Pituitary and Testicular Function in Spontaneously Hypertensive Rats

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Levels of plasma gonadotropins, prolactin (PRL), and testosterone, and the testicular concentration, total content, and affinity of hCG binding sites were measured in male spontaneously hypertensive rats (SHR) and in genetically matched normotensive (WKY) rats. Hypertensive rats had higher plasma PRL and FSH levels and lower plasma testosterone levels. The affinity of testicular hCG binding sites was similar in SHR and WKY rats, but SHR had considerably lower concentration and total content of hCG-binding sites than did WKY animals. The comparison of these findings with those obtained previously with pituitary-grafted rats and with tumor-bearing rats indicates that the endocrine effects of hyperprolactinemia may vary depending on the rate and magnitude of the increase in peripheral PRL levels. Species differences in the response to hyperprolactinemia and differences between SHR and other rat strains suggest that hormonal responses to PRL elevation depend also on the genetic characteristics of the animal.

Key words: spontaneously hypertensive rats, hyperprolactinemia, hypertension, hCG (LH) binding, gonadotropins, prolactin.

Prolactin (PRL) plays an important role in the regulation of reproductive functions in mammals. In the male, both a decrease and an increase in the levels of PRL can produce important changes in hormonal and reproductive functions (Bartke et al, 1977a; Bartke et al, 1977b; Bartke, 1978; Buvat et al, 1978; Zipf et al, 1978; Sharpe and McNeilly, 1979; Amador, 1980; Barenton and Pelletier, 1980; Amador, 1981; Klemcke et al, 1981; Klemcke and Bartke, 1981; Bartke et al, 1982). Most models for the study of hyperprolactinemia require experimental manipulation of the animal (Fang et al, 1974; Bartke et al, 1977b; Klemcke and Bartke, 1981; Bartke et al, 1982) and produce an abrupt elevation of peripheral PRL levels to a supraphysiologic range. In contrast, several investigators have reported that male spontaneously hypertensive rats (SHR) have a genetically determined progressive increase in plasma PRL levels during their life span (Iams et al, 1979; Hodson et al, 1981) and, therefore, might provide an interesting and different model for the study of hyperprolactinemia. The present study was undertaken to characterize the reproductive endocrine status of male SHR, and thus provide the basic data for future studies using this naturally occurring model for hyperprolactinemia. Genetically matched normotensive WKY rats were used as controls.

Materials and Methods

Animals

Spontaneously hypertensive (SHR) and normotensive WKY rats (17 weeks old and age matched) were purchased from Taconic Farms. All animals were maintained in a room with controlled temperature (22 ± 2 C) and illumination (14 hours light:10 hours darkness), with free access to commercial pelleted food (Wayne Breeder Blox) and tap water. The animals were sacrificed by decapitation immediately after removal from the cage and without the use of anesthetics. Trunk blood was saved for hormone measurements and the testes were removed for binding studies.

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Measurement of hCG Binding

Testicular ^{125}I-hCG binding was measured by radioreceptor assay using procedures described and validated for use in the mouse, hamster, and rat by Klemcke (Klemcke and Bartke, 1981; Bartke et al, 1981a; Klemcke et al, 1981). Testicular tissue was homogenized in phosphate-buffered saline (PBS) and the homogenate was centrifuged at 100 × g for 5 minutes at 4 C. The supernatant was then removed and recentrifuged at 1786 × g for 20 minutes at 4 C. After being washed with PBS, the pellet was resuspended in PBS, and served as the source of hCG binding sites. Binding of hCG (CR-121), previously iodinated via a chloramine-T procedure and purified on a concanavalin A-Sepharose column, was measured in triplicate by addition of 100 μl (20 mg tissue) aliquots of these testicular preparations to saturating quantities of ^{125}I-hCG (2.5 ng/tube). Nonspecific binding was measured in the presence of 100 IU hCG (Pregnyl, Organon). Incubations were conducted at 23 C for 20 hours. The specific activity of iodinated hCG was determined by self-displacement using unlabelled hCG (CR-121). The content of protein in testicular membrane preparations used for measurement of hCG binding was determined by a modification of Lowry's method, described by Markwell et al (1978), using bovine serum albumin as the standard.

Hormone Measurements

Testosterone levels were measured by radioimmunoassay (RIA) after extraction of plasma samples with hexane-ethyl acetate (9:1), using ^{3}H-testosterone as an internal trace to monitor losses during the procedure. The antiserum used (Endocrine Sciences, Tarzana, California) showed 44%, 41%, and 18% crossreactivity with dihydrotestosterone, Δ_1-testosterone, and Δ_1-dihydrotestosterone, respectively. Saturated ammonium sulfate was used as a gamma-globulin precipitating agent. Recovery of internal trace was 72 to 96%. The intra-assay coefficient of variation was 4.7%. The average sensitivity of this RIA was 0.14 ng/ml plasma.

Levels of FSH were measured by RIA using antiserum NIH-6 and RP-1 rat FSH standard. The intra-assay coefficient of variation was 1.4% and the average sensitivity was 29.1 ng/ml plasma.

Prolactin levels were measured by RIA using antiserum NIH-6 and RP-1 rat PRL standard. The intra-assay coefficient of variation was 10.1% and the average sensitivity was 0.32 ng/ml plasma.

Levels of LH were measured by RIA using NIH-RP-1 standard and an antiserum (Anti-ovine LH, #15, Niswender) which crossreacts with monkey, rat, mouse and hamster LH. The intra-assay coefficient of variation was 2.1% and the sensitivity of this RIA was 5.0 ng/ml plasma.

Statistics

Data obtained from SHR were compared to those obtained from WKY rats, using Student's *t*-test as described by Sokal and Rohlf (1969). The slopes of the Scatchard plots were compared statistically by the sum of squares simultaneous test procedure (Sokal and Rohlf, 1969).

Results

Testis Weights

Testicular weights in SHR were significantly greater than in WKY rats (2.82 ± 0.05 versus 2.24 ± 0.03 g; $P < 0.001$). Moreover, the ranges of testicular weights in these two types of animals did not overlap (SHR: 2.53 to 2.97 g; WKY: 2.13 to 2.36 g).

Plasma Hormone Levels

Plasma PRL levels were significantly elevated in SHR as compared with those measured in WKY rats (Table 1). Plasma FSH levels were also higher in SHR (Table 1), while no significant differences could be measured in plasma LH levels between the two groups of animals (Table 1). Plasma testosterone levels were slightly but significantly lower in SHR when compared with WKY rats (Table 1).

TABLE 1. Plasma Hormone Levels and Testicular hCG Binding in Spontaneously Hypertensive Rats (SHR) and Normotensive Control Animals

	SHR	WKY	*P*
PRL (ng/ml)	21.7 ± 3.33 (10)	12.04 ± 2.08 (8)	< 0.05
FSH (ng/ml)	504.2 ± 43.2 (10)	351.7 ± 18.6 (9)	< 0.01
LH (ng/ml)	22.36 ± 1.53 (10)	28.76 ± 3.31 (9)	NS
Testosterone (ng/ml)	4.45 ± 0.40 (10)	6.93 ± 1.16 (9)	< 0.05
hCG binding sites concentration*	30.68 ± 1.07 (10)	60.90 ± 3.00 (9)	< 0.001
Total hCG binding sites content†	8.69 ± 0.43 (10)	15.88 ± 3.81 (9)	< 0.001
Kd	1.46 × 10^{-11}M	1.26 × 10^{-11}M	NS

Means ± SE.
* Values expressed in fmoles/mg protein ± SEM.
† Values expressed in pmoles/testes ± SEM.

Testicular hCG Binding

Both concentration and total content of testicular hCG binding sites were lower in SHR than in WKY rats (Table 1). Scatchard plots were made from three different pools of testicular membrane preparation from each strain of rat. Statistical analysis of Scatchard plots revealed no differences in affinity of hCG binding sites between the two types of rats (Table 1).

Discussion

It is not known whether elevation of peripheral PRL levels in SHR represents a primary effect of their genetic constitution or is secondary to changes in the function of the vascular system. Factors which affect cardiovascular handling of blood were reported to alter serum PRL levels (Bruschi et al, 1981; Chap and Bedrak, 1981; Coruzzi et al, 1981); and thus elevated blood pressure, through its effects on cardiac function, could produce an increase in peripheral PRL levels. Regardless of its etiology, the spontaneous development of the hyperprolactinemic state in SHR offers an opportunity to examine the hormonal effects of progressive, mild elevation of serum PRL in animals not exposed to phrarmacologic or surgical manipulation.

Comparison of the results obtained in the present study with those derived from other models of hyperprolactinemia (Table 2) suggests that the effects of PRL on pituitary and testicular function in the male rat may depend on the rate and/or magnitude of the increase in plasma PRL levels. In rats in which hyperprolactinemia has been induced by transplantation of pituitaries under the renal capsules or by implantation of PRL-secreting tumors, peripheral gonadotropin levels are usually reduced (Bartke et al, 1977b; Sharpe and McNeilly, 1979; Hodson et al, 1980); while in SHR, FSH levels are elevated and LH levels are unchanged. These differences probably could be explained by a difference in the magnitude of hyperprolactinemia. The mild increase in PRL levels in SHR is accompanied by elevated plasma FSH levels, while the more severe increase in PRL levels in grafted or tumor-bearing rats suppresses FSH release. Thus, it would seem likely that PRL has a bimodal dose-related effect on FSH release. In hyperprolactinemic tumor-bearing rats studied by Fang et al (1974), serum FSH levels were unchanged and serum LH levels were significantly increased. In these animals, the testes were atrophic and peripheral testosterone levels were markedly reduced, suggesting that the changes in gonadotropin levels may have been due to the consequences of reduced testicular feedback superimposed upon the effects of hyperprolactinemia. The increase in the ratio of FSH to LH in SHR is unlikely to be androgen-dependent, as proposed by Denef et al (1980), since SHR have decreased plasma testosterone levels. Also, Bartke et al (1981b) showed a similar increase in castrated hyperprolactinemic hamsters. These results by Bartke and his co-workers also argue against the possibility of suppression of inhibin production by the testis as an explanation for increased FSH release. Differences in the magnitude of increase in plasma PRL levels could also explain the decrease in plasma LH levels observed in grafted and tumor-bearing rats, but not in SHR, as well as offer a reason for impaired fertility only in tumor-bearing animals.

Sharpe and McNeilly (1979) demonstrated that rats with pituitary grafts have elevated concentrations of testicular LH receptors. Similar results were found in pituitary grafted golden hamsters (Bartke et al, 1980; A. Amador, H. G. Klemcke, and A. Bartke, unpublished observations). However, SHR have dramatically decreased concentrations of testicular hCG-binding sites (LH receptors). This may be due to the rate of increase in PRL levels, in that hypertensive rats present a progressive increase while graft-bearing rats and hamsters have an abrupt increase. Another possibility is that these differences may be genetically determined, with the SHR phenotype including a decrease in hCG binding. The latter possibility is supported by findings in the mouse. In this species, several mutations alter the concentration

TABLE 2. Comparison Between Different Models for Hyperprolactinemia in the Rat*

	SHR‡	Grafts§	MtT-W15 Tumors‖	GH,B, Tumors¶
Plasma FSH	Increased	Decreased	—	Unchanged
Plasma LH	Unchanged	Decreased	Decreased	Increased
Plasma GH	—	†	—	Increased
Plasma T	Decreased	Unchanged	Decreased	Decreased
Testicular weight	Increased	Unchanged	Decreased	Decreased
LH receptors	Decreased	Increased	—	—

* All effects listed in this table are statistically significant.
† Pituitary grafts do not produce GH.
‡ Spontaneously hypertensive rats; present study.
§ Grafts of four pituitaries; data from Bartke et al (1977b) and Sharpe and McNeilly (1979).
‖ Transplants of PRL- and GH-secreting tumors; data from Hodson et al (1980).
¶ Transplants of PRL- and GH-secreting tumors; data from Fang et al (1974).
** T = testosterone.

of testicular hCG binding sites (Amador, 1981; Amador et al, 1981). Klemcke and Bartke (1981) found decreased testicular hCG binding in mice given pituitary transplants, but this may have been due to chronically elevated plasma LH levels in hyperprolactinemic mice.

The observation of unchanged plasma LH levels in SHR, combined with decreased hCG binding and decreased plasma T levels, indicate that regulation of hCG binding may be independent of prevailing LH levels. These observations suggest also that the reduction in plasma T levels in SHR may be causally related to a decrease in testicular hCG binding.

In summary, the endocrine effects of hyperprolactinemia may vary depending on the mode and magnitude of the PRL increase and on the genetic characteristics of the animal model.

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