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EFFECT OF ZIDOVUDINE (AZT) ON REPRODUCTIVE AND HEMATOPOIETIC SYSTEMS IN THE MALE RAT

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Abstract—The effects of Zidovudine (AZT) on parameters of the reproductive and hematopoietic systems of male rats were evaluated and compared to those of the controls. Young male Wistar rats were divided into three groups. The control rats (Group 1) received no drug. AZT was administered via drinking water to rats in Groups 2 and 3 (0.1 mg/mL Group 2, and 1.0 mg/mL Group 3) for 4 weeks. Daily water intake and weekly body weight were monitored. Serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone (T), prolactin (PRL) and intratesticular T levels were determined by specific radioimmunoassays. Serum AZT was measured by HPLC. Bone-marrow toxicity was monitored by colony-forming units of erythroid (CFU-E) and granulocyte-macrophage (CFU-GM) assays. The body weight of all rats increased 2-fold in four weeks, and no significant differences were observed between control and treated groups. Ventral prostate (VP) weight decreased significantly ($P < 0.05$) by 32% in Group 2 and 27% in Group 3 rats, compared to the control group. Seminal vesicle (SV) weight decreased by 20% in Group 2 (NS) and 30% in Group 3 ($P < 0.05$) rats. No significant differences were observed in testes weight or in the intratesticular sperm count between control and AZT-treated groups. Serum T levels (ng/mL) decreased significantly in Group 2 rats (2.8 ± 0.4 in control to 1.7 ± 0.2, $P < 0.05$) but recovered (2.6 ± 0.5) in Group 3 when compared to Group 1 (control). Serum LH and PRL levels showed a significant increase ($P < 0.05$) in Group 3 compared to control or Group 2 rats. Serum FSH and intratesticular T levels showed no significant change. A significant ($P < 0.05$) dose-dependent decrease in CFU-E and in CFU-GM was observed in AZT-treated rats compared to controls. These results demonstrate that the effects of AZT on the reproductive system in the male rat are more subtle and complex, compared to its significant effects on bone-marrow. This suggests that important interactions may exist between the endocrine and hematopoietic systems.

Zidovudine (AZT) is a thymidine analogue that suppresses human immunodeficiency virus (HIV) replication and is currently the only FDA-approved drug available for the treatment of patients with acquired immunodeficiency syndrome (AIDS) [1]. Administration of AZT, however, has led to significant dose-related effects in patients with AIDS, anemia and neutropenia being the most common toxicities observed [2–5]. Effects of AZT on the reproductive system have not been reported, although there is a high percentage (about 80%) of HIV-positive young men and women on AZT therapy who are both sexually active and in the reproductive age group. The Burroughs Wellcome Co. (the drug's manufacturer) has recently shown the development of vaginal tumors in female mice and rats given high doses of AZT [6].

The involvement of the brain in the late stages of AIDS is now a well-known factor of the disease. Alterations in the hypothalamic-pituitary-gonadal (HPG) axis in HIV-infected men have been reported, although there have been conflicting findings [7, 8]. The central nervous system, through complex networks of feedback control mechanisms, regulates endocrine, immune, and hematopoietic systems that are important, during normal development as well as during disease. Since AZT affects both the hematopoietic and the immune systems, its interactions on the reproductive axis may help in understanding the integrated communication between these components.

Using the rat as an animal model, we have evaluated the effects of AZT on the male reproductive axis in comparison to the effects on the hematopoietic system.

METHODS

Animals. Normal male Wistar rats (40 days old, 170–190 g) obtained from Charles River (Wilmington, MA) were used in these studies. The animals were given water and rat chow *ad lib.* and kept on a constant sleep–wake schedule throughout the study period. The daily water intake of all rats was monitored during the initial acclimatization period (1 week) and the 4 weeks of the study period.

Drug administration. AZT was synthesized in our laboratory according to the published procedure [9]. Rats were randomly divided into three groups (six rats in each group). Group 1 (control) received no drug, Group 2 received 0.1 mg/mL AZT, and Group 3 received 1 mg/mL AZT in drinking water for 4 weeks. This duration was selected in order to cover the pubertal period of the animals prior to sacrifice. These concentrations of AZT represented administered doses equivalent to approximately 15 mg/kg per day in Group 2 and 150 mg/kg per day

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in Group 3 based on an average water intake of 30 mL/rat per day.

At the end of 4 weeks, the animal was placed for 1 min in a large beaker containing an ether-impregnated cotton ball and a blood sample was drawn by cardiac puncture and centrifuged. Testes, ventral prostate (VP) and seminal vesicles (SV) were excised and weighed. Serum samples were kept frozen at −20° until assayed for hormone and AZT. The testes were homogenized gently in 5 vol. (1:5, w/v) of 0.05 M Tris–HCl containing 0.25 M sucrose and 5 mM dithiothreitol, pH 7.4 [10]. An aliquot (8 μL) was used for counting intratesticular sperm concentration using a Makler chamber (Zygotec Systems, Springfield, MA), and the remaining homogenate was kept frozen for intratesticular T measurement. Bone-marrow cells were aseptically aspirated after removal of the femur and processed for colony-forming units of erythroid (CFU-E) and granulocyte-macrophage (CFU-GM) assays.

Measurement of hormones. Rat serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone (T) and prolactin (PRL) levels were measured by specific radioimmunoassays (RIA) at the Hormone Assay Core Laboratory, UCLA Population Research Center, Torrance, CA. The reference preparations for RIAs of rat LH, FSH and PRL were rLH-RP-2, rFSH-RP-2 and rPRL-RP-3, respectively, supplied by the National Hormone and Pituitary Program. Serum and intratesticular T levels were determined by RIA procedures after extractions with 10 vol. of hexane:ethylacetate (2:3, v/v). All samples were analyzed in duplicates at the same time in each assay and the intra-assay coefficient of variation was less than 6%.

Measurement of AZT. AZT was extracted from serum samples with 3 vol. of methanol. The methanolic extracts were filtered and the concentration of AZT was determined by HPLC (Beckman model 340) using a reverse phase econosphere C-18 column (150 × 4.6 mm, 5 μm) and 15% acetonitrile and 85% 25 mM ammonium phosphate buffer, pH 2.2, as the mobile phase. Retention time for AZT in this system at a flow rate of 1.5 mL/min was 8 min. The minimum detection limit of AZT under these conditions was 10 ng/mL.

Preparation of bone-marrow cells. Femurs were aseptically removed at the time the animals were killed, and marrow was flushed with buffer using a 25 × 5/8 gauge needle. A single cell suspension was made with a pipette after repeated aspirations. The cells were resuspended in RPMI-1640 medium at a final concentration of 1×10^6 cells/mL.

CFU-E assay. Methylcellulose solution (1.3%, w/v) was prepared in RPMI-1640 containing L-glutamine (2.0 mM), heat-inactivated fetal bovine serum (20%), 2-mercaptoethanol (0.1 mM), penicillin (100 units/mL), streptomycin (100 μg/mL) and erythropoietin (200 units/mL) [11]. Bone-marrow cells (1×10^6/mL) were diluted 1:10 in the methylcellulose mixture. One millilitre of cell suspension (1×10^5 cells) was plated in duplicate in 35 mm petri dishes and incubated in 5% CO_2 and 95% humidified air at 37° for 48 hr. The plates were stained with 3,3-diaminobenzidine, and colonies containing eight or more benzidine-positive cells were counted under an inverted microscope (Olympus Corp., Tokyo).

CFU-GM assay. Methylcellulose solution (3%, w/v) was prepared in RPMI-1640 containing L-glutamine (2.0 mM), fetal bovine serum (15%), 2-mercaptoethanol (0.1 mM), penicillin (100 units/mL), streptomycin (100 μg/mL) and L-cell conditioned medium (15%). Bone-marrow cells (1×10^6/mL) were diluted 1:10 in the methylcellulose mixture. One milliliter of cell suspension (1×10^5 cells) was plated in duplicate in 35-mm petri dishes and incubated in 5% CO_2 and 95% humidified air at 37° for 7 days. Colonies that contained 50 or more cells were counted under an inverted microscope as above.

Data analysis. Statistical analyses between control and treated groups were done by one-way analysis of variance (ANOVA), and results were compared at the $P < 0.05$ level of significance.

RESULTS

There were no significant differences in body weight (mean ± SEM) between control and AZT-treated groups at weeks 1, 2, 3 and 4. An approximately 2-fold increase in body weight was observed in the control and AZT-treated groups after 4 weeks of treatment (Table 1). Ventral prostate weight (VPW) decreased significantly ($P < 0.05$) by 32% in rats treated with the lower dose of AZT (Group 2). However, this decrease in VPW was not dose dependent, since the decrease in rats treated with the high dose of AZT (Group 3) was not significantly different from the decrease in animals treated with the lower dose of AZT. In contrast, the decrease in seminal vesicle weight (SVW) was dose dependent; it was found to be 20% in Group 2 (NS) and 30% in Group 3 which was significantly ($P < 0.05$) different from the control. No significant differences were observed in testicular weight or in intratesticular sperm count between control and AZT-treated groups (Table 1). Serum T levels (ng/mL) decreased by 40% from 2.8 ± 0.4 in control to 1.7 ± 0.2 ($P < 0.05$) in rats treated with low dose AZT but recovered to 2.6 ± 0.5 in rats treated with high dose AZT. Serum LH and prolactin (PRL) levels (ng/mL) increased in a dose-dependent manner that was significant ($P < 0.05$) in rats treated with the high dose of AZT (Table 1). In contrast, serum FSH levels (ng/mL) showed no significant change between the control and AZT-treated groups, whereas intratesticular T (ng/g testis) levels showed a non-significant decrease in both low and high dose AZT-treated rats after 4 weeks. Serum AZT levels in both Groups 2 and 3 were found to be in a non-detectable range at the time of measurement, indicating lack of water intake during the preceding few hours before the animals were killed as plasma $T_{1/2}$ of AZT in rats was approximately 50 min (unpublished observations). A dose-related decrease in the ability of the bone-marrow progenitor cells to form colonies (CFU-E and CFU-GM) was observed in AZT-treated rats (Table 1) and showed a 50 and 60% decrease, respectively, after 4 weeks of high dose AZT administration (Fig. 1).

Table 1. Effect of AZT treatment on parameters of reproductive and hematopoietic systems in the male rat

Parameter	Control	0.1 mg/mL AZT	1 mg/mL AZT
Body weight* (g)	398 ± 11	389 ± 5	402 ± 3
Reproductive system:			
Testis weight (g) Left	1.6 ± 0.06	1.5 ± 0.02	1.5 ± 0.04
Right	1.6 ± 0.03	1.5 ± 0.02	1.5 ± 0.06
VPW (mg)	362 ± 50	250 ± 17†	263 ± 31†
SVW (mg)	417 ± 66	335 ± 26	293 ± 11†
LH (ng/mL)	0.98 ± 0.1	1.1 ± 0.1	1.7 ± 0.2†
FSH (ng/mL)	10.2 ± 0.8	9.8 ± 0.7	11.0 ± 0.4
T (ng/mL)	2.8 ± 0.4	1.7 ± 0.2†	2.6 ± 0.5
PRL (ng/mL)	8.6 ± 0.3	9.6 ± 0.4	10.6 ± 0.5†
Ti (ng/g testis)	361 ± 97	257 ± 82	311 ± 66
Sperm count ($\times 10^6$/g testis)	64 ± 3	58 ± 5	56 ± 6
Hematopoietic system:			
CFU-E (colonies/plate)	386 ± 13	317 ± 13†	193 ± 13†
CFU-GM (colonies/plate)	207 ± 12	151 ± 11†	82 ± 10†
Serum AZT (ng/mL)		ND‡	ND

AZT was administered via drinking water for 4 weeks. The results shown are means ± SEM (N = 6) for each group. Abbreviations: VPW, ventral prostate weight; SVW, seminal vesicle weight; LH, luteinizing hormone; FSH, follicle-stimulating hormone; T, testerone; PRL, prolactin; Ti, intratesticular T; CFU-E, colony-forming units—erythroid; and CFU-GM, colony-forming units—granulocyte macrophage.

* Average body weight at the beginning of the experiment was 190 ± 4 g (N = 18).

† $P < 0.05$, compared to control.

‡ ND = not detectable.

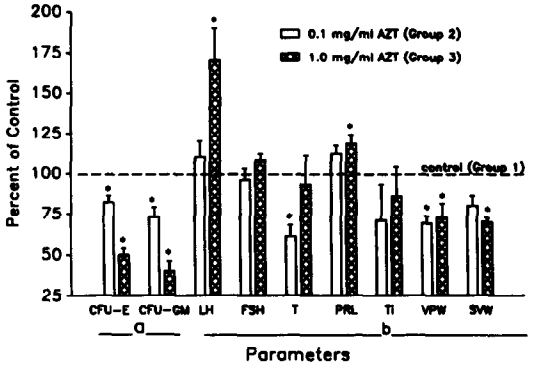

Fig. 1. Effects of AZT on the (a) hematopoietic and (b) reproductive axis in the male rat. AZT was administered via drinking water for 4 weeks. The data show percent change (mean ± SEM, N = 6) from control for (a) CFU-E and CFU-GM and (b) serum LH, FSH, testosterone (T), prolactin (PRL), intratesticular T (Ti), ventral prostate weight (VPW) and seminal vesicle weight (SVW). An asterisk (*) denotes $P < 0.05$, compared to control when analyzed by ANOVA. The absolute values are shown in Table 1.

DISCUSSION

The results of this study demonstrate that oral administration of AZT at low (15 mg/kg per day) and high (150 mg/kg per day) doses for 4 weeks caused subtle complex effects on the hypothalamic-pituitary-gonadal (HPG) axis compared to well-recognized dose-dependent toxic effects observed on the hematopoietic system of young male rats. These effects of AZT suggest that significant interactions may be involved among the hematopoietic, immune, and reproductive systems.

The biochemical mechanisms responsible for the hematopoietic toxicity of AZT may include perturbations of deoxyribonucleotide pool [12], and incorporation of AZT into DNA [13]. However, the exact mechanism of the hematopoietic toxicity of AZT has not been determined. Inhibition of globin gene expression has been shown recently to play a role in the cytotoxicity of AZT to the erythroid cells and this may affect host cell toxicity [14]. The importance of hematopoiesis in maintaining the immune system, which is the primary target for HIV, has been well established in cancer patients receiving cytotoxic drugs and radiation therapy. Thus, understanding of the drug-induced interactions between hematopoietic and immune systems may help not only in delineating the potential stimulatory or inhibitory effects of AIDS therapy but also in the development of better treatment regimens.

AZT has been shown to target rapidly proliferative erythroid hematopoietic cells by incorporating into DNA [13]. Spermatogenesis is also a rapidly proliferative phenomenon. However, we did not observe any reduction in sperm count during the 4 weeks of the study period, even in the high dose (150 mg/kg per day) group. However, the possibility of a significant antispermatogenic effect cannot be ruled out over a longer treatment period. AZT is activated to phosphorylated metabolites in human bone-marrow cells [15]. It is not clear if the drug or its metabolites can cross the blood–testis barrier. AZT, by interacting with the immune system, could possibly exert these effects on the male reproductive

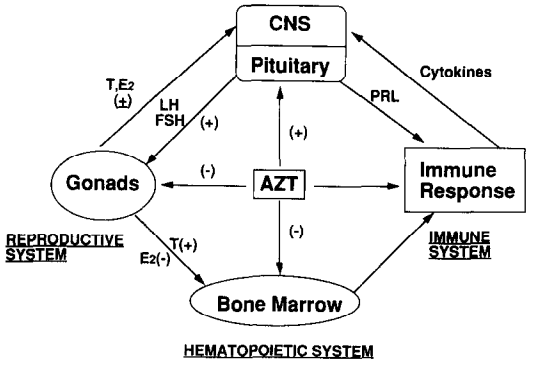

Fig. 2. Scheme representing interactions between hematopoietic, immune, and reproductive systems and the possible role of AZT. The effects of AZT as observed in the present study are depicted as (+) stimulatory, or (−) inhibitory. Key: LH, luteinizing hormone; FSH, follicle-stimulating hormone; E_2, estradiol; PRL, prolactin; and T, testosterone.

system in a complex manner. The immune system has been shown to be regulated by gonadal steroids, and the circulating levels of these steroids can be affected by immune-system function [16]. Such interactions appear to be mediated, at least in part, through the hypothalamic-pituitary-gonadal-thymic axis. However, involvement of the thymus in hypogonadism induced by AZT in our studies has not been directly tested. Further studies, including the effects of AZT on thymectomized rats, may help to clarify these complex interactions between the reproductive and immune systems.

Cytokines, specifically tumor necrosis-factor (TNF) and interleukin-1 (IL-1), have been shown recently to inhibit steroidogenesis in Leydig cells [17], in isolated rat adrenal glomerulosa cells [18], and in ovarian granulosa cells [19]. These cytokines are produced by macrophages, mediate many inflammatory and cellular responses, and have been shown to exert broad hormone-like effects on various cells [20]. An increased release of LH and other pituitary hormones in response to IL-1 has been shown *in vitro* [21]. An increased release of LH and decrease in serum T in response to AZT administration was observed in the present *in vivo* study in the young rats. One possible explanation of these *in vivo* AZT effects on the HPG axis may be via release of the cytokines from macrophages (Fig. 2). Measurement of these cytokines may determine their involvement and add to the growing evidence that a close relationship exists between the endocrine and the immune system.

Our results also suggest, for the first time, a role of the hematopoietic system in these possible immune–endocrine interactions and that AZT or other nucleoside analogues can be used as probes to further investigate such relationships. The increase in LH (hypergonadotropic) and PRL (hyperprolactinemia) by AZT in the present study appears to be dose-related and becomes significant in rats treated with high dose AZT. A significant decrease was observed in serum T in Group 2 rats, in addition to a decrease in ventral prostate weight in Groups 2 and 3 and in seminal vesicle weight in Group 3 (hypogonadal effect). No significant effect on intratesticular T or testicular weight was observed in any group. A recovery in serum T was noted in Group 3 rats. The hypergonadotropic effect in association with subtle hypogonadism as observed in our study can be explained as caused by feedback followed by compensatory mechanisms present in the HPG axis [22]. Assuming that the elevated serum immunoreactive LH levels reflected comparable elevations of bioactive LH, as is usually true in hypogonadism, the hormonal pattern implies primary testicular failure. However, the rise in serum T with increased LH after a larger dose of AZT explains this T secretory defect as being central and reversible. Serum FSH levels, testicular weight, and intratesticular sperm counts were not affected in the present study. It is possible that these changes in T levels may not be due to any direct central effects (on hypothalamus or pituitary) or peripheral effects (on gonads) (Fig. 2). Stress may also induce some of these hormonal changes [23]. We did not evaluate the hypothalamic-pituitary-adrenal axis to confirm this in the present study, although no significant body weight reduction was observed in any of the groups. The toxicity of AZT on the hematopoietic system, however, is well known [3, 11–15] and was also observed in the present study at both low and high doses. Fetal hepatotoxicity of AZT and other anti-HIV agents was only recently observed *in vitro* in murine fetal liver cells [11]. Acute renal toxicity because of AZT at these dose levels was not apparent in the present study, as the rats in all the groups had a significant weight gain (2-fold) during the 4-week period of treatment. It seems likely that the duration of the treatment period in the present study may not have been long enough to observe profound effects on the male reproductive tract. Thus, a chronic exposure to AZT in a larger population for longer durations may be needed to determine possible deleterious effects on the reproductive system.

It can be argued that the toxic effects of AZT on the reproductive tract may not be of basic concern to AIDS patients infected with HIV, since AZT therapy is the only hope of survival for them at present. However, considering the increase in the number of sexually active and reproductive age group patients, with the improved quality of life and life expectancy, any toxic effects of AZT when extrapolated to larger populations may have significant clinical implications for these sexually active young men and women undergoing long-term therapy for AIDS. In summary, these preliminary results demonstrate that the effects of AZT on the male reproductive axis under the present conditions are subtle and complex and suggest that important interactions may exist between the endocrine and hematopoietic systems, which warrant further investigation.

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REFERENCES

1. Yarchoan R, Weinhold KJ, Lyerly HK, Gelmann E, Blum RM, Shearer GM, Mitsuya H, Collins JM, Myers CE, Klecker RW, Marckham PD, Durack DT, Lehrman SN, Barry DW, Fischl MA, Gallow RC, Bolognesi DP and Broder S, Administration of 3'-azido-3'-deoxythymidine, an inhibitor of HTLV-III/LAV replication, to patients with AIDS or AIDS-related complex. *Lancet* **1**: 575–580, 1986.
2. Eichhoff TC, Acquired immunodeficiency syndrome (AIDS) and infection with the human immunodeficiency virus (HIV). *Ann Intern Med* **108**: 460–469, 1988.
3. Richman DD, Fischl MA, Grieco MH, Gottleib MS, Volberding PA, Laskin OL, Leedom JM, Groopman JE, Mildvan D, Hirsch MS, Jackson GG, Durack DT, Nusinoff-Lehrman S and the AZT Collaborative Working Group, The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex: A double-blind, placebo-controlled trial. *N Engl J Med* **317**: 192–197, 1987.
4. Mitsuya H and Border S, Strategies for antiviral therapy in AIDS. *Nature* **325**: 773–778, 1987.
5. De Vita VT, Border S, Fauci AS, Kovacs JA and Chabner BA, Developmental therapeutics and the acquired immunodeficiency syndrome. *Ann Intern Med* **106**: 568–581, 1987.
6. *Physicians' Desk Reference* (Publisher Barnhart ER), 45th Edn, pp. 788–794. Medical Economics Co., Oradell, NJ, 1991.
7. Dobs AS, Dempsey MA, Ladenson PW and Polk BF, Endocrine disorders in men infected with HIV. *Am J Med* **84**: 611–616, 1988.
8. Croxson TS, Chapman WE, Miller LK, Levit CD, Senie R and Zumoff B, Changes in the hypothalamic-pituitary-gonadal axis in human immunodeficiency virus-infected homosexual men. *J Clin Endocrinol Metab* **68**: 317–321, 1989.
9. Aggarwal SK, Gogu SR, Rangan SRS and Agrawal KC, Synthesis and biological evaluation of prodrugs of zidovudine. *J Med Chem* **33**: 1505–1510, 1990.
10. Sikka SC, Swerdloff RS and Rajfer J, Assay system for simultaneous measurement of steroidogenic enzyme activities in rat and human testis: Effect of hCG. *Anal Biochem* **149**: 72–86, 1985.
11. Gogu SR, Beckman BS and Agrawal KC, Anti-HIV drugs: Comparative toxicities in murine fetal liver and bone marrow erythroid progenitor cells. *Life Sci* **45**: iii–vii, 1989.
12. Frick LW, Nelson DJ, St. Clair MH, Furman PA and Krenitsky TA, Effects of 3'-azido-3'-deoxythymidine on the deoxynucleotide triphosphate pools of cultured human cells. *Biochem Biophys Res Commun* **154**: 124–129, 1988.
13. Sommadossi JP, Carlisle R and Zhou Z, Cellular pharmacology of 3'-azido-3'-deoxythymidine with evidence of incorporation into DNA of human bone marrow cells. *Mol Pharmacol* **36**: 9–14, 1989.
14. Weidner DA and Sommadossi JP, 3'-Azido-3'-deoxythymidine inhibits globin gene transcription in butyric acid-induced K-562 human leukemia cells. *Mol Pharmacol* **38**: 797–804, 1990.
15. Balzarini J, Pauwells R, Baba M, Herdewijn P, Clercq D, Broder S and Johns DG, The *in vitro* and *in vivo* anti-retrovirus activity and intracellular metabolism of 3'-azido-2', 3'-dideoxythymidine and 2',3'-di-deoxycytidine are highly dependent on the cell species. *Biochem Pharmacol* **37**: 897–903, 1988.
16. Grossman CJ, Interactions between gonadal steroids and immune system. *Science* **227**: 257–261, 1985.
17. Calkins JH, Sigel MM, Nankin HR and Lin T, Interleukin-1 inhibits Leydig cell steroidogenesis in primary culture. *Endocrinology* **123**: 1605–1610, 1988.
18. Natarajan R, Ploszaj S, Horton R and Nadler J, Tumor necrosis factor and interleukin-1 are potent inhibitors of angiotensis-II-induced aldosterone synthesis. *Endocrinology* **125**: 3084–3089, 1989.
19. Emoto N and Baird A, The effect of tumor necrosis factor/cachectin on follicle-stimulating hormone-induced aromatase activity in cultured rat granulosa cells. *Biochem Biophys Res Commun* **153**: 792–798, 1988.
20. Weignet DA and Blalock JE, Interactions between the neuroendocrine and immune systems: Common hormones and receptors. *Immunol Rev* **100**: 79–108, 1987.
21. Bernton EW, Beach J, Holaday JW, Smallridge RC and Rein HG, Release of multiple hormones by a direct action of interleukin-1 on pituitary cells. *Science* **238**: 519–521, 1987.
22. Swerdloff RS, Physiology of male reproduction. Hypothalamic-pituitary function. In: *Campbell's Urology* (Ed. Harrison JH), chap. 5, pp. 186–200. W. B. Saunders, Philadelphia, 1986.
23. Rivier C, Rivier J and Vale W, Stress-induced inhibition of reproductive functions: Role of endogenous corticotropin-releasing factor. *Science* **231**: 607–609, 1986.