



Title	Neuroendocrine Control of Anuran Anterior Preoptic Neurons and Initiation of Mating Behavior
Author(s)	Urano, Akihisa
Citation	Zoological Science, 5(5), 925-937
Issue Date	1988-10-15
Doc URL	<a href="http://hdl.handle.net/2115/43963">http://hdl.handle.net/2115/43963</a>
Type	article
File Information	ZS5-5_925-937.pdf



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## REVIEW

Neuroendocrine Control of Anuran Anterior Preoptic  
Neurons and Initiation of Mating BehaviorAKIHISA URANO<sup>1</sup>

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## INTRODUCTION

The preoptic area plays an important role in the evocation of sexual behavior in many vertebrate species [1-6]. In the anuran brain, the anterior part of the preoptic nucleus (APON) is considered to be the center for triggering male mate calling behavior. After careful re-examination of the lesion study by Aronson and Noble [7] on the brain of the leopard frog *Rana pipiens*, Schmidt [2, 8] proposed that the APON initiates an organizing activity of mating call patterns in the brain stem "call center" which is composed of the pretrigeminal nucleus and the laryngeal motor neurons. The same system may function in the brain of the Japanese toad, *Bufo japonicus* [9, 10]. The APON is further concerned with female orientation to conspecific mating calls in *R. pipiens* [11].

Anuran mating behavior is controlled not only by neural input signals but also by various hormonal signals. Testosterone seems to be not a sole, but a crucial hormonal factor in the initiation of male mate calling behavior [12-14]. In gravid female *Rana pipiens*, vasotocin, an anuran neurohypophysial hormone, suppressed female release calling behavior, and in turn elevated sexual receptivity [15]. Luteinizing hormone-releasing hormone (LHRH) also increased sexual receptivity in female *Xenopus laevis* [16].

The amphibian brain shows a fundamental organizational pattern common to the structure of the vertebrate brain [17]. Clarification of neuroendocrine control of mating behavior in amphibians is thus important for understanding phylogenetically fundamental control mechanisms of sexual behavior. In this paper, I first describe the morphological characteristics of the preoptic nucleus (PON), and then review recent studies concerning the neural and hormonal control mechanisms for the initiation of seasonal reproductive behavior in anuran amphibians.

## CYTOARCHITECTURE OF THE PON

*Subnuclear Organization*

The PON is a neuronal cell mass which surrounds the preoptic recess, and is considered to be an important neuroendocrine center. It is easily divisible into anterior and posterior halves by a relatively cell-poor zone along the sulcus preopticus (Fig. 1), and each half is composed of several subnuclei, some of which include many neurosecretory neurons. Recent physiological studies indicate that these subnuclei are functionally distinctive [18].

In the Japanese toad, the PON can be divided into seven subnuclei: the anterior part of the PON (APON), the dorsal and the ventral periventricular parts, the dorsal and the ventral magnocellular parts (*dmc* and *vmc*), the suprachiasmatic part, and the posterior part of the PON (PPON) [18]. The APON is composed of a rather compact cell

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Received May 27, 1988

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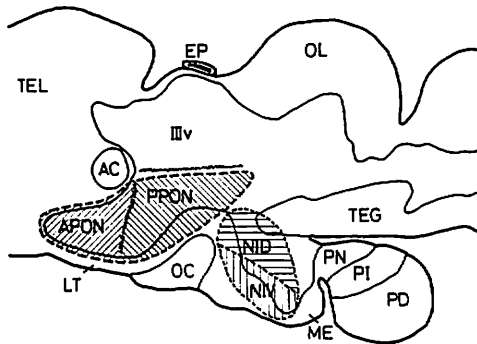


FIG. 1. Diagram of the parasagittal midplane of the toad diencephalon. Note that the preoptic nucleus which locates antero-dorsal to the optic chiasma (OC) is divisible into the anterior part (APON) and the posterior part (PPON). AC, anterior commissure; EP, epiphysis; LT, lamina terminalis; ME, median eminence; NID, nucleus infundibularis dorsalis; NIV, nucleus infundibularis ventralis; OL, optic lobe; PD, pars distalis of hypophysis; PI, pars intermedia of hypophysis; PN, pars nervosa of hypophysis; TEG, tegmentum mesencephali; TEL, telencephalon; IIIv, third ventricle. (from Urano and Ishihara [110])

mass surrounding the anterior portion of the preoptic recess in the toad brain, while, in many ranid species, APON neurons are well organized in a laminar pattern [19–21]. The *dmc* and the *vmc* consist of magnocellular neurosecretory neurons which contain immunoreactive (ir) vasotocin [18, 22], mesotocin [22], somatostatin [23] and other peptides [24]. The toad suprachiasmatic part in-

cludes small ir-vasotocin neurons and receives direct retinal innervation (Shimotoso and Urano, unpublished). Synapses between the optic terminals and the PON neurons were observed in *Rana temporaria* [25]. The PPN, the name of which follows nomenclatorial usage in the brain atlas of *Rana pipiens* [21], is a common preoptic structure in many anuran species.

#### Sexual Dimorphism

Differences in patterns of sexual behavior between males and females are partly due to differences between male and female neuronal circuitries, i. e., sexual dimorphism of nervous systems. As in songbirds [26], the Japanese quail [27], and some mammalian species [28–31], the brain loci concerning male mate calling behavior showed sexual dimorphism in *Xenopus* [32], *Rana pipiens* [33] and *Bufo japonicus* [34]. As for the toad PON, the nuclear volume of the APON in the male was significantly larger than that in the female (male/female, 1.25–1.39). The mean of the total cell number in the male APON was 1.20 times that of the female, while the cell sizes of male and female APON neurons were in the same range [35]. These results suggest that the sexual difference in the APON volumes was caused by a greater cell number in the male APON. In addition, the amygdala pars medialis (Am), which forms a morphological and probably functional complex with the APON [36], showed a similar

TABLE 1. Volumes ( $\times 10^{-3} \text{ mm}^3$ , mean  $\pm$  S.E.) of the anterior part of the preoptic nucleus (APON) and the amygdala pars medialis (Am) in 1-year old toadlets, and hibernating and post-breeding adult toads

Subnuclei	Male	Female	M/F
<b>APON</b>			
1-year old toad	103.6 $\pm$ 4.5	98.1 $\pm$ 3.2	1.06
hibernating	311.5 $\pm$ 23.0	224.1 $\pm$ 14.2	1.39*
post-breeding	250.0 $\pm$ 12.3	199.3 $\pm$ 9.7	1.25**
<b>Am</b>			
1-year old toad	45.9 $\pm$ 2.1	35.5 $\pm$ 3.1	1.30*
hibernating	153.8 $\pm$ 9.5	90.1 $\pm$ 4.2	1.71**
post-breeding	110.1 $\pm$ 8.4	58.9 $\pm$ 3.2	1.87**

\*,  $P < 0.02$ ; \*\*,  $P < 0.005$  by the t-test.

M/F, the ratio of the nuclear volume in the male to the female.

sexual difference. Ontogenetically, the sexual difference in the Am volumes appeared in yearling toadlets, preceding the development of the APON (Table 1).

The sexual dimorphism in the APON seems to be a fundamental part of vertebrate brain structure in general, since the preoptic area is sexually dimorphic in the quail [27], the rat [30] and the macaque monkey [29].

### MATE CALLING TRIGGER CENTER

#### *Location in the APON*

Mate calling can be induced by electrical stimulation of the APON. In freely moving *Rana pipiens*, the effective sites for electrical evocation of calling were located in the rostral extreme of the APON [37]. Using isolated *pipiens* brain preparations in which neural correlates of mate calling can be recorded, the area most sensitive to electrical stimulation was localized in the ventral half of the APON [38]. The ventrolateral border of the preoptic gray was much more sensitive than the ventricular surface. Stimulation of the same locus was also effective in inducing electrical activity

correlating to mate calling in *in situ* perfused brains of Japanese toads (Fig. 2).

#### *Scanning Electron Microscopy of the Ventricular Surface*

In the anuran brain, the ventricular wall of the dorsal part of the APON is ciliated as are the surfaces of the ventricular walls in many other brain loci. However, the wall of the ventral part is only sparsely ciliated; instead, it is studded with numerous large bulbous protrusions which are large cytoplasmic extensions of ependymal cells and intraventricular dendritic end bulbs of secretory neurons [36, 39–41]. This area includes almost all the portions of the ventricular wall of the rostroventral part of the APON, and corresponds topographically to the area mentioned above that is most sensitive to the initiation of calling behavior by electrical stimulation.

#### *Steroid Hormone Accumulating Neurons*

The APON has been considered to be a sex steroid-sensitive center that triggers sexual behavior in anurans. Autoradiographic studies of the brains in *Xenopus laevis* [42, 43] and *Rana pipiens* [44] showed that many APON neurons can

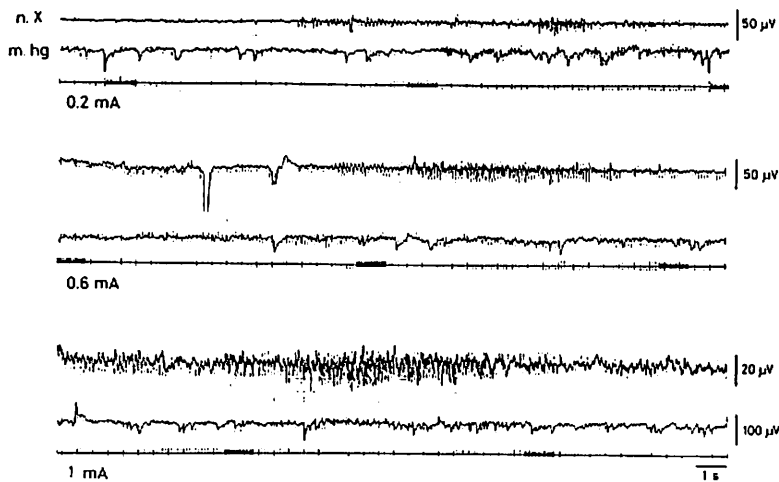


FIG. 2. Neural correlates of mate calling induced by electric stimulation (2 ms biphasic pulse, 50 Hz for 1 sec) of the APON through a fine bipolar electrode in a perfused toad brain. Electrical activity correlating mate calling was recorded at the root of the vagus nerve (n.X, upper trace) and the hyoglossal muscle (m. hg, lower trace). Magnitudes of the neural correlates increased when the intensity of stimulation was elevated from 0.2 mA to 0.6 and 1.0 mA.

accumulate sex steroid hormones. Such neurons were localized throughout the dorsal-ventral extent in the rostral part of the APON, but were found ventrally in the caudal part. This distributional pattern of sex steroid-accumulating APON neurons corresponds to the localization pattern of the ventricular bulbous protrusions. In mammals, the medial preoptic nucleus, which is the homologue of the APON, includes many sex steroid-accumulating neurons [45, 46].

Intracranial implantation of testosterone into or near the APON enhanced the incidence of mate calling which was evoked acoustically by play-back of tape-recorded conspecific mating calls in *Rana pipiens* [14]. In mammals, steroid hormones are reported to excite electrical activity of central neurons [47–50]. At present, it is not clear whether testosterone can modulate electrical activity of APON neurons in the amphibian brain due to the lack of any experimental data.

#### Projection of the APON to the Brain Stem Call Center

Mate calling behavior and its neural correlates could be evoked even after massive ablations or lesions to brain areas including almost all of the telencephalon, the dorsal thalamus, the torus semicircularis, the dorsal part of the isthmic nucleus and the infundibulum [38, 51]. These results indicate the presence of pathways at the ventro-lateral border of the central gray from the APON to the isthmo-trigeminal tegmentum.

The retrograde axonal transport study in which afferents to laryngeal motor neurons were traced in the brain of *Xenopus laevis* showed the presence of direct projection of APON neurons to the pre-trigeminal nucleus of the dorsal tegmental area (DTAM). DTAM neurons send efferent fibers to laryngeal motor neurons [52]. The projection from the APON to the DTAM is less flourishing in the female than in the male.

#### HUMORAL SIGNAL RECEPTIVITY

As is described above, the APON from which efferent fibers project to the brain stem call center is believed to be the androgen-sensitive center for male mate calling behavior. An electrophysio-

logical study of this locus in *Rana pipiens* showed the presence of neurons responsive to auditory stimulation by playback of conspecific mating calls, and injections of pituitary homogenate significantly increased the percentage of these units excited by the calls [53]. Activity of APON neurons thus can be modulated by humoral signals. A Golgi-electron microscopic study revealed that a portion of the APON neurons have the proper anatomical features for detecting humoral signals (Fig. 3) [36, 54].

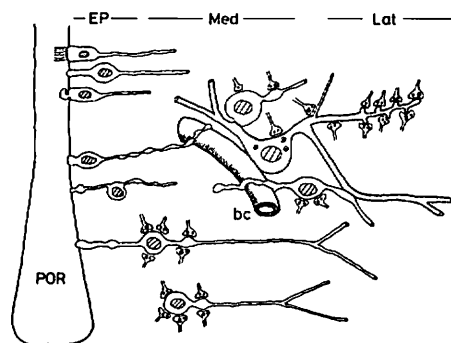


FIG. 3. Diagram showing CSF-contacting and BC-contacting neurons in the APON. These neurons have the proper morphological features to receive both humoral and neuronal synaptic, either chemical or electric, input signals. bc, blood capillary; EP, ependymal layer; Med, medial gray of the APON; Lat, lateral white of the APON; POR, preoptic recess. (from Urano and Ishihara [110])

#### Cerebrospinal Fluid (CSF)-Contacting Neurons

In many anuran species, the APON, especially its ventral half, contains CSF-contacting neurons [36, 39–41]. These neurons were rapid-Golgi stained and gold-toned, and then were examined by scanning electron microscopy after removal of surrounding tissues with hydrochloric acid and collagenase. The somata of stripped neurons bear debris of nerve terminals on their surfaces, suggesting that the neuronal activity of these cells can be modulated by various synaptic input signals.

Processes of CSF-contacting neurons, probably dendritic, protrude into the preoptic recess. The dendrites projecting into the preoptic recess from preoptic neurosecretory cells can be equipped to serve both secretory and sensory functions [55]. The CSF-ventricular system is thought to distri-

bute biologically-active hormonal substances within the brain, since many researchers have found various hormones in the CSF, such as LHRH and thyrotropin-releasing hormone (TRH) [56], oxytocin and vasopressin [57], and melatonin [58]. The concentrations of these hormones varied according to various physiological statuses. Further, single intraventricular injections of LHRH and TRH increased the amplitudes and frequency of electroencephalographic (EEG) activity recorded from the brain of a hibernating Japanese toad (Fig. 4) [59]. The effective dose of 1  $\mu$ g needed for enhancement of EEG activity through a single intraventricular injection of LHRH or TRH was much less than that needed for systemic injections. It is thus possible that the CSF-contacting neurons

whose dendritic processes protrude into the preoptic recess detect changes in ventricular hormonal status and motivate the neuronal circuitry in preparation for mating behavior in pre-breeding anurans.

#### Blood Capillary (BC)-Contacting Neurons

The presence of BC-contacting neurons is incompatible with the general concept of the relations between brain neurons and capillaries. Blood capillaries in the vertebrate brain are generally surrounded by astrocytic endfeet with an intervening basement membrane, so that brain neurons, even fish hypothalamic neurosecretory cells, are separated from the vascular endothelium [60]. Nonetheless, neurosecretory cells which directly come into contact with blood capillaries were shown in the toad preoptic nucleus [61]. Recently, it was found that a considerable number of peptidergic neurons come into contact with blood capillaries with only an intervening basement membrane in the APON of both the bullfrog and the Japanese toad [36].

BC-contacting neurons send their dendrites laterad toward the preoptic white matter. Although arborization is rather poor, the dendrites usually bifurcate several times and form dendritic fields. There, many axon terminals form synapses on the dendritic spines of these neurons. It is highly probable that APON neurons receive the input signals of the afferent fibers mainly through the dendritic synapses in the preoptic white matter along the border of APON cell mass, since Halpern [62] noted that terminal degeneration by the telencephalic lesions was located along the lateral edges of cell masses in the frog hypothalamus. The single BC-contacting neurons thus detect changes in titers of blood-born hormones, preferably sex steroid hormones which have activational effects on APON neurons, and further receive neuronal input signals through dendritic synapses to integrate hormonal and neural signals concerned with the initiation of sex behavior.

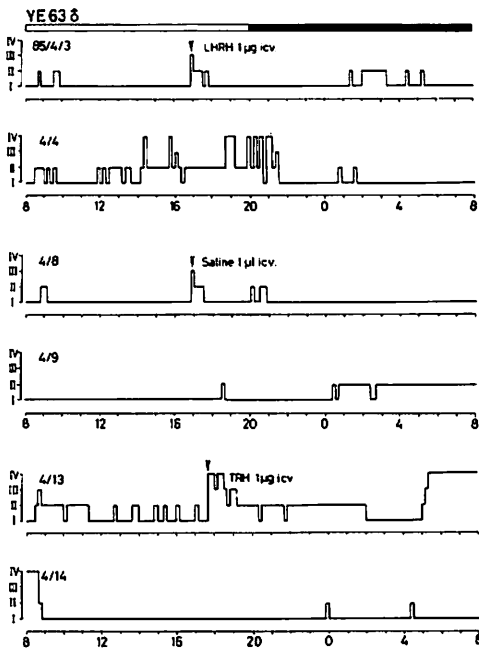


FIG. 4. Effects of intraventricularly injected LHRH and TRH on EEG activity of a hibernating Japanese toad. According to amplitudes and frequency, EEG activity was categorized into 4 levels: I, resting; II, awake; III, active; and IV, very active. Each level corresponds well to a behavioral state. Note that both LHRH and TRH induced dual fast and slow enhancements of EEG activity. Although the EEG record is not shown, the pattern of LHRH-induced fast response includes highly synchronized bursting waves that were not observed upon injection of control saline. (Fujita, thesis, Saitama University)

## AFFERENTS OF THE APON

### *Retrograde Horseradish Peroxidase (HRP) Study*

It is important to know what neuroanatomic afferent relations the APON has with other parts of the brain. Such information is requisite for better understanding of the sensory modalities and activating or inhibiting pathways that might trigger or modulate sexual behavior through the PON. Thus, the afferents of the APON were examined in *Rana pipiens* (Urano and Gorbman, unpublished) and *Bufo japonicus* [63] using the retrograde HRP method, which is a particularly useful tool in studies of neural connections.

Evidence of retrogradely transported enzymatic activity was observed in perikarya and neuropil in the following brain regions: the ventro-medial limbic cortex, the posterior part of the preoptic nucleus including the magnocellular part, the infundibular nuclei, the thalamic area, the subtectal and tegmental regions including the reticular formation, and the rhombencephalic central gray. Neurons in these regions appear to send their axons to the APON mainly *via* the medial and lateral forebrain bundles. Localization of some HRP-labeled perikarya and fibers coincides with that of immunoreactive perikarya and fibers containing either LHRH, vasotocin or TRH which have been considered to project to the APON [64, 65].

Particular HRP-labeled loci in the ventro-medial limbic cortex included the nucleus medialis septi, the nucleus lateralis septi, the nucleus accumbens septi, the amygdala pars medialis and the nucleus of the diagonal band of Broca. The amygdala-preoptic tract may exist in all vertebrate classes from cyclostomes to mammals [66]. The septal projection to the preoptic area in the leopard frog and the Japanese toad has an apparently homologous relationship to a similar pattern in the lizard [67] and the rat [68, 69]. Although in anurans, the physiological significance of amygdaloid and septal projections to the APON is not clear at present, it is possible that these projections are concerned with the control of sexual behavior as has been claimed in mammals [70, 71].

HRP-labeled structures in the subtectal and

tegmental regions were the nucleus anterodorsalis tegmenti mesencephali, the torus semicircularis, the nucleus posteroventralis tegmenti mesencephali, the nucleus isthmi and the mesencephalic reticular nuclei. Mesencephalic projections to the anterior hypothalamus are well known in amphibian brains [17, 19, 66, 72] as well as in other vertebrate classes [66, 73, 74]. The mammalian preoptic area is directly continuous with a vast nonspecific neuronal apparatus of the brain stem reticular formation [75]. In frog brains, the mesencephalic reticular system receives afferents from various parts of the brain, such as the telencephalon [62, 76], the optic tectum [77], and the superior olivary nucleus [78]. The presence of multimodal inputs suggests a nonspecific or generalized character of function of the anuran reticular formation as a possible activating or inhibitory regulatory system which may influence the neural substrate for mating behavior.

### *Chemical Neuroanatomy of the APON Afferents*

Information on the chemical nature of APON afferents is important for the examination of control mechanisms of APON neuronal activity at the cellular and molecular levels.

The HRP study mentioned above showed the presence of HRP-labeled neurons in the magnocellular part of the PON, and in the nuclei infundibularis dorsalis and ventralis in the toad brain. These regions are rich in vasotocinergic and mesotocinergic neurosecretory neurons [22], and TRH neurons [65, 79], respectively. Jokura and Urano [64] verified that varicose ir-vasotocin fibers are found in the ventrolateral region of the APON where the APON neurons have their dendritic fields. Some ir-vasotocin fibers from the *vmc* protrude into the APON cell mass, and appeared to come into contact with somata of APON neurons. In Japanese toads, ir-TRH neurons were localized mainly in the nucleus infundibularis ventralis (NIV) [65]. Ir-TRH fibers arising from the NIV neurons project to the median eminence to form the hypothalamo-hypophysial tract. In addition, a considerable number of ir-TRH fibers innervate into the APON. In the APON, varicose ir-TRH fibers are scattered widely among the neuronal cell mass and the white matter.

Other important loci in the toad brain where HRP-labeled neurons were found include the nucleus medialis septi and the nucleus of the diagonal band of Broca. These loci contained many ir-LHRH neurons which project to the APON [64]. Most ir-LHRH fibers emanating from the nucleus medialis septi form a loose fiber bundle with those arising from the diagonal band of Broca. These ir-LHRH fibers, which have typical beaded features, project to the ventrolateral border of the preoptic gray.

In mammalian brains, peptidergic axonic processes form ordinary synapses [80] and en passant synapses with dendritic profiles [81]. Therefore, it is highly probable that varicose ir-vasotocin, ir-TRH and ir-LHRH fibers form ordinary or en passant synapses in the dendritic fields of APON neuron in the toad brain.

#### *Functional Significance of the APON Afferents*

The retrograde HRP study indicates that there are multimodal inputs to the APON from various regions of the brain. The septal nuclei, which send ir-LHRH fibers to the APON, receive olfactory inputs through the medial olfactory tract [82, 83], and the amygdala is innervated by projections from the accessory olfactory bulb [84]. These limbic nuclei, from which afferents to the APON arise, may relay olfactory signals to the APON neurons. In addition, the terminal nerve, which may function in odor processing, sends an ir-LHRH-ergic projection to the preoptic region in the tiger salamander and the bullfrog [85].

Visual cues can be conveyed through direct retinal projection to the suprachiasmatic part of the PON. This was clarified in the Japanese toad by use of a cobaltic lysine method (Shimotoso and Urano, unpublished). The presence of direct retino-preoptic projection has also been supposed in the brain of *Rana temporaria* [25]. Acoustic signals which excite APON neurons may reach the preoptic region through at least two ascending pathways in the brain stem [78]. One is the pathway relayed through the nucleus olivae superior and the nucleus profundus mesencephali; the other is that relayed through the nucleus olivae superior and the torus semicircularis. The thalamo-preoptic connection is a possible pathway for

transmission of tactile signals. Thus, the APON neurons may be influenced by various kinds of sensory inputs, although almost all sensory modalities are relayed and may be regulated either by sex steroid hormones or by neurohormones released from extrahypothalamic terminals of neurosecretory neurons [86, 87]. Since the electrical activity of many APON units was excited by iontophoretically applied LHRH, TRH and vasotocin (Fujita and Urano, in preparation), the APON neurons probably integrate various sensory inputs under the influence of peptidergic neurosecretory neurons, and then generate neural signals for the initiation of mate calling behavior.

#### SEASONAL VARIATIONS

Many anurans, especially those in the temperate zone, are typical seasonal breeders which spawn in spring or early summer. The neuroendocrine systems associated with reproductive behavior also show seasonal changes in their synthetic and secretory activities. In bullfrogs, the plasma level of luteinizing hormone (LH), which can increase androgen secretion from the testes [88], was elevated during the breeding season [89]. In the *Xenopus* hypothalamus, the contents of LHRH, which can stimulate pituitary gonadotropin release in bullfrogs [90] and plasma androgen levels in Japanese toads [91], varied seasonally in correspondence to reproductive physiological states [92]. Ishii and his collaborators measured circannual changes in plasma levels of various pituitary hormones [93, 94], thyroid hormones [95], adrenal steroids [96, 97] and sex steroids [98]. Most of these hormones showed marked increases in their plasma titers prior to or during the breeding season. Further, classical histochemical and morphometric studies showed seasonal morphological changes in the hypothalamo-neurohypophysial neurosecretory system in *Rana temporaria* [99, 100]. The results of these studies suggest the possibility that the APON neurons do show some seasonal changes in their morphological and functional features, since the activity of APON neurons was modulated by administrations of testosterone [14] and pituitary homogenates [53].



### *Seasonal Changes in the Volumes of PON Subnuclei*

Seasonal variations in the volumes of several preoptic and amygdala subnuclei were found between hibernating and postbreeding Japanese toads [34]. The APON in the hibernating males was 125% larger than that in the post-breeding animals. The seasonal difference in the female APON was not statistically significant. In both sexes, the hibernating animals had larger ventral magnocellular parts of the PON, amygdala medialis and amygdala lateralis than the post-breeding animals did. The seasonal variation in the volumes of several subnuclei mentioned above may be due to hypertrophy of the neurons in these loci, since cell nuclear volumes of PON neurons increase prior to the breeding season [100]. Morphological changes in the APON and the amygdala thus precede physiological and behavioral changes in the breeding season. A similar result was observed in the song control nucleus in the brain of the male canary which is larger during breeding season than in the fall when the animal is sexually inactive [101], probably because sex steroids induce dendritic growth in this nucleus [102].

### *Immunoreactivity of Neuroendocrine Cells*

It is highly probable that neuronal input signals to APON neurons differ seasonally. Therefore, seasonal variations in LHRH, TRH and vasotocin that were localized in varicose afferent fibers to the APON were examined immunohistochemically in toad forebrains and neurohypophyses [65, 103]. The immunohistochemical technique utilized was the avidin-biotin-peroxidase complex (ABC) method, which is superior to the peroxidase-antiperoxidase method in a quantitative study. LHRH immunoreactivity (ir) was strong in both perikarya and fibers in animals captured in spring and autumn, while in summer animals, LHRH-ir was weak. Seasonal changes in TRH-ir were similar to those in LHRH-ir, while significant seasonal variations were not found in vasotocin-ir. The circannual changes in LHRH-ir appear to correspond with seasonal variations in plasma steroid levels reported by Inoue *et al.* [98]. This coincidence implies that LHRH and sex steroids

can have synergistic effects on the control of APON neurons.

### *Effects of Castration*

As is described above, testosterone may modulate neural activity of the APON to initiate reproductive behavior. Structures of the nervous system are modified by sex steroids during both fetal and adult periods in many vertebrate species [101, 102, 104]. In Japanese toads, the volumes of the APON and the amygdala in the male are larger than those in the female. Furthermore, the volumes of these nuclei and LHRH-ir also changed seasonally. These changes appear to correlate with the annual variation of plasma testosterone levels. Castration experiments, in which the role of testosterone in the control of the phenomena mentioned above was examined, showed that the effects of castration differ seasonally [105]. The volume of the amygdala medialis in autumn toads was significantly reduced by castration; however, the reduction in spring animals was not statistically significant. Meanwhile, castration did not modify LHRH-ir in the median eminence in either spring or autumn toads, although dense ir-LHRH fibers were observed in the mesencephalic tegmental region in the castrated spring toads but not in the autumn toads either intact or castrated. These results suggest that seasonal influences on the effects of castration were not uniform among the different brain loci.

## COORDINATION OF NEURAL AND ENDOCRINE ACTIVITY

Temporal coordination of neural and endocrine events is a crucial requisite for successful reproduction. Plausible candidates for coordinating the brain and endocrine functions are LHRH-ergic and vasotocinergic neurosecretory systems, both of which send fine varicose fibers to various extrahypothalamic brain loci other than the median eminence and the pars nervosa [86, 87]. Both LHRH-ergic and vasotocinergic fibers innervate either sensory or motor centers concerned with reproductive behavior.

LHRH applied by microiontophoresis increased discharge rates of individual neurons in the septal

preoptic area of the rat [106], and in the APON of the Japanese toad (Fujita and Urano, in preparation). An intracellular study showed that LHRH can mimic slow excitatory postsynaptic potentials when applied to the sympathetic neurons in the bullfrog [107]. LHRH at the synaptic level may play a role in increasing neuronal excitability in the loci where LHRH fibers innervate. On the other hand, LHRH applied systemically or intraventricularly can stimulate the pituitary-gonadal axis to elevate plasma androgen levels in male Japanese toads (Fig. 5). This evidence suggests that LHRH simultaneously affects both the neuronal activity of the APON neurons as an excitatory neurotransmitter or neuromodulator and the endocrine events of the pituitary-gonadal axis as a hypothalamic releasing hormone.

The endocrine functions of vasotocin in amphib-

ians are well documented in many endocrine textbooks. In addition, vasotocin and its homologues can excite unit-spike activity of neurons in the rat supraoptic and paraventricular nuclei [106, 108], the eel preoptic nucleus [109] and the toad APON (Fujita and Urano, in preparation). Vasotocin thus may facilitate the activity of many central neurons as a neuromodulator or a local hormone. The latter possibility is supported by the fact that  $10^{-9}$ M vasopressin, comparable to the effective dose of vasopressin necessary for peripheral targets, can excite rat paraventricular neurons [108].

At present, it is difficult to account for the temporal discrepancy between LHRH-induced neural events (Fig. 4) and endocrine events (Fig. 5). When LHRH functions as a neurotransmitter or a neuromodulator, its influence on target

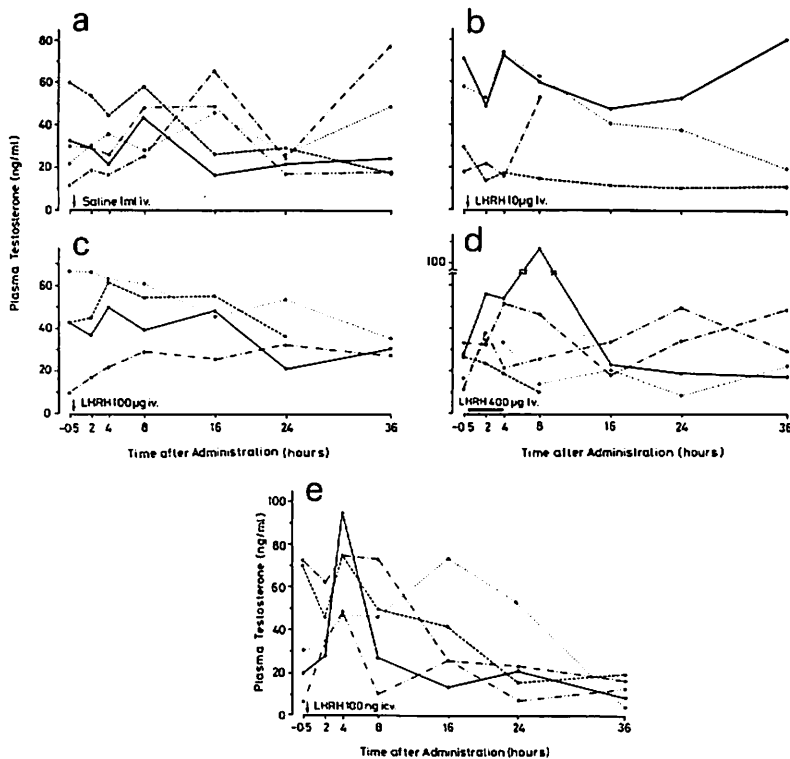


FIG. 5. Changes in the plasma testosterone levels after administrations of intravenous and intraventricular LHRH. Each curve represents a change in testosterone levels in an individual male toad. a, effects of intravenous saline as a control; b, single intravenous injection of  $10 \mu\text{g}$  LHRH; c, single intravenous injection of  $100 \mu\text{g}$  LHRH; d, continuous infusion of LHRH at a dose of  $100 \mu\text{g}/\text{hour}$  for 4 hours; and e, intracerebroventricular administration of  $100 \text{ ng}$  LHRH. Note that the dose of intracranial LHRH which markedly elevated plasma testosterone was much less than that of intravenous LHRH. (Fujita, thesis, Saitama University)

neurons lasted for within the order of seconds or minutes. However, endocrine events, e.g., the secretion of androgen, take a much longer time. Since the APON neurons are sex steroid-sensitive and are excited by LHRH, some unknown intrinsic cellular mechanisms within the APON neurons and neurons having the same characteristics may regulate the above temporal discrepancy in order to complete seasonal breeding successfully.

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