Induction of Feline Acquired Immune Deficiency Syndrome by Feline Leukemia Virus: Alteration in Response to Hormones in the Hypothalamic-Pituitary-Gonadal System (43869)

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Abstract. **Male kittens who were infected with the feline leukemia virus (FeLV) were found at various times after exposure to contain a sequence of dysfunction in their hypothalamic-pituitary-gonadal (HPG) system. To understand whether the involved endocrine glands in this system were damaged by FeLV, the hypothalamus, pituitary, and testes were tested for hormonal responsiveness *in vivo* and *in vitro*. The infected cats were administered with luteinizing hormone-releasing hormone (LHRH) and human chorionic gonadotropin (hCG). Their response to the treatment was studied, and they were then compared with untreated control cats. Normal response to LHRH for the synthesis of follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were found in the infected cats prior to 10 weeks of infection. After 10 weeks, the response was reduced by 25%, 38%, and 42%, respectively. Twelve weeks after infection, the response to hCG for testosterone synthesis was drastically reduced. The control cats, however, demonstrated normal prolonged biphasic patterns of response to hCG. The *in vivo* administration of the tropic hormones had no effect on the titer of FeLV gs antigen in the blood of the infected cats.**

The medial basal hypothalamus (MBH) from the control cats and cats in their 13th week of infection were cultured *in vitro*, with the presence of high K^+ ion (60 m*M*). The control MBH responded to K^+ ion stimulation for LHRH release. The K^+-stimulated release of LHRH in the control MBH was 99% higher than that of the infected MBH. In contrast, the amount of unreleased LHRH in the infected MBH was 74% higher than that of the control MBH. In *in vitro* culture, the control pituitary gland responded markedly higher to LHRH stimulation for the release of gonadotropins (FSH and LH) than that of the infected one (140% compared with 56% for FSH, and 70% compared with 28% for LH, respectively). Whereas, the amount of unreleased FSH and LH in the infected pituitary gland were 59% and 31%, higher than that of the control gland.

These results suggest that (i) the progressive development of neuroendocrine glands' dysfunction is related to viral replication time; (ii) the *in vivo* and *in vitro* responses to tropic hormones in the infected endocrine glands are drastically reduced; and (iii) this reduction in hormonal response may be caused by defective regulation of peptide hormonal secretion. [P.S.E.B.M. 1995, Vol 208]

In weanling kittens, infection with feline leukemia virus (FeLV, or feline immunodeficiency virus [FIV]) caused feline acquired immune deficiency syndrome (FAIDS) (1–3). Feline acquired immune deficiency syndrome was found to closely resemble human AIDS in that both are caused by retroviruses and are characterized by T-lymphocyte dysfunction, neutropenia, thymic atrophy, and a high susceptibility to opportunistic infections (4–8). In addition to immune dysfunc-

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tion, the disease affects multiple tissues and organs in the neurological, pulmonary, gastrointestinal, respiratory, dermatological, and reproductive systems (9–14).

Feline acquired immune deficiency syndrome induced by FeLV was associated with neuroendocrine dysfunction (i.e., alteration in the content of plasma adrenocorticotropic hormone (ACTH), growth hormone (GH), cortisol, and a sequence of dysfunctions in the hypothalamic-pituitary-gonadal (HPG) system (14–16) manifest in the reduction of luteinizing hormone-releasing hormone (LHRH), follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone in blood plasma (14). In addition to its effect on the neuroendocrine system, FeLV also causes a drastic reduction in the content of gonadotropin-like peptide hormones (i.e., FSH and LH) in lymphocytes (14).

Investigations on human male AIDS patients reveal that the disease suppresses reproductive function and leads to hypogonadism (17–20). One of the major clinical symptoms that pertain to hypogonadism is reduced testosterone secretion associated with hypothalamic and hypogonadotropic defect (20, 21). We discovered that similar symptoms appear with FAIDS and have been attributed to the abnormal function of the HPG system (14). At various times after FeLV exposure, male kittens displayed a sequence of dysfunctions in the HPG system. The alteration in this system appeared as a sequence of chain events. The time scale for the appearance of neuroendocrine dysfunction involving the hypothalamus, pituitary, and testes is 4, 6, and 11 weeks after FeLV infection respectively (14). From this information, two important questions need to be explored: (i) Does FeLV impair individual endocrine gland (i.e., hypothalamus, pituitary, and testes) response to their stimulants (i.e., high K^+ ion, LHRH, and hCG, respectively)? (ii) Does FeLV affect the release of LHRH and gonadotropins from the hypothalamus and pituitary in culture?

This communication is to report the following: (i) the *in vivo* effect of LHRH (or hCG) on the content of plasma gonadotropins and testosterone in the FeLV-infected as well as in uninfected kittens; (ii) the onset of impaired responsiveness toward tropic hormones in the FeLV-infected cats; (iii) the *in vitro* response of the FeLV-infected hypothalamus to potassium ions for the release of LHRH; (iv) the *in vitro* response of the pituitary glands (infected and uninfected) to the tropic hormone for the release of gonadotropins; (v) the ratio of stimulated-released to basal-released hormones in the infected and uninfected endocrine glands; and (vi) the comparison of unreleased hormones in the endocrine glands of the infected and uninfected kittens.

Materials and Methods

Animals and Their Inoculation with FeLV. Weanling male kittens (8 to 10 weeks old) with an average body weight of 0.8–1.0 kg (mean ± SEM, 0.9 ± 0.3 kg) (Pearcroft Cattery, Beaufort, NC) were used for observation. The kittens were all FeLV pathogen free, housed in individual cages under a 12:12-hr light: dark cycle, and provided with Purina Cat Chow (Ralston Purina Co., St. Louis, MO) and water *ad libitum*. For each experiment, a group of five kittens was used as the control, and the other six kittens were inoculated intravenously with 8×10^4 focus-forming units (FFU) FeLV "A" as assayed by clone 81 feline cell culture (22). (ATCC-717, prepared in feline embryo fibroblasts [FEF] tissue culture fluid; American Type Culture Collection, Rockville, MD). The control cats were inoculated with tissue culture fluid from uninfected FEF.

Hormone Administration. Five and ten weeks after FeLV infection, the control and infected cats were administered synthetic LHRH (D-Lys^6 LHRH; Sigma Chemical Co., St Louis, MO; 10 μg/kg body wt) intravenously. All experiments were performed between 1100 and 1200 hr, and each animal was serially bled by jugular venipuncture at time 0 (immediately before LHRH) and again at 20, 30, and 60 min later. Plasma was obtained by centrifugation and stored at −20°C until assayed. Twelve weeks after FeLV infection, the control and infected cats were administered with a single dose of hCG (Human hCG; Sigma) (100 IU/cat) intramuscularly. All experiments were performed between 1300 and 1400 hr. Blood samples were collected at time 0 and then at 1, 2, 24, 48, 72, and 96 hr after hCG infection. Plasma was collected and stored at −20°C until assayed.

Preparation of Endocrine Glands for *In Vitro* Study. The details of the endocrine gland preparation for superfusion culture were according to Azad *et al.* (23, 24). Freshly excised medial basal hypothalamus (MBH) and pituitary glands from the control and infected cats were sliced into 6 to 8 small pieces (approximately 1.0 mm in length and 3.0 mm in thickness). Two slices of the gland were pooled together and were then preincubated in 1.0 ml of standard Lock's medium (containing 154 m*M* NaCl, 6 m*M* $NaHCO_3$, 6 m*M* KCl, 0.8 m*M* $MgSO_4$, 1.2 m*M* $CaCl_2$, 10 m*M* glucose, 0.05% HEPES, and 0.03% bacitracin) for 90 min at 37°C with 95% air and 5% CO_2 in a constant shaker (100 cycle/min).

After preincubation, the medium was renewed, and the culture was resumed for 30 min. The peptide hormone released to the medium was assayed and defined as the "basal-released" hormone. Afterward, the hypothalamic slices were rinsed and continuously

cultured in Lock's medium containing 60 m*M* KCl and 15 m*M* NaCl (high K^+ and low Na^+ Lock's medium) for an additional 30 min at 37°C. The amount of LHRH in the medium was assayed and defined as the "K^+-stimulated release of LHRH." The pituitary slices were cultured in the standard Lock's medium containing 10 μ*M* LHRH for 30 min at 37°C. At the end of incubation, the medium was assayed for the content of FSH and LH (defined as LHRH-stimulated release of FSH [or LH]). After these series of incubations, the hypothalamic (or pituitary) slices were weighted and sonicated in 1.0 ml of standard Lock's medium with a Branson sonicator (at setting No. 6 for 1 min). The medium containing the sonicated hypothalamic tissue was extracted with 0.1 *N* HCl, neutralized with an equal amount of 0.1 *N* NaOH, and collected by centrifugation. The medium was then assayed for the content of LHRH. Similarly, the content of FSH and LH in the medium of the sonicated pituitary tissue was assayed with no acid extraction. The peptide hormones detected in the tissues after sonication were defined as "unreleased LHRH, FSH, or LH."

Quantitative Determination of FSH, LH, Testosterone, and LHRH in Plasma and Culture Medium. Determination of peptide and steroid hormone condition was done according to the procedure of Wang and Teng (14). The content of FSH and LH in plasma and medium was measured by direct radioimmunoassay (RIA) using an RIA kit (Simul TRAC LH [^{57}CO] FSH [^{125}I]) from Becton-Dickinson Co. (Orangeburg, NY). This assay consisted of a combined double antibody system. The percentage of the cross-reactivities for FSH antibody were 100% with FSH; 0.02% with LH: 0.9% with thyroid-stimulating hormone (TSH); and 0.2% with hCG. The specificities of the antibody for LH were 100% with LH; 5.5% with FSH; 4.8% with TSH, and 15.7% with hCG. The assay performance characteristics demonstrated intra-assay variations of 5.3% (FSH) and 4.9% (LH), and interassay variabilities of 9.3% (FSH) and 8.3% (LH). Testosterone levels were measured by RIA after ether extraction as outlined by Hodgson and Dekretser (25). Cross-reactivity of the antiserum with other steroids included 5α-dihydrotestosterone (7.5%), androstenedione (0.48%), androstenediol (0.096%), 5β-dihydrotestosterone (0.01%), and cholesterol (<0.01%). The inter- and intra-assay coefficients of variations were 7.6% and 4.3%, respectively. The minimum sensitivity for assay was 5 pg/tube. The amount of LHRH in medium was quantified by RIA (25). A diluted antiserum (1:15,000, rabbit anti-LHRH, Peninsula Lab, Inc., Belmont, CA) was used for the assay. Details of the procedures were described in a previous publication (14).

The standard of the test kit for gonadotropins (or LHRH) was developed for human clinical use. In order to verify its suitability for feline use, log-logit dose-response lines were constructed according to Wang and Teng (14). Increased volumes of plasma (or pituitary and hypothalamic culture media) from control or infected cats ran parallel to the standard in both gonadotropins, and LHRH radioimmunoassay (data not shown). Since there were no differences in the lines of gonadotropins (or LHRH) from the standard, plasma, and culture media, the commercial test kit was used for the rest of the studies.

FeLV Group-Specific Test. The presence of FeLV group-specific (gs) antigens in the blood of the control, infected, and hormone-treated cats was detected by an FeLV group-specific enzyme-linked immunosorbent assay (ELISA) kit (Virachek/FeLV; Synbiotics Corp., San Diego, CA). The details for blood sample testing (i.e., production of a stable color reaction) and the measurement of color at 630 nm on a Dynatech MR 700 micrometer were previously described (14).

Statistical Analysis. For experiments with MBH incubation, data were expressed as mean picograms of LHRH released per 30 min per milligram tissue weight ± SEM. Similar expression was applied to the experiment with pituitary gland culture. Results were expressed as mean μIU of FSH (or LH) released per 30 min per milligram tissue weight ± SEM. For most experiments, comparisons between hormone-treated groups were made using Student's *t* test (26). The results from dose-response experiments were analyzed by one- or two-way (ANOVA), and the differences between specific means were tested for significance by Scheffe's multiple range test (26). A difference between the two means was considered statistically significant when the *P* value was less than 0.05.

Results

LHRH Responsiveness. Basal levels of FSH, LH, and testosterone were quantified 4 weeks after FeLV exposure. Infected and control cats were given normal saline (0.25 ml/cat iv for 60 min) and plasma FSH, LH, and testosterone levels were quantified at 10-min intervals. The basal levels of FSH, LH, and testosterone secretion were 1.0 mIU/ml, 3.0 mIU/ml, and 150 pg/ml, respectively, with minimal fluctuation across sampling times (Fig. 1).

At 5 weeks postexposure, both control and infected cats increased plasma LH and testosterone 20, 30, and 60 min following LHRH administration. The plasma LH in the control and infected cats were stimulated by 36%–57% and 52%–72%, respectively (Fig. 2b). Whereas, the plasma testosterone was stimulated by 4- to 8-fold in control cats compared with 3- to 12-fold in infected cats (Fig. 2c). Notably, FeLV infection did not

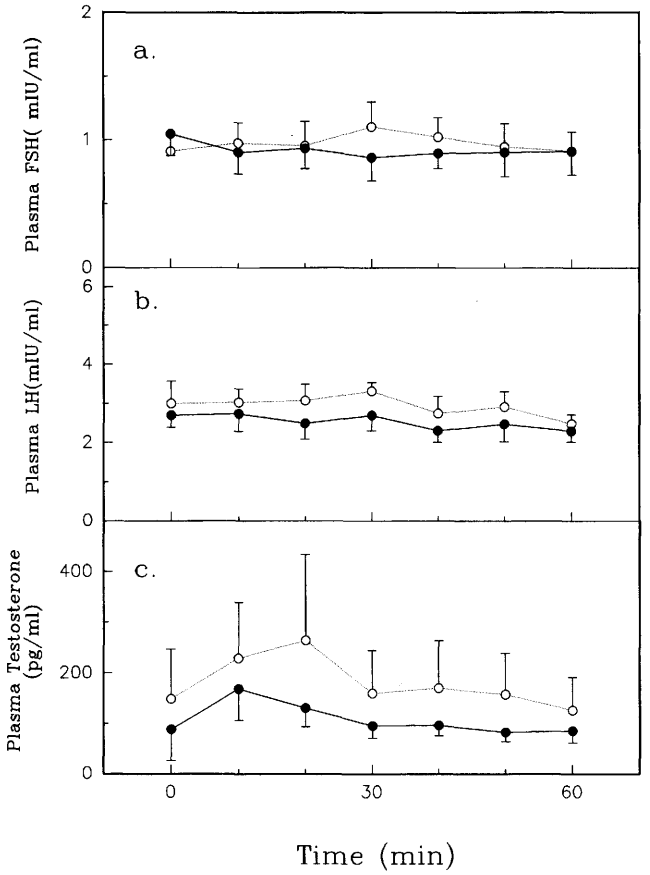

Figure 1. Plasma FSH (a), LH (b), and testosterone (c) content after administration with normal saline. The control cats (or infected cats at 4 weeks of infection) were administered a single injection of 0.25 ml normal saline for 60 min. Blood samples were collected once every 10 min. Plasma FSH, LH, and testosterone were detected by RIA. A difference between two means was considered to be statistically significant when P was less than 0.05. $^{*}P < 0.05$; $^{**}P < 0.01$ (compared to control group). (○), control cats (n = 5); (●), infected cats (n = 6)

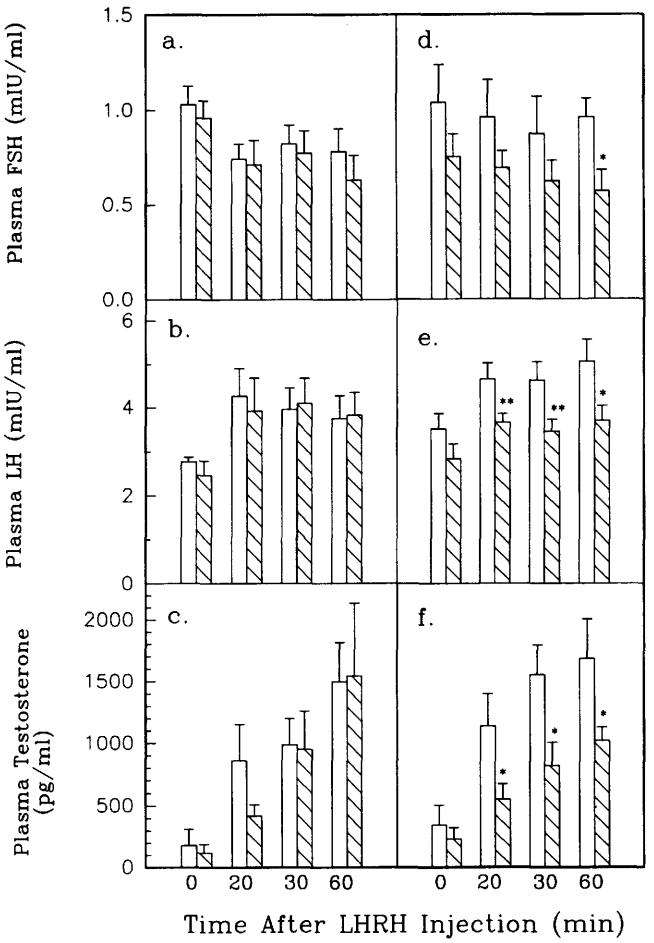

Figure 2. Plasma FSH (a and d), LH (b and e), and testosterone (c and f) content of cats treated with LHRH. The control and infected cats (at 5 and 10 weeks after infection) were treated *in vivo* with a single injection of 10 μg of LHRH/kg body wt in 0.25 ml normal saline for 60 min. Blood samples were collected once every 10 min. Plasma FSH, LH, and testosterone were detected by RIA. Left figures (a through c) represent the cats that were 5 weeks postinfection. Right figures (d through f) represent the cats that were 10 weeks postinfection. (□), control cats (n = 5); (▨), FeLV-infected cats (n = 6). Each value represents mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$ (infected cats compared with control cats)

alter responsiveness to LHRH administration. Furthermore, LHRH administration did not increase plasma FSH in either infected or control cats (Fig. 2a).

By 10 weeks postexposure, LHRH elicited decreased plasma LH and testosterone responses in FeLV-infected cats compared with control cats (Fig. 2, e and f). In FeLV-infected cats, LH and testosterone responses were 23%–28% and 41%–52% of their respective controls. Again, no significant response was found for FSH (Fig. 2b).

hCG Responsiveness. Twelve weeks after FeLV infection, both control and infected cats were given a single injection of 100 IU hCG/cat iv and plasma testosterone responses were quantified at 1, 2, 24, 48, 72, and 96 hr (Fig. 3). A biphasic response was seen in control cats. Plasma testosterone levels were elevated 2 hr after hCG administration, reached a maximum of 3000 pg/ml, declined to 1550 pg/ml by 24 hr, again increased to 2900 pg/ml at 72 hr, and again decayed to basal levels by 96 hr. In the infected cats, plasma testosterone responses to hCG administration were not biphasic. Rather, plasma testosterone increased linearly over the first 72 hr following hCG administration, but the increases were not statistically significant. Administration of tropic hormones (LHRH or hCG) did not change the titer of FeLV gs antigen present in the blood of infected cats at any time point evaluated (data not shown).

***In Vitro* Responses of the Hypothalamus to K^+ Stimulation.** The amount of basal-released LHRH (expressed as pg/MBH/30 min) did not differ between hypothalami from infected and control cats (Fig. 4). In contrast, K^+-stimulated LHRH release was twice basal-released LHRH from control cat hypothalami, while K^+-stimulated LHRH release from infected cat hypothalami was not increased over basal-released LHRH levels. The failure of K^+-stimulated LHRH

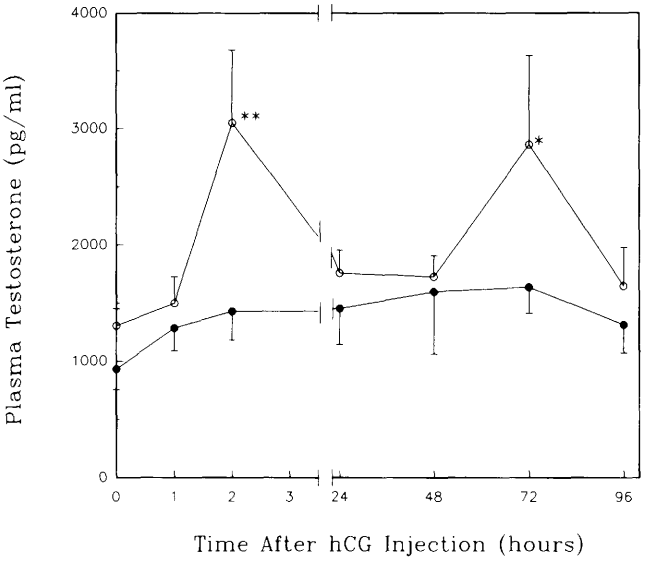

Figure 3. Plasma testosterone responses to hCG treatment. Control and infected cats (12 weeks after FeLV infection) received a single im injection of 100 IU hCG per cat for 96 hr. Blood samples were collected at various time periods, and the testosterone concentration was determined by RIA. The results presented in this figure are the average of five determinations. (○), control cats (n = 5); (●), infected cats (n = 6). *$P < 0.05$; **$P < 0.01$ (control compared with infected group).

release correlated with hypothalamic LHRH retention, as the amount of unreleased LHRH in the hypothalami of the infected and control cats were 1102 ± 224 and 633 ± 115 pg/mg tissue, respectively.

***In Vitro* Response of the Pituitary to LHRH Stimulation.** The release of gonadotropins with (or without) the stimulation of LHRH in pituitary culture was expressed as μIU/mg tissue/30 min. In control cats, the LHRH-stimulated FSH and LH release was 141% and 71% higher, respectively, than basal-released levels (Table I). In FeLV-infected cats, the stimulation of FSH and LH release were 40% and 39% of their respective control cats. The reduction of LHRH-stimulated gonadotropins release correlated with higher hypophyseal retention of gonadotropins in infected cats compared with those from controls (Table I).

Discussion

Current information compiled from the progression of human immunodeficiency virus (HIV) and FeLV infection indicates that, in the infected patient, the dysfunction in the neuroendocrine system is one of the early symptoms associated with the disease (14, 15, 17, 18). In FAIDS, this dysfunction is manifested in the presence of the FeLV in the endocrine glands of the HPG system and the reduction of LHRH, FSH, LH, and testosterone in blood plasma (14). In this regard, the effect of the FeLV may be to suppress endocrine gland response to tropic hormones, or it may reduce the glandular function for the release of peptide hormones. To understand the HPG system dysfunction in depth, we investigated the *in vivo* (as well as *in*

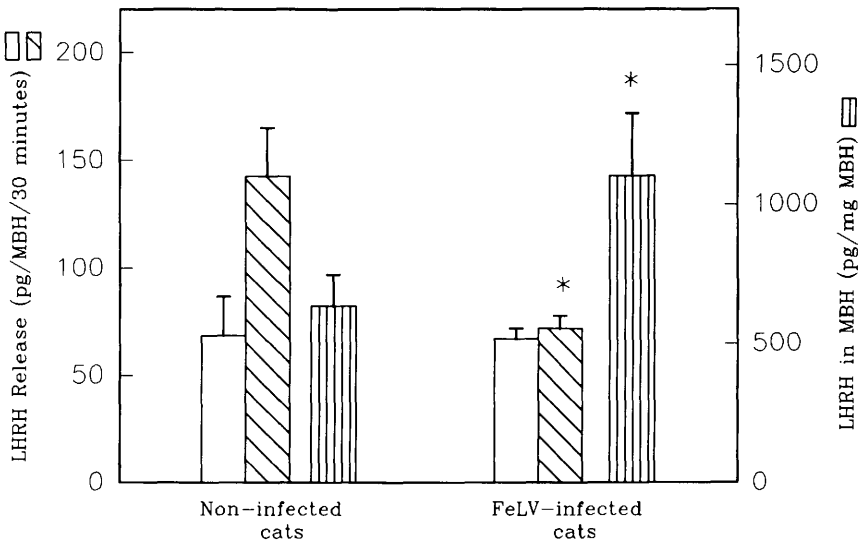

Figure 4. The *in vitro* response of hypothalamus to K^+ stimulation. Cats (five control and six 13-week-infected cats) were euthanized and the hypothalami excised. MBH portion of each hypothalamus was removed and sliced into the size as described in Materials and Methods. MBHs (two slices per culture) were preincubated in Locke's medium at 37°C for 90 min. They were then incubated for 30 min, and the LHRH released to the medium was assayed (□, basal-released LHRH). The slices were further incubated for 30 min with Locke's medium with high K^+ and low Na^+ ions (60 m*M* KCl and 15 m*M* NaCl). The LHRH released to the medium was assayed (▨, K^+-stimulated release of LHRH). Afterward, the slices of MBH were sonicated to release the LHRH for RIA assay (▥, unreleased LHRH). Each value represents the mean ± SEM. The data was analyzed by a two-way analysis of variance (ANOVA), and the differences between means were tested for significance using Scheffe's multiple range test. A difference between two means was considered to be statistically significant when P was less than 0.05. *$P < 0.05$ (compared with control group).

Table I. The *In Vitro* Response of Pituitary to LHRH Stimulation

Pituitary gland	FSH release				LH release			
	Basal release (μIU/mg/30 min)	LHRH-stimulated release (μIU/mg/30 min)	Percentage stimulated (L.S.R./B.R.)	Unreleased (μIU/mg)	Basal release (μIU/mg/30 min)	LHRH-stimulated release (μIU/mg/30 min)	Percentage stimulated (L.S.R./B.R.)	Unreleased (μIU/mg)
Control	22.7 ± 5	54.7 ± 2.3	141% ± 4.3%	94.5 ± 12	426 ± 40	728 ± 81	71% ± 4.3%	733 ± 98
Infected	36.2 ± 12	56.3 ± 13	56% ± 6.9%	151.0 ± 20[a]	350 ± 60	448 ± 35[a]	28% ± 2.3%	961 ± 81[a]

Note. The results presented in this table were based on experiments with five control cats and six 13-week-infected cats. Two slices of pituitary per group were preincubated in Lock's medium at 37°C for 90 min and then incubated for 30 min, the gonadotropins released to the medium was assayed (basal release, B.R.). The slices were incubated for 30 min with Locke's medium containing LHRH (10^{-5} *M*), the gonadotropins released in the medium were assayed (LHRH-stimulated release, L.S.R.). Finally, the slices were sonicated to release gonadotropins for assay (unreleased). Each value represents mean ± SEM.
[a] $P < 0.05$ compared with control value.

vitro) response of the endocrine glands to stimulants (i.e., hypothalamus to K^+ ion, pituitary and gonadal glands to the tropic hormones).

In the felid (i.e., cat and cheetah), the release of FSH, LH, and testosterone to the blood strain is not pulsatile in nature (27, 28). A short interval of blood sampling has proven to be desirable. Furthermore, within individual saline-treated male cats, there was little fluctuation in plasma FSH, LH, and testosterone concentration during the 60-min sampling period (Fig. 1). In addition, our experiments have shown that in male cats the plasma serum FSH, LH, and testosterone reaches its maximum level within 60 min of LHRH treatment (data not shown).

Information obtained from *in vivo* hormonal treatment indicated that a pituitary (or gonadal) refractory toward LHRH was demonstrated in the cats that had been infected for 10 weeks (but not those that had been infected for 5 weeks). The FSH, LH, and testosterone response to LHRH was quantitatively reduced in the cats infected for 10 weeks compared with that of the control cats. However, LHRH treatment in the 5-week-infected cats indicated that the pituitary and gonadal glands were still functioning normally. Since the titer of FeLV gs antigen in blood was not affected by LHRH (or hCG) administration in the 10-week-infected cats, the progressive development of pituitary and gonadal dysfunction is directly related to viral replication time. Whether or not this impairment is caused by an opportunistic infection is still unknown. The observed pituitary and gonadal dysfunction due to FeLV infection can, however, be closely compared with what has been reported in human AIDS patients. In HIV-infected men, dysfunction in the endocrine glands is rather common. Pathological changes in the pituitary include involvement with opportunistic infections (i.e., Toxoplasma, cytomegalovirus [CMV], and Pneumocystis) (17, 29, 30). Testicular opportunistic infections include CMV, *Mycobacterium avium-intracellulare* (MAI), *M. tuberculosis,* a reduction in testosterone secretion, and hypogonadism (31–33). Endocrine gland response to hormone treatment in men with AIDS has not been reported.

In normal male cats, we have demonstrated the biphasic and prolonged response to hCG for testosterone secretion. Similar findings have been reported in men and males of other species, e.g., bulls and rats (25, 34–36). The plasma testosterone peaks appear at 2 hr and then at 72 hr, and the intervening nadir suggests a period of Leydig cell refractoriness. It has been reported that the refractoriness was due to a lack of substrate for steroidogenesis (37). Furthermore, the early effects (2 to 12 hr) of hCG on the rat's mature Leydig cell involve a proliferation of smooth endoplasmic reticulum and an increase in the volume of mitochondria, Golgi ribosomes, and the enlargement of the cell (25, 38, 39). During this phase of growth, the Leydig cell may require all its energy and nutrients to be diverted toward synthetic mechanisms. This results in proliferation of cell organelles, initially at the expense of steroidogenesis. A refractory period is, therefore, required for the replenishment of energy. Finally, the mechanisms involved in the prolonged length of the refractory period in the cat and other species remain unknown. The Leydig cell's response for testosterone synthesis differs markedly from infected cats to that of normal cats. There is clearly no biphasic response to *in vivo* hCG stimulation. At present, no explanation is available for this anomaly, but it may be attributed to the changes as follows: (i) A concomitant decline in the Leydig cell number; (ii) The inhibition of Leydig cell maturation; (iii) The reduction in the number of hCG receptors; (iv) An impaired mechanism (or lack of substrate) for steroidogenesis.

We observed that the control hypothalamic tissue under *in vitro* culture conditions is able to release LHRH spontaneously. It also release LHRH under K^+ stimulation. Studies comparable in this aspect have been reported in different mammalian species (i.e., rodents, guinea pigs, and cats) (23, 24, 40–43). In

the cat, Micevych *et al.* (44) demonstrated that K^+ ion stimulated the release of a peptide hormone cholecystokinin (a pancreatic enzyme-releasing hormone) from hypothalamus in culture. Our *in vitro* study revealed that the basal release (or spontaneous secretion) of LHRH from the hypothalamus of the control and infected cat is comparatively negligible. The rate of LHRH release is 2.3 and 2.2 pg/min, respectively. In the control hypothalamus, the K^+-stimulated rate of release is 4.8 pg/min which is 110% higher than that of the basal-release value. However, in the infected tissue, its *in vitro* response to the K^+-stimulated release of LHRH is lacking. After stimulation, the LHRH rate of release is only 2.4 pg/min, which is statistically insignificant compared to the basal-released value. Thus, the content of the unreleased intracellular LHRH in the infected tissue is 74% higher than that in the control tissue. This observation coincided with what has been demonstrated in the uremic rat by Wibullaksanakul and Handelsman (45). They observed that a marked reduction in plasma FSH, LH, and testosterone in the male uremic rat was the result of a deficient LHRH secretion. Furthermore, the observed low LHRH release was accompanied with a high content of intracellular unreleased LHRH in the hypothalamus. Their observations along with ours demonstrated an impaired secretory function of the cells.

Our examination of the K^+-stimulated LHRH release could clarify the mechanisms of aberrant regulation of the hypothalamus LHRH secretion in FeLV-infected cats. In this regard, K^+ ion has been demonstrated in rats as an effective membrane depolarizing agent triggering an influx of calcium ion and leading to the exocytosis of intracellular LHRH (41, 46). Moreover, this stimulated release of LHRH is not only a Ca^{2+}-dependent process, it also involves a microtubular transport mechanism (41). The membrane depolarization and microtubular transport mechanism are the two major steps involved in the normal release of LHRH. Therefore, in the FeLV-infected hypothalamus, the lack of response to K^+-stimulated LHRH release could be attributed to the defective regulation of LHRH secretion.

The *in vitro* study of the response of the control and infected pituitary glands to LHRH for gonadotropins' release had confirmed the results obtained from a previous *in vivo* study (14). Both *in vivo* and *in vitro* investigations indicated a reduced response to LHRH in the infected pituitary. In addition, the *in vitro* study also revealed a higher content of the unreleased gonadotropins in the infected tissue than that of the control. The defective regulation of hormone secretion in pituitary gland caused by FeLV is parallel to what has been demonstrated in the hypothalamus. It is envisioned that the common defect in these two endocrine glands may be caused by the same factors. In the pituitary gland, the synthesis and secretion of FSH and LH are under the control of hypothalamic LHRH (47). Judging from the retention of a high content of intracellular FSH and LH in the infected pituitary gland, we propose that the inhibition of gonadotropins' secretion in the infected pituitary is due to a functional defect in the process of secretion. This defect could be attributed to an altered cellular membrane structure. Rojko and associates reported that, when the feline immune cells were infected with different strains of FeLV (i.e., R and A strain), it altered the proportions of fatty acid (FA) and arachidonic acid (AA) (48). Since both FA and AA are the major components of the cellular membrane, the depletion of FA and AA could cause the perturbation of the membrane structure. In this regard, a parallel finding indicated that, in FeLV-infected feline immune cells, the calcium-mediated membrane signal transduction system has also been altered (49). This, in turn, may initiate a cytopathic effect that has been reported to be the consequence of FeLV and HIV infection (50–52). The FeLV-caused perturbation in the membrane structure could account for the cellular defects that we have discovered in the infected endocrine glands. Unfortunately, the mechanisms involving these functional defects in regulating the gonadotropins' secretion by FeLV are still unknown. Further studies are needed to clarify the mechanisms in detail.

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