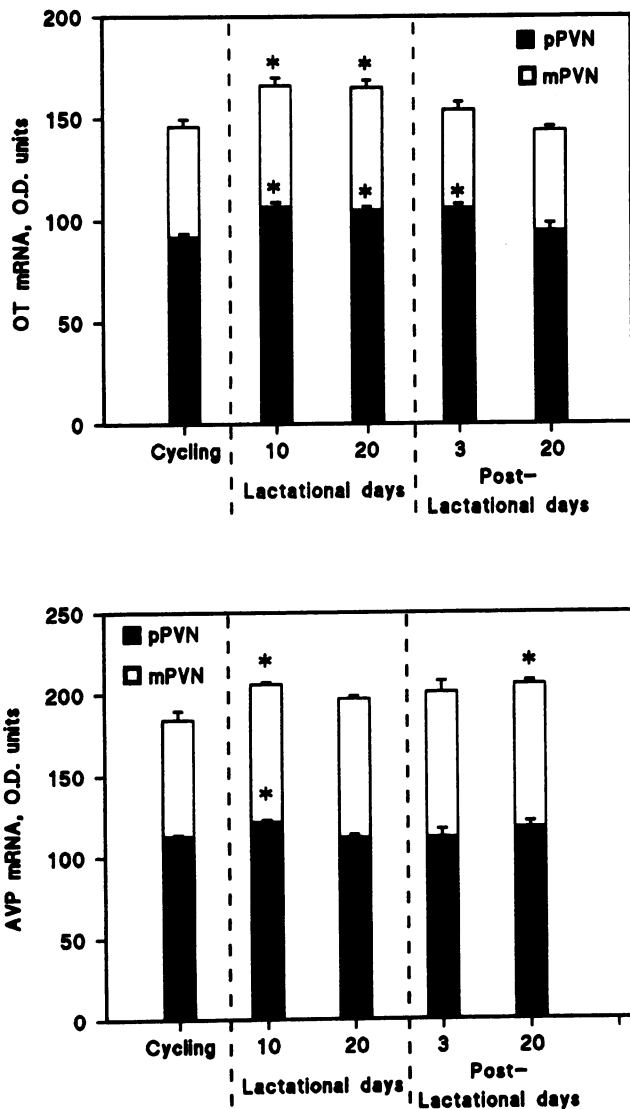
Figure 8. Expression of OT (*top*) and AVP (*bottom*) genes in the pPVN (*closed bars*) and mPVN (*open bars*) of cycling virgin (control), lactating, and postlactating rats. Means±SEM are shown (*n* = 8–12 rats per group). After appropriate tests of variance, data were pooled as follows: early and late lactation (up to day 10 and up to day 20, respectively), and early and late postlactation (up to day 3 and up to day 20, respectively). Asterisks denote values that are significantly different from those obtained in cycling rats (*P* ≥ 0.05).

might permit lactators to fluctuate between states of hyper- and normocorticalism. However, it would be premature at present to assign a single hormone (PRL, OT, or AVP) to this role; for example, it is conceivable that lactation is also accompanied by adaptive processes in other brain and adrenal neurochemical systems. In addition to providing a pretext for further studies into the biological basis of neuropsychiatric disorders associated with corticosteroid hypersecretion, the clinical importance of our findings is underscored by the fact that pregnancy and lactation are recurrent life events, raising the exciting possibility that a mother's corticosteroid buffering capacity may increase with parity. Indeed, there are grounds to believe this is the case: increasing parity has been associated with a gradual decline in neural and endocrine responses (52).

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