Anterior Pituitary Proopiomelanocortin Expression Is Decreased in Hypertensive Rat Strains*

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ABSTRACT

Studies comparing neuroendocrine differences between the spontaneously hypertensive rat (SHR) and the normotensive Wistar-Kyoto (WKY) strains have suggested altered anterior pituitary corticotrope expression of POMC associated with the development of hypertension in SHR animals. One major difficulty in comparing the SHR and WKY strains is that the two strains exhibit genetic differences unrelated to blood pressure status, because inbred in the SHR genome is a profile of behavioral characteristics different from those in the WKY, including hyperactivity in a novel environment and hyperreactivity in responding to stress. The present studies examine two new inbred rat strains, the WKHT and WKHA, which independently express the hypertension and behavioral traits, respectively. Together with the SHR and WKY, these genetically related, homozygous strains permit a more definitive means of examining the neuroendocrine correlates of either hypertension or behavior.

The adult (5-month-old) male anterior pituitary gland content of the POMC peptides β-endorphin and ACTH was decreased approximately 50% in the SHR and WKHT strains compared to that in the WKY strain, whereas hormone levels in the WKHA strain were not significantly different from those in the WKY strain. Reduced POMC peptide levels were, therefore, specifically associated with the hypertensive trait. Hormone content in prehypertensive weanling (5- to 7-week-old) SHR and WKHT animals was also reduced approximately 35% compared to that in WKY animals. Northern blot analysis identified a 45% decrease in POMC mRNA expression in the hypertensive SHR and WKHT strains, which paralleled the changes in tissue hormone content. Using both immunocytochemistry and in situ hybridization histochemistry, the number of labeled cells per unit area of tissue section was reduced approximately 45% in anterior pituitary tissues from SHR and WKHT rats compared to that in WKY tissues. The levels of POMC mRNA per cell, determined by quantitative densitometry, were not statistically different in the anterior pituitaries of WKHT, SHR, and WKY rats. The decrease in hormone content and POMC mRNA levels may, thus, reflect decreased anterior pituitary gland corticotrope populations. Although POMC peptide levels in the anterior pituitaries of adult WKHA animals were not significantly different from those in WKY animals, the morphological studies demonstrated a 30% increase in the corticotrope population in the WKHA strain. In contrast, POMC mRNA levels in WKHA animals were decreased 30%, and the amount of POMC mRNA per corticotrope was decreased approximately 35% compared to that in WKY, SHR, and WKHT tissues.

Using four related rat strains, SHR, WKY, WKHA, and WKHT, changes in anterior pituitary gland corticotrope POMC expression associated with the hypertensive phenotype in both adult hypertensive and weanling prehypertensive rats suggest a possible involvement of the hypothalamic-pituitary-adrenal axis in both the development and maintenance of the hypertensive state. The distinct profile of POMC expression in WKHA rats suggests altered corticotrope function associated with the hyperactive/hyperreactive to stress responses. (Endocrinology 134: 196-205, 1994)

AMONG numerous factors, the physiological responses to stress are thought to initiate and sustain a variety of pathological states, including behavioral abnormalities, neuroendocrine dysfunction, and cardiovascular disorders. Among many animal models used to study the relationship between stress and the physiological basis of these disorders, the spontaneously hypertensive rat (SHR) has been widely used as a model of essential human hypertension (1, 2). The SHR strain develops hypertension spontaneously between 9 and 15 weeks of age, which subsequently persists throughout life. Inheritance of the hypertensive trait in the SHR is additive, involving multiple genes (3). The hypothalamic-pituitary-adrenal axis (HPA) is activated during stress, and among many physiological changes, heightened neuroendocrine responses including alterations in POMC expression, have been implicated to participate in the hypertensive process in the SHR (4–8). Studies have demonstrated that hypophysectomy or adrenalectomy of SHR rats before the onset of hypertension ameliorates the hypertensive process (1, 7). Anterior pituitary gland corticotrope cell populations are altered in the SHR (1), and basal plasma POMC peptide and corticosterone levels exhibit abnormalities in SHR animals during development (7, 9, 10). SHR rats under a number of stress paradigms display aberrant POMC peptide and corticosterone responses (7, 11). Furthermore, iv administration of hypothalamic CRH I vasopressin to SHR animals elicits altered ACTH responses compared to those in WKY animals (6, 7, 12). These observations are consistent with the hypothesis that abnormalities in the HPA axis have central roles in the development of spontaneous hypertension.

It may be inappropriate to attribute all of these changes in the HPA axis to the hypertension in the SHR strain. The SHR is a highly inbred strain and displays numerous other characteristics different from those of the WKY control animals apart from blood pressure. In particular, SHR rats...
exhibit behavioral differences from WKY rats, including increased spontaneous activity in a novel environment, impaired habituation to novelty, increased cardiovascular and sympatho-adrenal medullary responses to stress, increased avoidance and operant responding, and increased aggression (13–17).

Holden and Ohlsson (18) initiated a recombinant inbreeding program to dissociate the two most prominent characteristics of the SHR strain, hypertension and hyperactivity. Cross-breeding of the SHR and WKY strains followed by selected recombinant inbreeding led to development of the WKH1 and WKHA rat strains. WKH1 rats are hypertensive, but not hyperactive, whereas WKHA rats are hyperactive, but not hypertensive (18). Together with the parental strains, SHR, which are both hyperactive and hypertensive, and WKY, which are neither, the four strains were used in the present study to determine neuroendocrine changes in anterior pituitary gland corticotropes associated with either of these two traits. It was hypothesized that changes relevant to hypertension should be observed in SHR and WKHT rats, but not WKY or WKHA rats. Similarly, changes relevant to hyperactivity should be observed in WKHA and SHR rats, but not WKY or WKHT rats. It was reasoned further that because WKHA rats express the hyperreactivity to stress observed in the SHR strain in addition to hyperactivity (19, 20), any changes in WKHA and SHR relative to the other two strains may also be relevant to hyperreactivity to stress. It should be noted, however, that the WKHA strain does not express some of the other identified behavioral traits of the SHR strain, such as impaired habituation to novelty (18) or aggression (21).

To investigate whether altered anterior pituitary gland POMC expression is associated with hypertension, we examined tissue POMC peptide content, POMC mRNA levels, and corticotrope populations in subjects from the inbred SHR, WKY, WKHA, and WKHT strains. We have identified decreased anterior pituitary gland POMC peptide and mRNA levels and apparent decreases in corticotrope populations in the hypertensive SHR and WKHT rat strains.

**Materials and Methods**

**Animals**

WKY, SHR, WKHA, and WKHT rats are homozygous strains bred and housed in a closed colony in the Animal Care Facility of the University of Vermont. All protocols were approved by the Institutional Animal Care and Use Committee. The SHR and WKY rats were descended of the original Wistar-Kyoto strains, inbred at the University of Vermont using breeding stock from the NIH and maintained genetically uniform with the NIH lines (WKY/N and SHR/N) by periodically introducing NIH breeding stock into the colony.

The WKHA and WKHT strains have been developed since 1980, starting from a cross-breeding of the SHR with the WKY strains. WKHA rats were selectively inbred for the traits of hyperactivity without hypertension, and WKHT rats were selectively inbred for hypertension without hyperactivity. In the present study, WKHA rats were from the F18 to F21 generations, and WKHT rats were from the F16 to F19 generations. Systolic blood pressure and locomotor activity measurements verified significantly elevated blood pressure in the SHR and WKHT and increased spontaneous activity in the SHR and WKHA.

For each experimental paradigm, adult (5- to 7-month-old) male rats from each of the four strains were matched for age. Among the hypertensive strains, the weanlings are considered prehypertensive, as hypertension develops from 9-15 weeks of age and persists throughout adult life (18). Among the hyperactive strains, increased locomotor activity is observed as early as weaning age (4 weeks), and this increase persists throughout adult life (18). Rats were housed communally, no more than four per cage, under a 12-h light, 12-h dark cycle, with access to food and water ad libitum.

**RIAs**

Rats from the four strains were killed by decapitation within 15 sec after removal from their cages between 0900–1100 h when pituitary POMC levels are at their nadir. Anterior pituitary lobes were separated from neurointermediate lobes under a dissecting microscope, weighed, and individually extracted in 5 μl acetic acid containing 2 mg/ml BSA and 30 μg/ml phenylmethylsulfonylfluoride using a ground glass microhomogenizer. The homogenates were frozen and thawed three times; the resulting extracts were lyophilized and subsequently reconstituted in 100 μm sodium phosphate buffer, pH 7.5, containing 1% Triton X-100 and 30 μg/ml phenylmethylsulfonylfluoride. Double antibody RIAs for POMC-related peptides were performed, as previously described (25), in 50 mM sodium phosphate, pH 7.6, containing 0.025% Triton X-100 at a final assay volume of 200 μl. Anterior pituitary gland extracts from individual animals were assayed in quintuplicate. Assays for β-endorphin used antisera JH2, which recognizes all forms of β-endorphin (26). Amino-terminal ACTH-specific antisera JH93 was used in assays for ACTH and αMSH-related peptides (27). Antiserum JH3 was used in assays for neuropeptide-Y (NPY)-related material. Antisera JH3 was raised against human NPY conjugated to keyhole limpet hemocyanin with glutaraldehyde. At a dilution of 1:100,000, the assay had a midpoint of 10 fmol. Maximum net binding was approximately 20% with [3H]NPY (2,000 Ci/mmol: Amersham Corp., Arlington Heights, IL); nonspecific binding was less than 2%. Antisera were generously provided by Drs. Richard Mains and Betty Epper (Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD).

**Immunocytochemistry**

Animals from all four strains were killed on the same day by decapitation between 0900–1100 h. The pituitary glands were rapidly removed, hemisected or blocked (<1 mm³), and immersed in 1% glutaraldehyde in 0.15 M sodium phosphate buffer, pH 7.5. Glutaraldehyde fixation was previously shown to provide best preservation of antigenic and tissue structure in studies using antisera to specific regions of the POMC molecule (25, 28). The tissues were subsequently washed in 0.15 M sodium phosphate buffer (pH 7.5). dehydrated, infiltrated, and embedded in Araldite 6005. Semithin plastic sections (0.5 μm) taken throughout the gland were heat mounted onto each slide; the sections were immunocytochemically stained using the avidin-biotin-peroxidase complex technique (Vectorstain Elite ABC Kit; Vector Laboratories, Burlingame, CA) with diaminobenzidine and hydrogen peroxide as peroxidase reaction substrates (25, 28–30). The tissue sections were incubated in dilute antiserum to specific POMC peptides in 1:200 normal goat serum for 48 h at 4°C. The antiserum to POMC-related peptides used for immunocytochemistry included a 1:100,000 dilution of antiseraum Kathy for ACTH and a 1:50,000 dilution of antiseraum Georje for the 16K fragment, as described previously for immunocytochemical studies (25, 26).

Morphometric analyses were performed as previously described (25, 29). Immunocytochemically stained anterior pituitary tissue sections were viewed under a x10 objective and a x10 ocular lens with a calibrated grid reticle positioned in one eyepiece (29). Stained nucleated cells within random grid areas were enumerated. Data are expressed as the mean number of cells per unit area from three animals per strain ± SEM.
In situ hybridization histochemistry

Rate of each strain were killed by decapitation, and anterior pituitary glands were rapidly removed, embedded individually in Tissue-Tek (Miles Laboratories, Elkhart, IN), and frozen in a dry ice-ethanol slurry. All pituitaries were stored at −70°C before processing for in situ hybridization histochemistry. Cryosections (16 μm) of anterior pituitary glands from each of the four strains were mounted on the same gelatin-chrom-alum-coated glass slides and stored at −70°C. Radiolabeled riboprobes were prepared using uridine 5′-[α-35S-thio]triphosphate (DuPont-New England Nuclear Research Products, Boston, MA) and T3 or T7 RNA polymerase (Promega Corp., Madison, WI) to synthesize, respectively, full-length antisense and sense transcripts from rat POMC cDNA (31) (kindly provided by Drs. Mains and Eipper). In situ hybridization histochemistry was performed as previously described (30, 32). Briefly, tissue sections were fixed with 4% paraformaldehyde, acetylated, and dehydrated. Hybridization was performed for 48 h in a moist chamber under unsealed cover slips at 45°C, using 1 × 106 cpm probe in 10 μl Tris-HCl, pH 7.4, containing 50% formamide, 600 μM sodium chloride, 1× Denhardt’s solution, 10% dextran, 10 mM dithiothreitol, and 1 mM EDTA (hybridization solution) for each section. The tissues were subsequently washed and incubated in RNase-A (Boehringer Mannheim Biochemicals, Indianapolis, IN) to synthesize, respectively, full-length antisense and sense transcripts from the rat POMC cDNA. Tissue sections were exposed to Kodak NTB-2 autoradiographic emulsion (Eastman Kodak, Rochester, NY) for 14 days at room temperature (32). POMC mRNA levels were quantitated on emulsion-coated sections using computer-assisted densitometry with a Matrox Image processing board (Summagraphics, Seymour, CT). Grain densities over 2-fold above background were evaluated.

Anterior pituitary levels of β-endorphin and ACTH were determined using RIA in tissue extracts from male WKY, SHR, WKHA, and WKHT rat at 5–7 weeks of age (weanling prehypertensive) and 5 months of age (adult hypertensive). The levels of β-endorphin immunoreactivity in the adult rat anterior pituitary glands were decreased significantly in the hypertensive strains [F(3,15) = 9.71; P < 0.001]; levels were reduced 45–50% in the SHR and WKHT strains compared to those in the WKY strain (Fig. 1A). Anterior pituitary levels of β-endorphin immunoreactivity in adult WKHA rats were not significantly different from those in the WKY control animals. Corticotrope ACTH immunoreactivity levels were similarly decreased approximately 40% in the WKHT and SHR strains relative to those in the WKY strain [F(3,19) = 56.01; P < 0.001; data not shown].

Hormone content in prehypertensive weanling SHR and WKHT animals was also significantly reduced [F(3,21) = 35.59; P < 0.001] approximately 30–35% below normotensive WKY levels (Fig 1B). Accordingly, a reduction in POMC peptide levels was apparent before the onset of hypertension in the SHR and WKHT strains. In contrast to those in adult tissues, the levels of β-endorphin immunoreactivity were significantly reduced approximately 10% in the anterior pituitary glands of weanling WKHA animals.

In addition to POMC, the anterior pituitary gland expresses a number of other bioactive peptides. Previous studies demonstrated that anterior pituitary enkephalin levels were decreased in hypertensive rat strains.

Northern blot analysis

The expression of POMC mRNA was determined using Northern blot analysis. Male rats from each of the four strains were killed by decapitation between 0900–1100 h. The pituitary glands were rapidly dissected, the anterior lobe was separated from the neurointermediate lobe, and the tissues were frozen at −70°C. Total RNA from individual anterior pituitary lobes was prepared using RNA STAT-60 total RNA/mRNA isolation reagent (Tel-Test B, Inc., Friendswood, TX), which includes guanidium isothiocyanate and phenol in a monophase solution. Each anterior pituitary gland was homogenized in 800 μl RNA STAT-60 reagent by repeated passage through a 27-gauge needle. After the addition of 160 μl chloroform, the sample was centrifuged at a relative centrifugal force of approximately 15,000 × g for 20 min at 4°C. Approximately 400 μl of the upper aqueous phase were transferred to a sterile tube; 400 μl isopropanol were added, and the RNA was allowed to precipitate on ice for 30 min. The samples were centrifuged to pellet the RNA precipitate. The pellet was washed once in 75% alcohol and redissolved in sterile distilled water. RNA was quantitated spectrophotometrically.

Total RNA (5 μg/lane) was denatured and electrophoresed on 1.5% agarose gels containing 2.2 M formaldehyde, 20 mM morpholinopropanesulfonic acid, 5 mM sodium acetate, and 1 mM EDTA, pH 7.0. The RNA was transferred to a Nytran membrane (Schleicher and Schuell, Keene, NH) by capillary action in 20 × SSC (3 M NaCl and 0.3 M sodium citrate, pH 7.0). The rat POMC cDNA probes were labeled with [α-32P]dideoxy-CTP to a specific activity of 475 μCi/μg by random prime synthesis (Amersham Corp., Arlington Heights, IL) and used in the Northern analysis at 1 × 106 cpm/ml. The Nytran filters were prehybridized, hybridized, and washed, as previously described (33). The blots were exposed to x-ray film at −70°C with an intensifying screen, and multiple autoradiographic exposures were obtained to permit accurate measurements of POMC mRNA. To correct for the actual amount of RNA applied to each lane, blots were rehybridized to cDNA probes (570 μCi/μg) derived from 18S and 28S frog ribosomal RNA (33). Total integrated optical density for autoradiographic bands was determined by densitometry using a Quantity One image analysis system (pdi, Huntington Station, NY).

Data analysis

Analysis of variance (ANOVA) was used to determine differences among the four strains. Tukey’s or Newman-Keuls test was used in post hoc analysis to identify which strains differed from the others; P < 0.05 was considered significant. All values are expressed as the mean ± SEM.

Results

Anterior pituitary POMC peptide levels are decreased in hypertensive rat strains

The levels of the anterior pituitary gland POMC peptides β-endorphin and ACTH were determined using RIA in tissue extracts from male WKY, SHR, WKHA, and WKHT rat at 5–7 weeks of age (weanling prehypertensive) and 5 months of age (adult hypertensive). The levels of β-endorphin immunoreactivity in the adult rat anterior pituitary glands were decreased significantly in the hypertensive strains [F(3,15) = 9.71; P < 0.001]; levels were reduced 45–50% in the SHR and WKHT strains compared to those in the WKY strain (Fig. 1A). Anterior pituitary levels of β-endorphin immunoreactivity in adult WKHA rats were not significantly different from those in the WKY control animals. Corticotrope ACTH immunoreactivity levels were similarly decreased approximately 40% in the WKHT and SHR strains relative to those in the WKY strain [F(3,19) = 56.01; P < 0.001; data not shown].

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DECREASED CORTICOTROPE POMC IN HYPERTENSION

**FIG. 1.** Anterior pituitary gland 

β-endorphin immunoreactivity levels are decreased only in hypertensive rat strains. 

Data represent mean picomoles of β-endorphin immunoreactivity per lobe from four to seven animals per strain ± SEM. One-way ANOVA demonstrated a significant effect of strain on β-endorphin levels in both adult [F(3,15) = 9.71; \( P < 0.001 \)] and weanling [F(3,21) = 32.59; \( P < 0.001 \)] animals. *, Significantly different from WKY by ANOVA and Newman-Keuls test (\( P < 0.05 \)). **, Significantly different from WKY, SHR, 

and WKHT by ANOVA and Newman-Keuls test (\( P < 0.05 \)).

**FIG. 2.** Anterior pituitary gland POMC mRNA is decreased in SHR, WKHA, and WKHT animals compared with that in WKY animals. Total RNA samples (5 μg) from individual anterior pituitary glands from adult WKY, SHR, WKHA, and WKHT rats were electrophoresed on 1.5% agarose gels and examined by Northern blot analysis using \(^{32}\)P-labeled rPOMC cDNA. The blots were rehybridized with radiolabeled ribosomal RNA probes. Individual POMC mRNA bands on the autoradiograms were densitized, and the integrated optical densities were normalized to sample ribosomal RNA levels. Data represent the mean relative optical density from four or five animals per strain ± SEM. ANOVA demonstrated a significant effect of strain on POMC mRNA levels \[F(3,14) = 32.40; \( P < 0.001 \); Fig. 2\]. The reduced levels of POMC mRNA in the hypertensive strains paralleled the trait-specific changes in POMC peptide content. Anterior pituitary POMC levels in the adult WKHA strain, determined by RIA, were not statistically different from those in the WKY strain. In contrast, POMC mRNA levels in the anterior pituitaries of adult WKHA animals were diminished approximately 30% compared to those in WKY animals (Fig. 2).

Decreased anterior pituitary gland corticotrope cell numbers are associated with hypertension, whereas increased corticotrope cell numbers are associated with hyperactivity/hyperreactivity

Immunocytochemical studies were performed to examine the cellular basis for altered anterior pituitary gland POMC peptide content and mRNA expression among the four rat strains. The stained anterior pituitary cells from all four strains were primarily stellate in shape, with dense immunoreactive material at the cell periphery characteristic of anterior pituitary corticotropes. Immunocytochemically stained anterior pituitary corticotropes in adult male tissues from SHR and WKHT rats demonstrated two major differences from WKY animals (Fig. 3). Firstly, with respect to the apparent relative staining intensity in individual cells, SHR and WKHT corticotropes exhibited a broader range of staining intensities than WKY tissues. Secondly, quantitation of the ACTH-immunoreactive anterior pituitary cells among the anterior pituitary gland POMC mRNA expression in the WKY, SHR, WKHA, and WKHT rat strains was assessed using Northern blot analysis. Total RNA from anterior pituitary glands from individual animals of each strain was fractionated, blotted, and hybridized using radiolabeled rat POMC cDNA. The resulting autoradiograms were quantitated using computer-assisted densitometry, and the levels of POMC mRNA in each sample were normalized to ribosomal RNA levels. Adult rat anterior pituitary POMC mRNA expression was decreased approximately 45% in the SHR and WKHT strains compared to that in the WKY \([F(3,14) = 32.40; \( P < 0.001 \); Fig. 2]\). The reduced levels of POMC mRNA in the hypertensive strains paralleled the trait-specific changes in POMC peptide content. Anterior pituitary POMC levels in the adult WKHA strain, determined by RIA, were not statistically different from those in the WKY strain. In contrast, POMC mRNA levels in the anterior pituitaries of adult WKHA animals were diminished approximately 30% compared to those in WKY animals (Fig. 2).

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Anterior pituitary POMC mRNA levels are decreased in hypertensive and hyperactive rat strains

To determine whether the changes in tissue POMC peptide content reflected altered POMC mRNA levels, anterior pituitary gland POMC mRNA expression in the WKY, SHR, WKHA, and WKHT rat strains was assessed using Northern blot analysis. Total RNA from anterior pituitary glands from individual animals of each strain was fractionated, blotted, and hybridized using radiolabeled rat POMC cDNA. The resulting autoradiograms were quantitated using computer-assisted densitometry, and the levels of POMC mRNA in each sample were normalized to ribosomal RNA levels. Adult rat anterior pituitary POMC mRNA expression was decreased approximately 45% in the SHR and WKHT strains compared to that in the WKY \([F(3,14) = 32.40; \( P < 0.001 \); Fig. 2]\). The reduced levels of POMC mRNA in the hypertensive strains paralleled the trait-specific changes in POMC peptide content. Anterior pituitary POMC levels in the adult WKHA strain, determined by RIA, were not statistically different from those in the WKY strain. In contrast, POMC mRNA levels in the anterior pituitaries of adult WKHA animals were diminished approximately 30% compared to those in WKY animals (Fig. 2).

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FIG. 3. Immunocytochemical staining of anterior pituitary tissues from hypertensive and hyperactive rat strains revealed altered ACTH immunoreactivity staining patterns. Anterior pituitary tissue sections (0.5 μm) from 6-month-old WKY, SHR, WKHA, and WKHT rats were immunocytochemically stained for ACTH using a 1:100,000 dilution of antiserum Georgie. In contrast to tissues from WKY and WKHA animals, tissues from SHR and WKHT animals contained a smaller population of ACTH-immunoreactive cells. In addition, corticotropes in tissues from the SHR and WKHT rat strains exhibited a broader range of staining intensities compared with normotensive animals. Bar = 50 μm.

FIG. 4. Anterior pituitary gland corticotrope populations are decreased in SHR and WKHT animals and increased in WKHA animals. The number of anterior pituitary gland cells labeled for POMC peptide immunoreactivity (A) and POMC mRNA (digoxigenin-labeled POMC riboprobes) (B) were quantitated using an ocular grid over a 0.16-mm² area under brightfield microscopy. Two to four tissue sections from each animal were enumerated. Data represent the mean cell number per unit area ± SEM (n = 3–4 animals/strain). ANOVA demonstrated a significant effect of strain on corticotrope cell number in immunocytochemical [F(3,8) = 51.15; P < 0.001] and in situ hybridization histochemical [F(3,12) = 75.64; P < 0.001] studies. *, Significantly different from WKY by ANOVA and Newman-Keuls test (P < 0.05). **, Significantly different from WKY, SHR, and WKHT by ANOVA and Newman-Keuls test (P < 0.05).

four rat strains revealed that the number of corticotropes per unit area of tissue section was reduced in the hypertensive SHR and WKHT animals compared to that in the WKY tissue (Fig. 4A). Cell numbers were decreased 40–45% relative to those in WKY tissue [F(3,8) = 51.15; P < 0.001], which is consistent with the decreased levels of anterior pituitary POMC peptides determined by RIA. Taken together, the RIA, Northern blot, and immunocytochemical studies suggested that the decreased anterior pituitary POMC expression in hypertensive animals was a consequence of reduced numbers of corticotropes.

In contrast to SHR and WKHT rats, WKHA exhibited a significant increase in the number of corticotropes in adult anterior pituitary glands. The number of immunoreactive cells in the WKHA tissue was increased approximately 30% compared to that in the WKY control rats. As the POMC peptide levels in adult WKHA anterior pituitary glands, determined by RIA, were not significantly different from
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FIG. 5. In situ hybridization histochemical photomicrograph of the relative distribution of anterior pituitary gland POMC mRNA among WKY, SHR, WKHA, and WKHT rats. Cryosections (16 μm) of anterior pituitary tissues from 6-month-old WKY, SHR, WKHA, and WKHT animals were hybridized to 35S-labeled POMC riboprobes, as described in Materials and Methods. The darkfield micrograph reveals an apparent decrease in the number of hybridizing cells in the hypertensive SHR and WKHT strains. Bar = 50 μm.

WKY levels, but the number of corticotropes was increased, it appeared that POMC peptide content per corticotrope was decreased in the WKHA.

POMC mRNA per corticotrope is not changed in hypertensive rats, but is decreased in hyperactive/hyperreactive rats

A number of physiological processes could contribute to the apparent decreased levels of corticotrope ACTH immunoreactivity and cell number in hypertensive SHR and WKHT animals. Although lower anterior pituitary POMC peptide content may be a result of decreased corticotrope number, the diminution could also reflect hypersecretion from increased corticotrope stimulation, leading to decreased pituitary cell peptide content. Chronic stimulation of anterior pituitary corticotrope cells with CRH either in vivo or in vitro has been shown to deplete cellular secretory granules (25, 29, 35). The degranulation process decreases tissue POMC content and produces an apparent decrease in the corticotrope number observed immunocytochemically.

To determine whether the decreased anterior pituitary POMC peptide levels in the hypertensive rats were the result of either decreased corticotrope number or increased corticotrope secretin, in situ hybridization histochemical studies were initiated, using both isotopic and nonisotopic methods. The enzymatic detection of digoxigenin-labeled probes methods permitted higher cellular resolution for cell enumeration, whereas hybridization with 35S-labeled riboprobes allowed densitometric measurements to assess changes in cellular mRNA levels. The digoxigenin and autoradiographic labeling pattern in the anterior pituitary tissues among the four rat strains determined by in situ hybridization histochemistry were similar to those determined by immunocytochemistry (Fig. 5). The number of anterior pituitary cells expressing POMC mRNA was decreased approximately 40% in the hypertensive SHR and WKHT rat strains compared to the WKY strain \( F(3,12) = 75.64; P < 0.001; \) Fig. 4B); the number of corticotropes in WKHA animals was increased approximately 30%. The changes in corticotrope cell numbers observed by in situ hybridization histochemistry correlated well with the immunocytochemical studies. Combined, these studies suggest that the decreased anterior pituitary POMC expression associated with the hypertensive trait was not a reflection of hypersecretory processes, but may result primarily from decreased corticotrope cell numbers.

Although the number of corticotropes was elevated in adult WKHA animals, the levels of POMC peptides were similar to those observed in WKY rats, and POMC mRNA expression was reduced. The relative changes in POMC mRNA levels in individual corticotropes in tissue sections hybridized to 35S-radiolabeled riboprobes among the four strains were quantitated using autoradiographic densitometric methods. The cell population profiles, as a function of integrated optical density, were similar for WKY, SHR, and WKHT cells (Fig. 6). In contrast, the cell population profiles were shifted to lower optical densities in WKHA tissues,
indicating lower POMC mRNA content per corticocyte. The mean optical densities over corticocytes from WKHT and SHR animals were not significantly different from those in WKY animals (Fig. 6), supporting the hypothesis that the decreased POMC expression in the anterior pituitaries from the hypertensive strains were primarily a result of changes in the population of corticocytes. On the other hand, the mean POMC mRNA per corticocyte in the WKHA strain was decreased approximately 35% compared to that in the WKY, SHR, and WKHT strains (Fig. 6).

Discussion

Despite early studies implicating defects in the HPA axis in the ontogenesis of hypertension, there has been little consensus on the role of the POMC system in the development of hypertension. Hypophysectomy or adrenalectomy before the onset of hypertension has been shown to ameliorate hypertension in SHR animals (1, 7). Transplantation of hypothalamic tissue from SHR to WKY leads to increased blood pressure in the WKY animal (36). Anterior pituitary gland and plasma POMC peptide levels have been reported to be higher, unchanged, or diminished in SHR animals compared to those in WKY (9, 34, 37–42). Our present data coincide with recent studies demonstrating significantly lower levels of POMC peptides in the anterior pituitary gland of the SHR compared to the WKY strain (34). We identified decreased anterior pituitary gland POMC peptide levels in the adult SHR and WKHT rats compared to those in the normotensive WKY and WKHA animals, indicating that reduced hormone levels were specifically associated with the hypertensive trait. Although lower in magnitude, the changes were also observed in the prehypertensive young animals, suggesting that decreased corticotrope hormone levels preceded the onset of hypertension and were exacerbated during the progression of the hypertensive process. Northern blot analysis demonstrated decreased POMC mRNA levels in parallel with decreased POMC peptide levels in the hypertensive animals and indicated that the lower anterior pituitary gland hormone levels were a direct reflection of tissue POMC mRNA expression. Morphological studies, including immunocytochemistry and in situ hybridization histochemistry, demonstrated that these changes in tissue POMC peptide content represented an apparent decrease in the anterior pituitary corticocrope cell population. Indeed, the number of anterior pituitary gland cells that exhibited POMC peptide immunoreactivity was decreased in the SHR and WKHT strains. As the depletion of cellular hormone stores under chronic stimulatory conditions can produce an apparent decrease in the number of immunocytochemically identifiable cells (25, 29, 35), the populations of anterior pituitary cells over individual cells in four random fields per section from three animals per strain were digitized. The cell population frequencies are plotted as a function of integrated optical densities for each strain. The mean optical densities (O.D.) from the tissues ± SEM are indicated for each strain. ANOVA demonstrated a significant effect of strain on optical density [F(3,8) = 6.11; P = 0.018]. *, Significantly different from WKY, SHR, and WKHT by ANOVA and Newman-Keuls test (P < 0.05).
expressing POMC mRNA were examined using in situ hybridization histochemistry. In the hypertensive animals, the POMC mRNA-expressing corticotrope populations were decreased in parallel with the cell population expressing POMC peptide immunoreactivities. Thus, the decreased corticotrope populations identified immunocytochemically were not biased by hypersecretory events, and the reduction in the corticotrope population may have contributed to the decreased anterior pituitary POMC expression associated with the hypertensive trait. The broader range of corticotrope POMC peptide immunoreactivity staining intensities in the SHR and WKHT animals, however, may reflect altered corticotrope secretory patterns in the hypertensive rat strains and will require further regulatory studies. In contrast to the present study, Hauser et al. (37) demonstrated no change in the volume density of ACTH-immunoreactive cells between WKY and SHR strains. However, these studies examined the volume density of ACTH-immunoreactive cells in 4-week-old prehypertensive SHR animals, whereas our morphological studies examined cell numbers per tissue unit area in 6-month-old hypertensive animals, which may contribute to the apparent differences between the two studies.

Although POMC peptide levels in the anterior pituitary glands of adult WKHA animals were not significantly different from WKY levels, the population of corticotropes was increased in both the immunocytochemical and in situ hybridization histochemistry studies. Furthermore, tissue and cellular POMC mRNA levels were decreased in the anterior pituitary of WKHA compared to WKY. Homeostatic regulatory mechanisms may be involved in the maintenance of tissue POMC production levels by regulating biosynthesis in a larger population of corticotrope cells; this may play a role in the hyperactivity/hyperreactivity to stress responses observed in the WKHA strain. The mechanisms underlying these processes will require additional studies of corticotrope POMC expression, biosynthesis, and secretion in the WKHA strain under normal and stress conditions.

Several physiological studies have demonstrated abnormal corticotrope responsiveness to hypothalamic regulators in SHR animals. Intravenous injection of CRH elicits lower plasma ACTH levels in SHR animals compared to WKY rats, although there are no apparent differences in anterior pituitary gland CRH receptor binding between the two strains (6, 7, 12, 43). Although the blunted corticotrope ACTH response to the releasing factor CRH observed in adult SHR animals could be interpreted as a consequence of a reduced corticotrope population resulting in a diminished maximal stimulatory ACTH response, the reduced ACTH response to CRH in the SHR has been suggested to be associated with the hyperactivity/hyperreactivity trait (43). The mechanisms underlying these changes in corticotrope responses to hypothalamic factors will require further examination of the four strains.

The developmental and physiological events that alter anterior pituitary corticotrope function in the hypertensive SHR and WKHT strains remain unclear. Our current results are consistent with suggestions that the abnormal corticotrope responses may be secondary to augmented adrenal function in hypertension. Pituitary corticotrope function is tightly regulated by circulating corticosteroids in negative feedback pathways to the pituitary gland and central nervous system (44). Corticotropes chronically treated with glucocorticoids or dexamethasone in vivo or in vitro exhibit diminished POMC peptide production and transcription of POMC mRNA (44-48). Compared to the WKY strain, prehypertensive SHR animals display earlier expression of plasma corticosterone during development (6, 7, 9-11). Young SHR animals exhibit elevated baseline levels of corticosterone and greater corticosterone responses to stress (6, 7, 10). Several considerations suggest that the elevated plasma corticosterone levels in young SHR animals may have significant consequences on the developing HPA axis and the hypertensive process. Along with other endocrine systems, the HPA axis undergoes rapid developmental changes during the first 2 weeks of life in the rat, and the functional capacity to respond to stressors or physiological regulators is present throughout development, exhibiting age and stressor/regulator specificity (49, 50). As adult levels of glucocorticoid receptors are present in neonatal rat pituitary glands, corticotropes exhibit increased steroid sensitivity (45). Furthermore, low circulating levels of corticosterone-binding globulin in neonatal animals may also result in higher circulating levels of free steroid, thereby enhancing the inhibitory feedback process on the HPA axis during development (51). Inappropriate or transient changes in plasma corticosterone levels in developing animals have thus been implicated to elicit dramatic long-term effects on a variety of neuroendocrine functions. Whether elevated corticosterone from hyperfunctioning adrenal cortical cells is involved in the altered corticotrope development in the hypertensive SHR and WKHT strains will require further study. The decreased corticotrope population and POMC expression in the SHR and WKHT animals are consistent with inhibitory regulatory mechanisms and concur with studies suggesting adrenal function defects in hypertensive animals. The physiological events leading to augmented adrenal function and elevated corticosterone production during development of hypertension await more detailed study. The altered accelerated development of adrenal cortical function in the SHR has been suggested to be a consequence of increased hypothalamic CRH expression and anterior pituitary corticotrope POMC peptide production in the developing animal (7). Sustained activation of the adrenal cortex may subsequently contribute to the physiological changes leading to the development of hypertension. In contrast, other studies have suggested that glucocorticoid and mineralocorticoid receptor function are altered in some strains of hypertensive rats (52, 53). Whether steroid receptor dysfunction, variations in steroid receptor expression, or changes in adrenal cortical function contribute to altered anterior pituitary POMC production and blood pressure control remains uncertain. Additional studies of the hypothalamic-pituitary-adrenal axis in the hypertensive rat strains during development may clarify some of these issues.

The present studies demonstrate that altered anterior pituitary corticotrope function is correlated with hypertension and that the reductions in tissue POMC expression most
likely reflect decreased corticotrope cell numbers. The four strains of rats have allowed identification of specific changes associated with the hypertensive and hyperactive traits. Further studies using these animals should help define the contributions of altered hypothalamic-pituitary-adrenal function to the hypertensive process.

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