Laboratory Studies

Hyperparathyroidism and Abnormal Calcitriol Metabolism in the Spontaneously Hypertensive Rat

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Abnormalities of calcium metabolism and of its two principal regulating hormones, parathyroid hormone and 1,25-dihydroxyvitamin D$_3$ (calcitriol), have been reported in the spontaneously hypertensive rat (SHR). Reports of abnormal calcitriol metabolism in the SHR by several groups have not provided measurements of tissue calcitriol receptors. Similarly, few data are available as to the parathyroid status of the SHR. In the present study, circulating calcitriol levels and intestinal and parathyroid gland calcitriol receptor status were determined in male SHR and in Wistar-Kyoto (WKY) rats. Parathyroid status was investigated by determination of parathyroid gland mass together with tissue micromorphometry and by quantitative histology of bone as a measure of the biological action of parathyroid hormone. Circulating calcitriol levels were reduced in the 11-week-old SHR compared with the WKY rat (165±23 vs. 194±28 pmol/1, p<0.01, mean±SD). Calcitriol-free ratio was diminished and maximal specific binding capacity for calcitriol was increased in the SHR in parathyroid tissue (172±4.9 vs. 123±6.6 fmol/mg protein, p<0.01) and in intestinal mucosa with no change of receptor affinity. Plasma ionized calcium (1.29±0.05 vs. 1.45±0.35 mmol/1, p<0.05) and phosphate (1.5±0.26 vs. 2.4±0.03 mmol/1, p<0.05) were significantly lower in the SHR. Parathyroid gland mass was increased in the SHR (59±12 vs. 11±1 pg/100 g body wt, p<0.001) as a result of hyperplasia and not hypertrophy. Higher osteoclast numbers were observed in SHR bone (27.6±0.79 vs. 23.9±0.66 osteoclasts/mm$^2$, p<0.01), suggesting increased parathyroid hormone activity. In summary, in the 11-week-old SHR we observed reduced circulating calcitriol levels together with increased tissue calcitriol receptor numbers, increased parathyroid gland mass, and histological evidence of hyperparathyroidism. It is possible that these abnormalities influence the development of hypertension in the SHR. (Hypertension 1989;13:233-242)
blood pressure\textsuperscript{17} and altered pressor responsiveness\textsuperscript{18} in vitamin D-deficient rats is of obvious relevance.

Few data are available on the parathyroid status of the SHR. Primary hyperparathyroidism is associated with hypertension\textsuperscript{19,20} but the mechanism relating the two conditions is far from clear.

In the present study, we have sought to clarify the abnormalities of the SHR calcium endocrine system by examining both the vitamin D and parathyroid axis. We determined both circulating calcitriol levels and intestinal and parathyroid gland calcitriol receptor status in male SHR and WKY rats. Parathyroid status was investigated by determination of parathyroid gland mass together with tissue micromorphometry. The biological effect of PTH was determined by quantitative histology of its major target organ, bone.

**Materials and Methods**

**Animals**

Male SHR and their normotensive controls (WKY rats) were obtained from the Department of Pharmacology, Heidelberg University, Heidelberg, FRG (courtesy of Dr. U. Ganten).\textsuperscript{21} SHR were of the stroke-prone strain, bred in Heidelberg by brother-sister mating. WKY rats were also from the original Okamoto strain, a gift of Professor K. Okamoto, Kyoto, Japan, and were brought to Heidelberg with the SHR in 1975. Stroke-prone SHR consistently exhibit lower body weight than WKY rats. After weaning at 3 weeks of age, seven rats from each group were kept in plastic cages in a controlled environment with a 12-hour light/dark cycle and constant temperature (22°C) and humidity (70%). Rats received a diet that contained 2.0 IU/g vitamin D3, 0.66% phosphorus, and 0.75% calcium (% dry weight). The rats had free access to food and deionized water. The rats used for histological analysis of bone were obtained and reared as previously described.\textsuperscript{7}

**Receptor Studies**

Four series of rats were studied. The first three series comprised seven SHR and seven WKY rats that were fed the above diet until the age of 5, 11, or 24 weeks. Those studied at 11 weeks were kept in metabolic cages for a 24-hour urine collection to measure creatinine and cyclic adenosine 3',5'-monophosphate (cAMP). Then, unless indicated otherwise, rats were exsanguinated under ether anesthesia, and tissues were used for receptor studies. In the fourth series, SHR and WKY rats were given 2×10\textsuperscript{11} ng/day calcitriol or vehicle by intraperitoneal injection for a total period of 4 days. Animals were subsequently used for receptor studies.

**Chemicals**

1,25-Dihydroxy[26,27-methyl-\textsuperscript{3H}]cholecalciferol (158 Ci/mmol) was obtained from Amersham/Buchler Co. (Braunschweig, FRG). Radiochemical purity, as checked by high-performance liquid chromatography (HPLC), was 93-98% with no other demonstrable vitamin D metabolites. Radioinert, chromatographically pure calcitriol and calcifediol were obtained from Hoffman-La Roche AG Laboratories (Grenzach, FRG). [\textsuperscript{14}C]Methylated bovine serum albumin (20 μCi/mg protein), [\textsuperscript{14}C]methylated ovalbumin (20 μCi/mg protein), and [\textsuperscript{3}H]methylated gammaglobulin (20 μCi/mg protein) were purchased from NEN Laboratories (Dreieichenhain, FRG). Hydroxyapatite, dithiothreitol, calf thymus DNA (type I), Whatman CF-11 cellulose, Triton X-100, sodium molybdate, and L-1-tosylamide 2-phenylethyl chloromethyl ketone (TPCK) were obtained from Sigma Laboratories (München, FRG).

**Micromorphometric Analysis of Parathyroid Glands**

One parathyroid gland per animal was randomly chosen and carefully excised for morphometric investigation. All specimens were fixed for 24 hours in ice-cold phosphate buffer (0.1 mol) that contained 1.5% paraformaldehyde and 1.5% glutaraldehyde and were subsequently transferred for 30 minutes to 1% OsO\textsubscript{4} at room temperature, dehydrated in ethanol, and embedded in Epon-Araldite. Semithin sections (1 μm) were stained with methylene blue and basic fuchsin\textsuperscript{22} and were examined by light microscopy (Zeiss, Oberkochen, FRG) with use of oil immersion and phase contrast.

The volume densities (volume per unit tissue volume) of epithelial cells \(V_{V(e)}\) and interstitial tissue \(V_{V(i)}\), and the volume density of epithelial cell nuclei \(V_{V(n)}\) were estimated by means of the point counting procedure.\textsuperscript{23} Two random sections of each parathyroid gland were selected for morphometric analysis. The test areas were obtained by random systematic subsampling. Eight test areas (58,000 μm\textsuperscript{2}) per gland were analyzed with a Zeiss eyepiece containing 100 test points. Reference volume was the total volume of the parathyroid gland.

Mean nuclear volumes \(\bar{V}_{V(n)}\) were determined as proposed by Gundersen and Jensen.\textsuperscript{24} The average of the third power of observed point-sampled intercept lengths \(\langle l_0\rangle^3\) multiplied by \(\pi/3\) is an unbiased estimator of the mean particle volume in the volume-weighted distribution of individual particle volumes:

\[
\overline{V}_{V} = \frac{\pi}{3} \times \langle l_0 \rangle^3
\]

Numerical density \(N_{V}\) of epithelial cell nuclei cannot be obtained from two-dimensional sections without assumptions about the size and shape of the particles. \(N_{V}\) was estimated according to the equation\textsuperscript{25}:

\[
N_{V} = K \times 1/\beta \times N_{A}^{3/2} \times \bar{V}_{V}^{1/2}
\]

where \(K\) is the correction for assumed size distribution of particles (\(=1.05\)), \(\beta\) is the shape coefficient (sphere \(=1.38\), prolate ellipsoid with axes 2:1:1 = 1.38), prolate ellipsoid with axes 3:1:1 = 1.95), and \(N_{A}\) is the number of profiles per unit sectional area.\textsuperscript{23} Since parathyroid epithelial cell nuclei tend to resemble prolate ellipsoids with unknown ratios of the axes,
we calculated NV, both for spherical particles N\text{S}\text{P} and prolate ellipsoid axes 3:1:1 N\text{E}\text{P} to obtain an estimate for the potential range of NV. From the NV estimates of nuclei and V\text{c} of epithelial cells we deduced mean epithelial cell volume per nucleus V\text{c}(e)=V\text{c}/N\text{c} and V\text{c}(e)=V\text{c}/N\text{c}. In the case of mononuclear epithelial cells this parameter reflects mean cell volume.

**Bone Histology**

Oxytetracycline was administered to 20 SHR and 20 WKY rats (20 mg/kg Terramycine, Pfizer Inc., New York, New York) as a single intravenous dose 14 days and 1 day before exsanguination. Quantitative histology was carried out as previously described. In brief, proximal tibial metaphyses were fixed in 2% glutaraldehyde and 1.2% formaldehyde, decalcified in 10% EDTA and embedded in ester-wax. Four-micron-thick frontal sections were stained with toluidine blue (pH 6.4).

Osteoclasts were identified as large multinucleated cells with foamy cytoplasm, located on or near bone or cartilage surfaces. Osteoclasts with a single nucleus were identified by their cytoplasmic appearance and their typical brush border. The number of osteoclasts was measured at 500× magnification in the central part of the metaphysis in a 1,200-μm large (along the growth cartilage) and 1,500-μm deep area (below the growth cartilage) and expressed as number of cells per millimeter squared.

Tibial diaphyses were fixed in absolute ethanol, and two cross sections perpendicular to the longitudinal axis were taken from the segment proximal to the fibular junction. The sections were hand-ground to a thickness of 50 μm and examined by fluorescent microscopy. The periosteal calcification rate, expressed in μm/day, was obtained by dividing the mean distance between the two subperiosteal fluorescent tetracycline labels by the time interval between the two tetracycline injections. The values obtained for each animal represent the mean of about 100 measurements made on one section at regular intervals along the circumference of the periosteal surface.

Periosteal bone formation was obtained by multiplying the periosteal calcification rate by the total periosteal surface. All measurements were performed by using a semiautomatic technique (Morphomat 10, Zeiss) at 250× magnification.

**Measurements**

Serum and urine chemistry was determined with Autoanalyzer technique. Serum ionized calcium was measured by Electrolyte Analyzer 980 (Gesellschaft für Medizintechnik, Bad Homburg, FRG). Calcitriol was measured by radioimmunoassay after HPLC of serum extracts, and 25-hydroxyvitamin D\text{3} (calcidiol) was measured by a binding assay described previously. Absolute free ratio of calcitriol was calculated as described before from the measured molar concentrations of calcitriol and total D binding protein with the known association constant of D binding protein. Urinary cAMP was measured with the kit obtained from the NEN Company. Protein was measured after the method of Lowry et al.

**Preparation of Tissues**

Intestinal mucosa was scraped off the intestine with glass slides and homogenized in four volumes (wt/vol) of homogenization buffer TED: (10 mM Tris-HCl; 1.5 mM EDTA; 1 mM dithiothreitol; 10 mM sodium molybdate; pH 7.4; 4°C) with a Polytron homogenizer (Jahnke Co., Stauffen, Breisgau, FRG). Parathyroid glands were harvested by microsurgery under ether anesthesia, put immediately into 4°C TED buffer and homogenized as described above.

**Analytical Techniques**

**Nuclear preparation.** The methods used for nuclear preparations were as previously described. In brief, pooled parathyroid glands of 30 rats were homogenized in four volumes (wt/vol) of potassium chloride (KC1)-free TED buffer with a high speed Polytron homogenizer (10 bursts/10 sec) at the temperature of melting ice. Homogenate was centrifuged at 5,000g for 10 minutes at 4°C to yield the nuclear pellet. A crude chromatin fraction was prepared by resuspending the pellet three times in the above buffer with 0.5% Triton X-100 (pH 7.4) followed by centrifugation at 10,000g for 10 minutes. The resulting crude chromatin pellet was then extracted with 0.4 M KCl-TED for 45 minutes with frequent mixing. Chromatin extract was centrifuged at 5,000g for 10 minutes and the resulting supernatant subsequently centrifuged at 100,000g for 1 hour at 4°C.

**Saturation analysis according to Scatchard.** Aliquots (0.1 ml) of nuclear extracts (3.0 mg protein/ml) were incubated with varying concentrations (0.1 to 10 nmol) of \([^{3}H]\)calcitriol for 3 hours in the absence (total binding) or presence (nonspecific binding) of 100-fold molar excess of unlabeled calcitriol for 16 hours at 4°C. Bound \([^{3}H]\)calcitriol was determined by using the hydroxyapatite assay.

**Sucrose density gradient analysis.** Linear 5% to 20% sucrose density gradient in 0.4 M KCl-TED buffer (4 ml) was made by using a self-designed gradient former. Nuclear samples (0.2 ml) were incubated with 1 nM \([^{3}H]\)calcitriol alone or after addition of 100-fold molar excess of cold calcitriol. They were then carefully layered on top of pre-equilibrated (2 hours, 4°C) gradients and centrifuged at 255,000g for 21 hours at 4°C (SW 60 rotor, Beckman Instruments Co., Fullerton, California). Seven-drop fractions were collected. The sedimentation rate in Svedberg units (S) for proteins was calculated by using \([^{14}C]\)ovalbumin (3.7 S) or gammaglobulin (7.3 S).

**Binding of calcitriol receptors to deoxyribonucleic acid cellulose.** Deoxyribonucleic acid (DNA)-cellulose polymerized calf thymus DNA (type I, Sigma), and Whatman CF-11 cellulose (Sigma), were used.
Each column (1.5 × 6 cm) was equilibrated in TED buffer for 16 hours at 4°C before use and run at a flow rate of 5 ml/hr/cm². Samples were loaded on the columns, washed with three volumes of TED and eluted in 10 volumes with a linear gradient from 0.1 M to 0.8 M KCl in TED buffer. Nuclear extracts (0.6 ml/1.8 mg protein) of cell fractions were incubated for 2 hours at 4°C with 1 nM [3H]calcitriol plus 100-fold molar excess of radioinert calcitriol before performing chromatographic analysis of the DNA cellulose.

Quantitation of occupied and unoccupied receptors. Crude chromatin pellet was suspended (10% wt/vol) in TED buffer containing 10 mM sodium molybdate and 500 KIU/ml of trasylol. Aliquots (200 μl) of the chromatin suspension were pipetted into polypropylene tubes containing 3 nM [3H]calcitriol with or without 200-fold excess of radiionert calcitriol. The tube was incubated without addition of TPCK at 4°C for 4 hours (unoccupied receptor) or with addition of TPCK, dissolved in ethanol, to a final concentration of 100 μM. After incubation at 4°C for 30 minutes, 200 μl aliquots were pipetted into tubes containing the steroid as above, followed by incubation at 37°C for 30 minutes to determine occupied receptors. Measurements were carried out with the hydroxyapatite technique as described by Hunziker et al. and Walters et al.

Statistics. All data have been expressed as mean±SD, and Wilcoxon’s test for unpaired data was used. For the stereological evaluation, analysis of variance was used.

Results

Baseline Plasma and Urine Biochemistry

Baseline data on calcium metabolism in SHR and WKY rats of 5, 11, and 24 weeks of age are shown in Table 1. At 11 weeks of age, plasma ionized calcium and plasma phosphate were significantly reduced in the SHR compared with the WKY rats. At 5 and at 11 weeks of age, serum calcitriol levels were significantly lower in the SHR despite no difference in calcidiol or serum creatinine concentrations. In addition, stimulated parathyroid function at 11 weeks of age was suggested by high urinary cAMP in the SHR (3.6±0.19 nmol cAMP/mmol creatinine) versus the WKY rat (1.4±0.45 nmol cAMP/mmol creatinine). After 4 days of treatment with 2x10 ng/day calcitriol i.p., urinary cAMP returned toward the normal range (3.6±0.9 to 1.7±0.2 nmol cAMP/mmol creatinine) in the 11-week-old SHR, whereas it remained unchanged in the WKY rat (1.4±0.45 vs. 1.5±0.5 nmol cAMP/mmol creatinine). In the SHR, serum phosphate levels were raised by calcitriol (in the SHR 1.5±0.26 mmol/l after vehicle vs. 2.0±0.4 after calcitriol and in the WKY rat 2.4±0.3 after vehicle vs. 1.95±0.3 after calcitriol).

Study of Parathyroid Glands

Table 2 demonstrates increased weight of parathyroid glands in the SHR at 11 weeks of age. Morphometric studies indicate unchanged volume density of epithelial cells or interstitial tissue, and unchanged mean epithelial cell volume per nucleus. The latter finding suggests the presence of hyperplasia and not hypertrophy. The calcitriol receptor is a nuclear receptor, yet the nuclear volume (density as well as mean nuclear volume) remained unchanged. Qualitative ultrastructural investigation of SHR and WKY rats (Figure 1) revealed normal appearance of glandular epithelium without regressive changes, which indicated that increased weight of the gland in the SHR is caused by an increase of the mass of functionally intact cells. Quantitative alterations of cell organelles are not excluded, but an ultrastructural morphometric analysis was not performed.

Receptor Studies

All measurements shown in Figure 2 were carried out in animals of 11 weeks of age. As indicated in Figure 2A, [3H]calcitriol was bound in the nuclear fraction of parathyroid gland by a macromolecule sedimenting at 3.5 S. Binding was specific, since it...
was almost completely obliterated by a 100-fold molar excess of radioinert calcitriol. The area under the curve suggests greater binding capacity in SHR.

As shown in Figure 2B, after preincubation with \[^{3}H\]calcitriol, binding of the calcitriol holoreceptor complex to DNA, as assessed by DNA cellulose affinity chromatography, revealed unchanged DNA affinity for SHR and WKY.

Figure 3 shows saturation analysis in a homogenate of pooled parathyroid glands. Because of limited availability of tissue, Scatchard plots could not be obtained for individual rats. Figure 3 demonstrates, however, that the plateau of binding occurred at 3–5 nmol/l. Therefore, \(N_{\text{max}}\) as measured by incubation at 3 nmol/l, was examined in parathyroid tissue of 30 SHR and 30 WKY rats. Higher \(N_{\text{max}}\) was found in the 11-week-old SHR than in the corresponding WKY controls (172±4.9 vs. 123±6.6 fmol/mg protein; \(p<0.01\)). In 5-week-old rats, a similar difference was noted (157±1.3 vs. 130±4.6 fmol/mg protein; \(p<0.01\)). Scatchard plots in intestinal mucosa demonstrated qualitatively similar deviations as in parathyroid tissue (see data below).

To quantitate receptor saturation by endogenous ligand and the proportion of occupied and unoccupied receptor, TPCK displacement studies were performed.\(^{33}\) In the nuclear fraction of intestinal mucosa of SHR, \(N_{\text{max}}\) at 3 nmol was 250±9 fmol/mg protein versus 173±4 fmol/mg protein in WKY controls (\(n=8\) rats per group). In both groups, the equilibration constant \(K_{D}\) was 1.5×10\(^{-10}\) M. The calculated proportion of total receptor occupied by endogenous ligand was 18% in the SHR versus 15% in the WKY rats.

To examine regulation of calcitriol receptors in response to in vivo administration of calcitriol (2×10 ng/day i.p. for 4 days), \(N_{\text{max}}\) at 3 nmol/l was examined in the chromatin fraction of intestinal mucosa. In the SHR, pretreatment of calcitriol increased \(N_{\text{max}}\) from 250±9 to 274±9.5 fmol/mg protein (eight rats per group; \(p<0.01\)). In the WKY, \(N_{\text{max}}\) increased from 173±4 to 182±5.5 fmol/mg protein (\(p<0.01\)). This observation suggests intact regulation in the SHR.

### Discussion

The present study was designed to investigate aspects of the calcium endocrine system in the male Okamoto-Aoki SHR. In the SHR, we observed reduced circulating calcitriol concentrations and free calcitriol ratio in association with an increased number of calcitriol receptors, both in the intestine and in the parathyroid gland. We also observed hyperplasia of the parathyroid gland with the functional correlates of increased numbers of osteoclasts present in SHR bone and increased urinary cAMP.

The finding of reduced circulating calcitriol in the 5- and 11-week-old male SHR confirms our previous observations\(^{6,7}\) and those of others.\(^{10}\) Reduced active duodenal calcium absorption by the Ussing chamber method,\(^{4}\) reduced calcium absorption by in situ perfusion methods,\(^{4,10}\) and by balance study methodology\(^{5}\) indicate its probable functional significance. Some controversy remains concerning the precise nature of the abnormality of vitamin D metabolism in the SHR in that calcitriol levels have been observed unchanged in the 11-week-old SHR\(^{4}\) and elevated in the 5-week-old SHR.\(^{8}\) Regulation of the calcitriol receptor has not been investigated in great detail. It is of note, however, that administra-
Figure 1. Panel A, photomicrograph showing ultrastructure of parathyroid gland in a Wistar-Kyoto (WKY) control rat. Note regular appearance of nuclei, mitochondria, and endoplasmic reticulum of epithelial cells (EM magnification 12,200:1). Panel B, photomicrograph showing ultrastructure of parathyroid gland in a spontaneously hypertensive rat. No qualitative ultrastructural differences compared with WKY rat. (EM magnification 12,200:1).

tion of calcitriol upregulates the calcitriol receptor. Consequently, the mechanisms causing calcitriol receptors to be high despite low circulating calcitriol concentrations are not immediately apparent. At any rate, artifacts resulting from altered receptor occupancy have been excluded by our measurements of occupied and unoccupied receptors with the TPCK technique.

It is also of importance that there was no difference in the physico-chemical characteristics of the
FIGURE 2. Panel A, line graphs showing sucrose density gradient sedimentation profile of the calcitriol receptor in the nuclear fraction of parathyroid glands. Nuclear fraction (0.2 ml) in 0.4 M KCl-TED buffer (10 mM Tris-HCl; 1.5 mM EDTA; 1 mM dithiothreitol; 10 mM sodium molybdate; pH 7.4; 4°C) was incubated for 2 hours at 4°C with calcitriol as indicated and sedimented in linear 5−20% (wt/vol) sucrose density gradient by ultracentrifugation (255,000g for 21 hours at 4°C). Incubation occurred with 1.0 nM tritiated 1,25-dihydroxyvitamin D$_3$ ([3H]calcitriol) in the absence (●) or presence (○) of a 100-fold molar excess of [3H]calcitriol. Arrow, [14C]ov albumin at 3.7 Svedberg units (S). Panel B, line graphs showing elution of specific calcitriol receptors from deoxyribonucleic acid (DNA) cellulose (affinity chromatography). Nuclear fraction of parathyroid gland. The nuclear fraction (3 mg protein/ml) was preincubated with 1 nM [3H]calcitriol alone (●) or with 100-fold molar excess of unlabeled calcitriol (○) and placed on 1.5x6 cm column of DNA cellulose. After extensive washing of unabsorbed material, the column was eluted in 10 volumes with a linear gradient between 0.1 to 0.6 M KCl (—). Aliquots (0.25 ml) of column fractions were counted for [3H]calcitriol to determine the distribution of the calcitriol holoreceptor complex. A 100-fold molar excess of calcitriol completely obliterated the radioactive peak at 0.27 M KCl. WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats.
Receptors between the SHR and WKY either in terms of sedimentation rate constant (as measured by sucrose density gradient analysis) or of DNA affinity (as measured by DNA affinity chromatography). It is not clear whether increased receptor numbers in the SHR are due to enhanced synthesis of receptor protein, decreased degradation, or both.

The increase in intestinal calcitriol receptors did not compensate for reduced circulating calcitriol in terms of calcium absorption, a situation parallel to that of the aluminum-intoxicated rat. Because of as yet unresolved technical difficulties, the presently available means of determining circulating PTH are of questionable validity for the rat in vivo. Therefore, to address the issue of parathyroid status of the SHR, we elected to examine the parathyroid gland itself and then to determine the functional effects of circulating PTH on a classical target organ, bone. To our knowledge, parathyroid gland weights in SHR and WKY have not previously been compared. The increased weight of each gland in the SHR resulted from hyperplasia, as confirmed by morphometry, which indicated unchanged volume density of epithelial cells and interstitium in the SHR. In addition, the nuclear volume per epithelial cell did not differ between the strains, a fact of importance given the predominant nuclear location of calcitriol receptors in parathyroid tissue. Reports on parathyroid status of the SHR have suggested that circulating PTH is normal or increased. The question is further complicated by evidence of resistance to some of the actions of PTH in the SHR. It is thus noteworthy that histological evidence of hyperparathyroidism was observed in SHR bone in the present study. Furthermore, increased osteoclast number, the most abnormal parameter we observed, has been established to reflect increased PTH activity.

The mechanism underlying parathyroid hyperplasia in the SHR has not been established in the present study. The reduced serum levels of ionized calcium and calcitriol may both play a role. Recent evidence has suggested that calcitriol may exert an effect inhibiting PTH synthesis and secretion and that this effect may be independent of that of ambient calcium. Our finding of parathyroid hyperplasia despite increased numbers of calcitriol receptors in the parathyroid glands of the SHR, suggest that increased calcitriol receptors alone cannot compensate for reduced calcitriol levels. In this context it is noteworthy that the higher levels of urinary cAMP observed in the SHR and consistent with hyperparathyroidism were reduced to normal after calcitriol administration in high doses.

The relation between the observed abnormalities of the calcium endocrine system and raised arterial blood pressure in the SHR deserves comment. The presence of specific receptors for calcitriol in preparations derived from vascular smooth muscle suggests that calcitriol may play a physiological role, although the nature of that role is as yet entirely a matter for speculation. However, vitamin D-deficient rats have recently been reported to develop significantly elevated blood pressure and to exhibit altered pressure responsiveness when compared with vitamin D-replete controls. As far as hyperparathyroidism in the SHR is concerned, the situation is no less complex. The association of hyperparathyroidism and hypertension has been recognized for many years, although the underlying mechanism remains obscure. While a hypotensive effect of acute intravenous administration of PTH has been demonstrated in SHR, the mechanism by which this effect occurs is not fully understood. Additional studies are needed to determine the role of calcitriol in the hypertrophic response of the SHR.

FIGURE 3. Plots showing saturation analysis (Scatchard) with [1H]calcitriol in parathyroid glands in Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR). Insert (top) gives Scatchard plot for specific binding. At 5 nM, nonspecific binding was 15% of total binding. Nuclear fraction in 0.4 M KCI-TED buffer (10 mM Tris-HCl; 1.5 mM EDTA; 1 mM dithiothreitol; 10 mM sodium molybdate; pH 7.4; 4°C) was incubated for 16 hours at 4°C with increasing concentrations (0.1-5 nM) of [1H]calcitriol in the presence and absence of a 100-fold molar excess of radioinert calcitriol. The equilibrium constant Kd calculated from the regression curve was unchanged in the SHR (1.7 vs. 2.4x10^-10 M in the WKY). Extrapolation of the curve to the abscissa yielded Nmax of 119 fmol/mg protein in SHR versus 62.3 fmol/mg protein in WKY. Each value represents the mean of four replicate measurements of a tissue pool of 30 glands.
both rats and dogs, chronic ablation of the parathyroid glands has been shown to attenuate experimental mineralocorticoid-induced hypertension. In a recently reported study with SHR, we demonstrated that parathyroidectomy was associated with a significant attenuation in the rise in blood pressure in SHR, but had no effect on that of the WKY rat.

In summary, we have provided evidence of hyperparathyroidism in the SHR, having observed parathyroid hyperplasia and histological evidence of its effect on bone. In the same model, reduced circulating calcitriol levels and increased tissue calcitriol receptors were demonstrated. Both calcitriol and PTH have been implicated in the regulation of calcium entry into the cell, a process involved in determining the amount of cytosolic calcium available to the contractile apparatus of vascular smooth muscle.

Since submission of this paper, Patel et al (Kidney International 1988;34:224–228) reported on age-related mineralocorticoid-induced hypertension. In a recent study with SHR, we demonstrated that parathyroid hyperplasia and histological evidence of reduced circulating calcitriol and PTH have been implicated in the regulation of calcium entry into the cell, a process involved in determining the amount of cytosolic calcium available to the contractile apparatus of vascular smooth muscle.

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References


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