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Sexual Dimorphism of Gonadotropin-releasing Hormone Type-III (GnRH3) Neurons and Hormonal Sex Reversal of Male Reproductive Behavior in Mozambique Tilapia

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In tilapia, hormone treatment during the period of sexual differentiation can alter the phenotype of the gonads, indicating that endocrine factors can cause gonadal sex reversal. However, the endocrine mechanism underlying sex reversal of reproductive behaviors remains unsolved. In the present study, we detected sexual dimorphism of gonadotropin-releasing hormone type III (GnRH3) neurons in Mozambique tilapia *Oreochromis mossambicus*. Our immunohistochemical observations showed sex differences in the number of GnRH3 immunoreactive neurons in mature tilapia; males had a greater number of GnRH3 neurons in the terminal ganglion than females. Treatment with androgen (11-ketotestosterone (11-KT) or methyltestosterone), but not that with 17β-estradiol, increased the number of GnRH3 neurons in females to a level similar to that in males. Furthermore, male-specific nest-building behavior was induced in 70% of females treated with 11-KT within two weeks after the onset of the treatment. These results indicate androgen-dependent regulation of GnRH3 neurons and nest-building behavior, suggesting that GnRH3 is importantly involved in sex reversal of male-specific reproductive behavior.

Key words: sex reversal, GnRH, behavior, brain, androgen, sexual dimorphism, sex-changing fish

INTRODUCTION

Gonadal steroid hormones are key regulators of reproduction and differentiation of secondary sexual characteristics including sexual dimorphism of the brain across vertebrate animals. It is well known in rodents that sexual differentiation of the brain area related to the reproduction is induced by gonadal steroids during a single sensitive perinatal period known as the 'critical period', and that the sexually differentiated neural circuits lose their plasticity after this period. On the other hand, certain fish species undergo complete sex reversal during their life. Some can naturally change sex from female to male or from male to female; others can change their sex by an impact from social environments or artificial hormonal treatment even after initial maturation. In those fishes changing gonadal sex as well as behavioral one, neural circuits involved in sex difference are considered to maintain its plasticity even in adult stages (Munakata and Kobayashi, 2010). However, it is yet to be elucidated how the sex reversal takes place in the brain.

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Tilapias, commercially important fish species in aquaculture, are often subjected to artificial sex reversal during their early life stages. Because male fish grow faster than females, sex hormone administration has been adopted for production of monosex (all male) tilapia. Clemens and Inslee (1968) produced all male populations of Mozambique tilapia (*Oreochromis mossambicus*) larvae by adding 17α-methyltestosterone (MT), a synthetic androgen, into the diet. Adrenosterone, a steroid with androgenic effects, was administrated to fertilized eggs of Nile tilapia (*Oreochromis niloticus*) to induce sex reversal from female to male (Katz et al., 1976). Nakamura et al. (1998) also masculinized Mozambique tilapia larvae by feeding them 11-ketotestosterone (11-KT), a major androgen in teleosts. Complete feminization of genetically-male Mozambique tilapia was also induced by oral administration of ethinylestraiol during gonadal sex differentiation (Nakamura and Takahashi, 1973). Hormone treatment during the period of sexual differentiation does not alter the genotype, but the phenotype of the gonads (ovary or testis) as well as secondary sexual characteristics and sexual behaviors. After maturation, Mozambique tilapia, for example, shows sex-specific reproductive behaviors such as male-specific nest-building and female-specific mouth breeding. A mature male establishes a territory and builds a round nest (30–60 cm in diameter, depending on the size of the male), where spawning occurs.

Females treated with androgen during sexual differentiation become to display nest-building behavior when they mature. The mechanisms underlying sex differentiation and hormone-induced sex reversal of gonads during early development have been well studied in tilapia (Kobayashi et al., 2008; Ijiri et al., 2008; Wang et al., 2007, 2010). In contrast, the mechanisms of sex differentiation and sex reversal of the brain area controlling reproductive behaviors remain still unclear.

Previous studies on sex reversal of reproductive behaviors in fishes have mainly examined neuropeptides including gonadotropin-releasing hormone (GnRH) in sex-changing fishes (Grober and Bass, 1991; reviewed in Foran and Bass, 1999; Goodson and Bass, 2001), because neuropeptides are considered as key regulators of reproductive function and behavior in fishes, as is the case in tetrapod vertebrates. Advanced teleosts of tilapia have three GnRH subtypes; that is, seabream GnRH (GnRH type I, GnRH1), chicken GnRH (GnRH type II, GnRH2) and salmon GnRH (GnRH type III, GnRH3) (White et al., 1995; Fernald and White, 1999; Parhar, 1996), which are expressed in the preoptic area (or hypothalamus), midbrain, and terminal ganglion, respectively. The different localization of these three subtypes indicates that different GnRH subtypes may exert different functions. Among three GnRHs, Ogawa et al. (2006) showed that injection of GnRH3 antiserum into the intracerebroventricular region of male Nile tilapia significantly suppressed nest-building and aggressive behaviors specific to males, suggesting the involvement of GnRH3 in induction of male-specific behaviors.

In the present study, we aimed to reveal possible sexual dimorphism of GnRH3 neurons in the brain and hormonal sex reversal of male reproductive behavior in Mozambique tilapia. We first examined sexual dimorphism in the number of GnRH3 neurons in the brain. Next, we studied effects of androgen on the number of GnRH3 neurons in females. Finally, we investigated whether androgen treatment could induce male-specific nest-building behavior in females. Our findings clearly indicated androgen-dependent regulation of GnRH3 neurons and male-specific reproductive behavior in Mozambique tilapia.

MATERIALS AND METHODS

Fish and hormone treatments

Experiments were conducted according to the principles and procedures approved by the Institutional Animal Care and Use Committee of the University of Tokyo. Mozambique tilapia *Oreochromis mossambicus* were maintained in a 3000-L tank supplied with recirculating fresh water at 25°C. Sexually mature male and female fish of almost equal size (approximately 30 g in body weight (BW) and 15 cm in standard length) were used in the present study. As the intact controls, six males and six females were anesthetized with 0.1% 2-phenoxyethanol, and transcardially perfused with saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Brains were then removed and further fixed in the same fixative overnight. Three females were injected intraperitoneally with 17β-estradiol (E2, 5 μg/g BW), 11-ketotestosterone (11-KT, 5 μg/g BW), or methyltestosterone (MT, 5 μg/g BW). All sex steroid hormones (Sigma Aldrich Chemical, St. Louis, MO) used in the present study were disolved in sesame oil at the concentration of 1.0 mg/ml. As sham-injected controls, three females and three males were injected with sesame oil alone. After injections, fish were kept individually in observation tanks (50-L glass tanks) for 4 days. Thereafter, the brains were fixed in the same manner as described above. The sexual maturity was confirmed by the macroscopic and histological analysis of ovaries and testes.

GnRH3 immunohistochemistry

Fixed brains were washed in 0.1 M phosphate-buffered saline (PBS), and then immersed in 0.1 M PB containing 20% sucrose overnight. Serial frontal sections of 20 μm thickness were cut on a cryostat (Leica CM1100, Wetzlar, Germany), thaw-mounted onto polysilane-coated MS slides (Matsunami Glass, Osaka, Japan), and stored at –80°C. The cryosections were first incubated with PBS containing 10% Block Ace (Dai-Nihon Seiyaku, Japan) at room temperature for 30 min to block nonspecific binding. After a brief wash with PBS, they were incubated overnight at room temperature with a primary antibody against GnRH3 (Okuzawa et al., 1990) diluted 1:10,000 with 10% Block Ace in PBS. After rinsing in PBS, sections were incubated for 2 h at room temperature with Alexa Fluor 488-conjugated goat anti-rabbit IgG (Invitrogen, Eugene, OR) diluted 1:500 with 10% Block Ace in PBS, followed by nuclear staining with Hoechst 33258 (Sigma-Aldrich). Finally, after rinsing several times in PBS, coverslips were mounted on slides with an antifade reagent (FluoroGuard, Bio-Rad Laboratories, Hercules, CA).

Stained sections were observed under a confocal laser scanning microscope system (LSM5 Pascal, Zeiss, Oberkochen, Germany). The wavelengths of excitation and recorded emission for fluorescent dyes were as follows: Alexa Fluor 488, 488 nm and 515/30 nm; and Hoechst 33258, 352 nm and 461 nm. Quantitative analyses of GnRH3-immunoreactive neurons observed in the brain through serial sections were conducted with the aid of the NIH image. The number of GnRH3 neurons was counted, and the soma size of GnRH3 neurons was determined by measuring the long diameter of the soma with a nucleus. As a negative control to confirm the specificity of the immunoreaction, the sections were processed without the incubation with the primary antibody, or the sections were incubated with normal rabbit IgG in place of the specific antibody.

Hormone treatment and behavior observation

Thirty mature females were injected with 11-KT (5 μg/g BW) in the same manner as described above, whereas four males and four females were injected with sesame oil as controls. After the first injection with 11-KT or sesame oil, fish was kept separately in 50-L glass tanks and observed for nest-building behavior for one week. Then, those fish received the second injection of the same dose of 11-KT. After the first nest was destroyed, the fish were returned to the same tank, and checked for nest-building behavior for another one week. Nest-building behavior was observed according to the previously described method (Uchida et al., 2005; Ogawa et al., 2006). Briefly, a gravel bed (> 3 cm thick) was laid in the tanks, allowing the fish to make a nest. During nest building, the fish picked up a large amount of gravels with the mouth and deposited them away from the cleaned surface, resulting in a clear depression in the gravel substrate. The nest was generally circular, and the glass bottom of the tank usually became visible.

Measurement of hormone concentration in the serum

After the observation of nest-building behavior for two weeks, fish were anesthetized with 0.1% 2-phenoxyethanol and weighed. Then, the blood was collected from the caudal vessels with a heparinized syringe and needle. The blood was centrifuged to obtain plasma, which was stored at -20°C. Later, plasma concentrations of 11-KT and E2 were measured by using EIA kits (Cayman Chemical, Ann Arbor, MI), according to the manufacture's protocol.

Observation of GnRH3-immunoreactive cells and ovaries after behavioral test

After the observation of nest-building and blood sampling, fish

were transcardially perfused with saline, followed by 4% paraformaldehyde in 0.1 M PB. Brains and ovaries were then removed and immersed in the same fixative overnight. Serial frontal sections of the brains were cut at 20 μm thickness on a cryostat, processed for GnRH3 immunohistochemistry, and observed under a confocal laser scanning microscope, as described above.

After being weighed, ovaries were dehydrated in a graded ethanol series, cleared in xylene and embedded in paraffin. Sections were cut at 10 μm thickness and mounted on egg albumin-coated slides. After the paraffin was removed with xylene, sections were stained with hematoxylin and eosin, and observed under a light microscope.

Statistical analysis

Significant differences in the number as well as size of GnRH cells and the concentration of plasma E2 or 11-KT were determined by one-way ANOVA followed by the post-hoc Newman-Keuls multiple comparison test. Significant differences in nest-building behavior between 11-KT- and oil-injected females were statistically tested using Fisher's exact probability test.

RESULTS

Sexual dimorphism in GnRH3 neurons

The localization of GnRH3 neurons was immunohistochemically examined on sections of female and male brains from the olfactory bulb to the end of the midbrain. GnRH3 neurons were evidently localized in the terminal nerve ganglia (nucleus olfactoretinalis) of both sexes (Fig. 1A–D), and there were prominent sex differences in the incidence of these neurons. Only a few GnRH3 neurons were detected in females, whereas the GnRH3 neurons in males were more numerous than in females (Fig. 1C). The total number of GnRH3 neurons in this brain region was significantly greater in males than in females ($P < 0.001$, Fig. 1E).

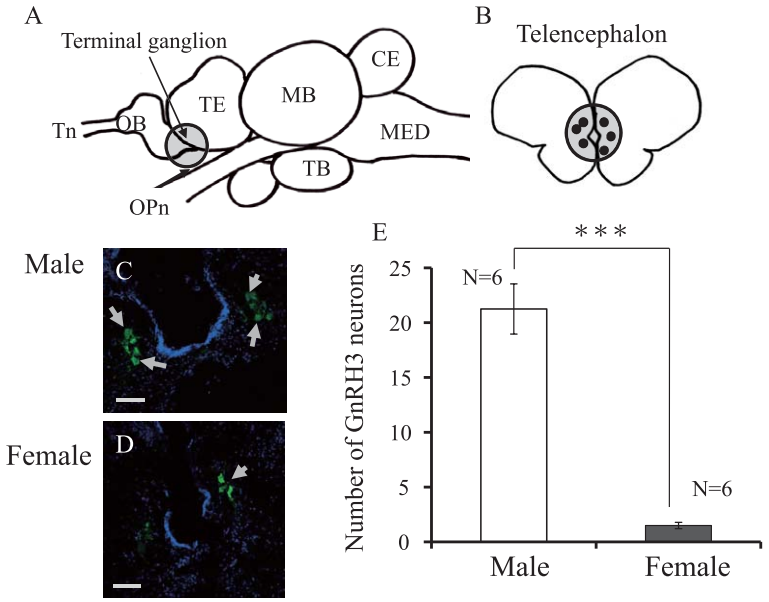

Fig. 1. Localization of GnRH3 neurons in the terminal ganglion of Mozambique tilapia and sexual dimorphism of GnRH3 neurons. **(A)** Schematic lateral view of tilapia brain, indicating the position of the terminal ganglion. **(B)** A schematic representation of GnRH3 neurons in a cross section of the terminal ganglion. **(C)** and **(D)** Representative micrographs of GnRH3 neurons (green, arrows) in male (C) and female (D) tilapia. Nuclei were stained with Hoechst 33258 in blue. Scale bar: 50 μm. **(E)** Difference in the number of GnRH3 neurons in the terminal nerve ganglion between sexes. White and gray columns represent male and female, respectively. Bars represent the mean ± SD for each object. ***, $P < 0.001$ (F).

GnRH3-immunoreactive neurons in fish treated with sex steroids

In order to examine the influence of sex steroids on the sexual dimorphism of GnRH3 neurons, females were injected intraperitoneally with either E2, 11-KT or MT. The localization of GnRH3 neurons in the terminal nerve ganglia remained unchanged (Fig. 2A). However, the total number of GnRH3 neurons in females was significantly increased by the treatment with 11-KT or MT ($P < 0.001$), compared to that of control or E2-injected females. The total number of GnRH3 neurons in females treated with 11-KT or MT was elevated to a similar level of control males (Fig. 2B). The average size of GnRH3 neurons was signifi-

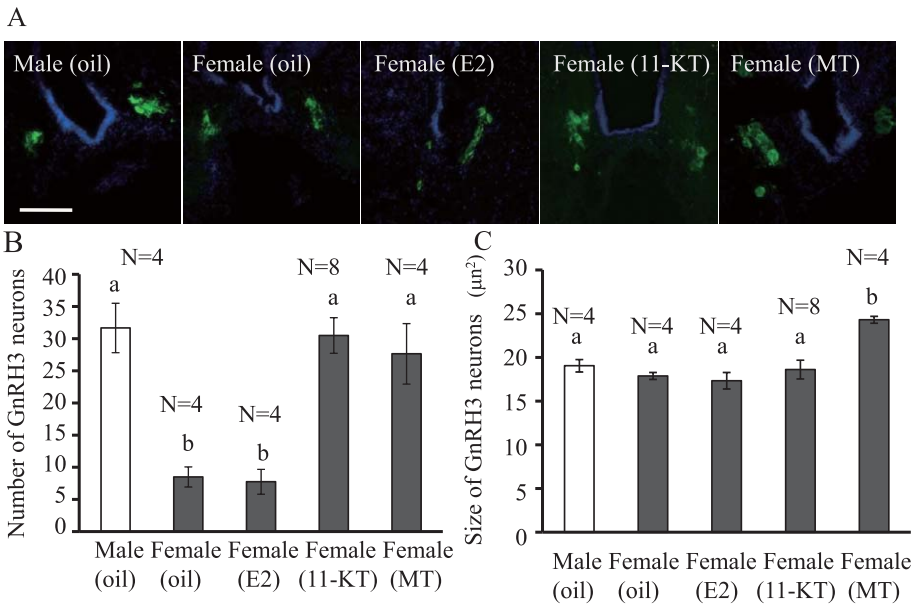

Fig. 2. Effects of sex steroid hormones on GnRH neurons in female Mozambique tilapia. **(A)** Representative micrographs of GnRH3 neurons (green) in the terminal nerve ganglion of control (oil-injected) male and females, and 17β-estradiol (E2)-, 11-ketotestosterone (11-KT)- and methyltestosterone (MT)-injected females. Nuclei were stained with Hoechst 33258 in blue. Fish were fixed four days after hormone treatment. Scale bar: 100 μm. **(B)** Hormonal effects on the number of GnRH3 neurons. Different letters indicate significant differences at $P < 0.001$. **(C)** Hormonal effects on the size of GnRH3 neurons. (B) and (C) White and gray columns represent male and female, respectively. Bars represent the mean ± SD for each object. Different letters indicate significant differences at $P < 0.05$.

cantly increased only after MT treatment ($P < 0.05$, Fig. 2C), whereas no significant effects of 11-KT and E2 on cell size were observed.

Hormone treatment and male-specific nest-building behavior

Nest-building is a well known male-specific reproductive behavior in tilapia. We next examined whether 11-KT could induce male-specific nest-building behavior in female mature tilapia. We checked for nest-building behaviors of females treated with 11-KT (n = 30) or oil (n = 4) for two weeks. All control males (n = 4) dug a nest during the first and second weeks after oil treatment, whereas no control females built a nest during the period of two weeks (Table 1). Among 30 females treated with 11-KT, 5 females (16.7%) showed nest-building behaviors in both first and second weeks, and 16 females (53.3%) dug a nest in either first or second week: some dig a nest only in the first week and the others did only in the second week (Table 1). On the other hand, nine females (30.0%) failed to build a nest for two weeks. Thus, 70% of females treated with 11-KT displayed one or two nest-building behaviors during the experimental period of two weeks. The occurrence of nest-building behavior was statistically different between 11-KT- and oil-treated females ($P < 0.05$, Fisher's exact probability test).

Figure 3 shows the mean plasma concentrations of 11-KT and E2 in control females and those treated with 11-KT or oil. Plasma 11-KT concentration was 41.7 ± 4.6 ng/ml in control males, while it was 0.06 ± 0.02 and 0.82 ± 0.18 ng/ml in control females and 11-KT-treated females, respectively (Fig. 3A). Plasma 11-KT concentration was significantly higher in 11-KT-treated females than in control females ($P < 0.05$), though this was still lower than that in control males ($P < 0.001$).

In some fishes, androgen treatments have been shown to induce female-to-male sex change of the matured gonad (Kroon and Liley, 2000; Yeh et al., 2003). In order to examine the possibility of ovary-mediated effects, we investigated plasma levels of E2 and the microscopic structure of the ovaries two weeks after the first injection. Plasma E2 concentration was not significantly different between 11-KT-treated and control females, whereas E2 concentration in control males was significantly lower than those in control and 11-KT-treated females (Fig. 3B, $P < 0.001$). Histological observation showed that ovaries contained numerous vitellogenic oocytes ready for final maturation in both control and 11-KT-treated females (Fig. 3C, D).

Finally, we investigated the number of GnRH3 neurons in the terminal ganglion of control males, control females and 11-KT-treated females. After two injections of 11-KT, the number of GnRH3 neurons was significantly increased in 11-

Table 1. Nest-building behavior performed by controls and females treated with 11-KT.

	Nest-building		n	%
	First week	Second week		
Control males (n = 4)	+	+	4	100
Control females (n = 4)	–	–	0	0
Females	+	+	5	16.7
treated with 11-KT (n = 30)	+	–	10	33.3
	–	+	6	20
	–	–	9	30
	total		30	100

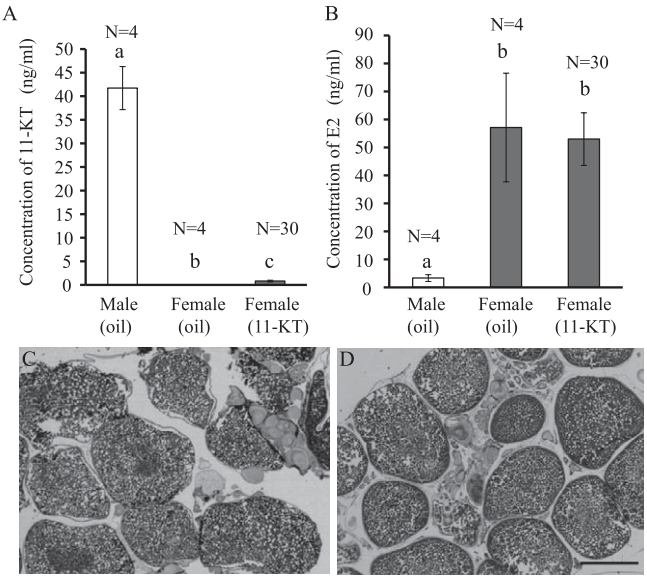

Fig. 3. Concentrations of 11-KT and E2. **(A)** Concentrations of plasma 11-KT in control males, control females and 11-KT-treated females. a vs b, a vs c, $P < 0.001$; b vs c, $P < 0.05$. White and gray columns represent male and female, respectively. Bars represent the mean ± SD for each object. **(B)** Concentrations of plasma E2 in control males, control females and 11-KT-treated females. a vs b, $P < 0.001$. C, D; Representative micrographs of vitellogenic oocytes in the ovaries of control **(C)** and 11-KT-treated **(D)** females. Sections were stained with hematoxylin and eosin. Scale bar: 100 μm.

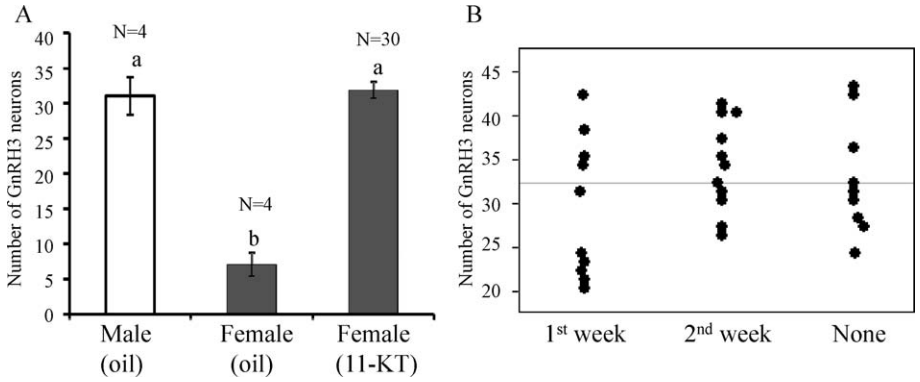

Fig. 4. Effects of 11-KT on GnRH3 neurons in two weeks after the onset of the treatment. **(A)** Effects of 11-KT on the number of GnRH3 neurons in the terminal nerve ganglion. White and gray columns represent male and female, respectively. Bars represent the mean ± SD for each object. Different letters indicate significant differences at $P < 0.001$. **(B)** The number of GnRH3 neurons in fish in the following three groups of 11-KT-injected females: females which dug a nest in the first week, those which dig a nest in the second week, and those which did not dig any nest in the first and second weeks.

KT-treated females compared to control females, and reached a level comparable to control males (Fig. 4A). To further examine the difference in the number of GnRH3 neurons between 11-KT-treated females that built a nest and those that did not, we compared the numbers of GnRH3 neurons among females classified into three groups; females which dug a nest in the first week, those which dug a nest in the second week, and those which did not dig any nest in the first and second weeks (Fig. 4B). There was no difference in the number of GnRH3 neurons among the three groups; the number ranged from 20 to 45 neurons, regardless of whether they displayed a nest-building behavior or not (Fig. 4B).

DISCUSSION

The present study is the first to reveal the sexual dimorphism in the number of GnRH3 neurons in the terminal ganglion of sexually matured Mozambique tilapia. Androgen (11-KT and MT) treatment to females increased GnRH3 neurons and cancelled the sexual difference. Furthermore, 50% of 11-KT-treated females showed a nest-building behavior, a male-specific reproductive behavior, within a week after the onset of 11-KT treatment, and another 20% came to dig a nest within a week after the second treatment. Therefore, we succeeded in inducing a male-specific nest-building behavior in 70% of matured females within two weeks after the onset of 11-KT treatment.

In tilapia, an array of previous studies have shown that endogenous estrogens act as natural inducers of ovarian differentiation, whereas doublesex and mab-3 related transcription factor 1 (Dmrt1), which is required for normal activation of androgen receptor in Sertoli cells, may be important in testicular differentiation (Wang et al., 2010). Contrary to the sex determination of the gonads in tilapia, to our knowledge, there are as yet no reports addressing sex reversal of the brain in mature tilapia. This study clearly indicated two sex reversal phenomena of the brain induced by 11-KT in mature female tilapia. One is the cancellation of sexual difference in GnRH3 neurons, and the other is the induction of a male-specific nest-building behavior in females. The relation between these two effects of 11-KT was not directly shown in the present study. By administrating anti-GnRH3 antibody into the intracerebroventricular region, Ogawa et al. (2006) demonstrated that immunoneutralization of GnRH3 inhibited nest-building and aggressive behaviors in tilapia, suggesting that GnRH3 is a potent neuromodulator of male reproductive behavior. Taken together, our results suggested that 11-KT induced the increase of GnRH3 neurons, which in turn might cause a nest-building behavior in females. The widespread distribution of GnRH3 fibers in the tilapia brain also suggests its possible neuromodulatory function (Soga et al., 2005). Koyama et al. (1984) and Kyle and Peter (1990) have shown that brain regions rich in GnRH3 fibers are important in male reproductive behavior in goldfish, supporting the involvement of GnRH3 neurons in male reproductive behavior. However, as shown in Fig. 4B, the number of GnRH3 neurons did not directly determine whether 11-KT-treated female dug a nest or not. It could be possible that the GnRH3 peptide was not secreted from neurons in the females that did not show the male-specific behavior. On the other hand, there may be other factors affecting the male-specific behavior, since the nest-building behavior has been shown to be affected by environmental factors (e.g., water quality) through the olfactory system and/or other GnRH (GnRH1 and GnRH2) systems (Uchida et al., 2005). Although we did not identify any factor except for 11-KT, our present study supported a neuromodulatory role of GnRH3 in nest-building behavior, and provided the idea that GnRH3 plays an important role in androgen-induced sex reversal of nest-building behavior in females.

Changes in the number of GnRH3 neurons and nest-building behavior raise a question about a role of the ovary and its endocrine products in regulating those processes. Histological observation showed no distinct difference in the ovaries between control and 11-KT-treated females. Both ovaries contained numerous vitellogenic oocytes ready for final maturation (Fig. 3C, D). Furthermore, plasma E2 levels in 11-KT-treated females were also similar to those of control ones (Fig. 3A, B). These results suggested that the effects of 11-KT on the number of GnRH3 neurons and nest-building behavior were not mediated by the change in ovaries.

The present study demonstrated that androgens (11-KT and MT) increased the number of GnRH3 neurons in females. In Nile tilapia, in situ hybridization showed that mRNA expression levels of GnRH3 in the terminal nerve ganglion were significantly elevated in testosterone-treated males (Soga et al., 1998). In other teleosts species, it has been shown that exposure to testicular androgens leads to an increase in GnRH3 mRNA in male masu salmon *Oncorhynchus masou* (Amano et al., 1994). However, cloning and functional analysis of promoter regions of three GnRH genes in Nile tilapia showed the occurrence of putative binding sites for glucocorticoid receptors, thyroid hormone receptors, retinoid X receptors, and some other transcription factors (Kitahashi et al., 2005). It was somewhat surprising; however, that the GnRH3 promoter sequence lacked putative binding sites for androgen receptors (ARs), estrogen receptors (ERs), or progesterone receptors (Kitahashi et al., 2005). The absence of AR and ER binding sites in the GnRH3 promoter sequence suggests that androgens regulate GnRH3 genes indirectly through androgen-responsive neurons, as is the case in rodents (Zwain et al., 2002). On the other hand, localizing ARs in another species of tilapia *Astatotilapia burtoni* by in situ hybridization, Harbott et al. (2007) have shown that GnRH1 neurons express ARs. Although they did not examine the colocalization of GnRH3 and ARs, they showed that some cells in the terminal nerve ganglion expressed AR mRNAs. Thus, it can not be ruled out the possibility that 11-KT directly influences GnRH3 neurons.

Neuropeptides including GnRH are considered to be key regulators of reproductive functions and behaviors in sex-changing fishes (Foran and Bass, 1999; reviewed in Goodson and Bass, 2001). An et al. (2010) have found that GnRH analogue treatment increased mRNA expression levels of gonadotropin (GTH) subunits in the pituitary of cinnamon clownfish *Amphiprion melanopus*, whose sex is controlled by the social rank in the group, suggesting that GnRH plays an important regulatory role in the sex-changing processes. Among the studies on sex difference of GnRH neu-

rons in sex-changing fishes, much attention has been paid to GnRH1 neurons in the preoptic area (POA) of the brain. For example, bluehead wrasse (*Thalassoma bifasciatum*) males at the terminal phase exhibited a greater number of GnRH neurons in the POA than females or initial-phase males (Grober and Bass, 1991), and the number of GnRH neurons was increased in females and initial-phase males implanted with 11-KT (Grober and Bass, 1991). Males of ballan wrasse (*Labrus berggylta*) also had more GnRH1 neurons in the POA than females, but no difference was found in populations in the ventral telencephalon or the terminal nerve ganglion (Elofsson et al., 1999). Male anemonefish, which display protandrous sex change, have a greater number of GnRH neurons in the POA than females (Elofsson et al., 1997). Thus, the GnRH neurons in the POA, presumably GnRH1 neurons, are considered as the key regulator of the hypothalamic-pituitary-gonad axis in sex-changing fishes. However, a mechanistic model for sex reversal of reproductive behavior is yet to be determined.

Our present immunohistochemical observations showed sex difference in the number of GnRH3 immunoreactive neurons in mature tilapia. Furthermore, this study clearly showed the sex reversal of GnRH3 neurons and reproductive behavior, suggesting that GnRH3 is a key regulator in sex reversal of male-specific reproductive behavior in Mozambique tilapia. Mozambique tilapia will serve as another unique model for sex reversal of the brain in teleosts.

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