Long-term GnRH-induced gonadotropin secretion in a novel hypothalamo-pituitary slice culture from tilapia brain

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Organotypic cultures, prepared from hypothalamo-pituitary slices of tilapia, were developed to enable long-term study of secretory cells in the pituitary of a teleost. Values of membrane potential at rest were similar to those recorded from acute slices, and cells presented similar spontaneous spikes and spikelets. Some cells also exhibited slow spontaneous oscillations in membrane potential, which may be network-driven. Long-term (6 days) continuous exposure to GnRH induced increases in LH and FSH secretion. FSH levels reached the highest levels after 24 h of exposure to GnRH, and the highest secretion of LH was observed in days 4 and 5 of the experiment. Since slices were viable for several weeks in culture, maintaining the original cytoarchitecture, electrical membrane properties and the ability to secrete hormones in response to exogenous GnRH, this technique is ideal for studying the mechanisms regulating cell-to-cell communication under conditions resembling the in vivo tissue organization.

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1. Introduction

The hypothalamic neuropeptide gonadotropin-releasing hormone (GnRH) induces the expression and secretion of the gonadotropins LH and FSH from the pituitary. In mammals, GnRH is secreted from the hypothalamus in a pulsatile fashion (Carmel et al., 1976; Clarke and Cummins, 1982; Levine and Ramirez, 1982). Pulsatile administration of GnRH evokes gonadotropin release from the pituitary (Belchetz et al., 1978; Valk et al., 1980; Wu et al., 1987), and gonadotropin secretion is differentially regulated by changes in pulse frequency and amplitude (Wildt et al., 1981). Prolonged GnRH treatment, in contrast, is unable to sustain elevated levels of gonadotropin (Belchetz et al., 1978). Studies in live animals and in perfused pituitary cells have shown that continuous exposure to GnRH leads to decreased gonadotropin secretion and a blunted secretory response to subsequent pulses of GnRH, a process referred to as homologous desensitization (Weiss et al., 1995). Desensitization in response to continuous GnRH exposure was similarly observed in chickens (King et al., 1986) and turtles (Licht and Porter, 1985), but not in frogs (Porter and Licht, 1985).

In teleost fish, GnRH is released directly at the distal pituitary by hypothalamic nerve fibers and not via a hypothalamic–hypophysial portal system (Batten and Ball, 1977; Ball, 1981; Batten et al., 1999). Although pulsatile gonadotropin (probably LH) secretion has been observed in female rainbow trout (Zohar et al., 1986), the lack of a portal system renders the direct measurement of GnRH secretion patterns in fishes very difficult. However, commonly used protocols for spawning induction of many farmed fish species include a long-term exposure to GnRH, in slow release implants, even up to several weeks (reviewed by (Mylonas et al., 2010). Hence, data from in vitro studies on GnRH desensitization, at the gonadotropin secretion level are important. Moreover, such studies can indicate whether pulsatile secretion of GnRH is required to evoke gonadotropin secretion over time. In goldfish, sGnRH or cGnRH-II, administered either continuously (60 min) or at high frequency pulses, significantly desensitized pituitary gonadotropin release, and this desensitization was reduced when GnRH was given at longer intervals (Habibi, 1991). In tilapia, in contrast, LH secretion continued to rise in the first 3 h of continuous exposure to GnRH, and reached basal secretion level only after 17–19 h (Avitan et al., 2007).

Unlike mammals, in which the different types of cells are scattered throughout the pituitary, the cells of the anterior pituitary of teleosts are segregated into distinct regions according to the characteristic hormone they secrete (Yaron et al., 2003; Levavi-Sivan...
et al., 2010). Since direct cell-to-cell communication via gap junctions may affect secretion patterns (Levavi-Sivan et al., 2005; Le Tissier et al., 2012), it may be important to retain the original organization of the various cell types in the pituitary in order to preserve the dynamics of gonadotropin secretion. This requirement is not met by the dispersed cells preparation frequently used in in vitro studies on GnRH desensitization in teleosts (e.g., Levavi-Sivan and Yaron, 1989, 1992). Pituitary fragments preparations, in contrast, partially maintain the organizational structure of the anterior pituitary, enabling – to some degree – the original cell-to-cell contact existing in the pituitary. These preparations, however, cannot be maintained over an extended period of time, and experiments examining GnRH effect on gonadotropin secretion in perfused pituitary fragments are limited to a period of about 24 h (Levavi-Sivan and Yaron, 1989; Avitan et al., 2007). Since recovery from refractoriness has been reported to take 2–4 days in mammals (Jinnah and Conn, 1985; Gorospe and Conn, 1988), preparations that would enable the examination of long-term effect of GnRH on gonadotropin secretion would be advantageous.

In this study, we developed an organotypic hypothalamo-pituitary slice culture, which preserves the basic structural and connective organization of the various cell types in the fish pituitary, and which can be sustained for up to 3 weeks. We characterized the electrical properties of the cells in the cultures and examined the lasting effects of sGnRH analogue on LH and FSH secretion.

2. Materials and methods

2.1. Preparation of hypothalamo-pituitary slice cultures

Hypothalamo-pituitary slices were prepared according to (Levavi-Sivan et al., 2005). Young (9–10 months old) tilapia fish of both sexes were anesthetized with 2-phenoxethanol (1 ml/liter; Sigma–Aldrich Corp., St. Louis, MO) and decapitated. Brains in (Stoppini et al., 1991). The plates were incubated at 26.5°C. Cell types in sagittal sections. The sagittal angle of the slice was compared with the previously established arrangement of the different cell types in sagittal sections. The sagittal angle of the slice was similar with the previously established arrangement of the different cell types in sagittal sections. The sagittal angle of the slice was similar with the previously established arrangement of the different cell types in sagittal sections.

2.2. Hematoxylin–eosin stain

LH cells were localized in a peripheral region of the proximal pars distalis of the tilapia pituitary (Aizen et al., 2007a). In order to obtain electrophysiological recordings from the area containing mostly LH cells, a sagittal section had to be obtained, and compared with the previously established arrangement of the different cell types in sagittal sections. The sagittal angle of the slice was verified using hematoxylin–eosin (H&E) stain. Several slices were fixed in 10% formalin and were embedded in paraffin, and 6-µm sections were mounted on slides. For H&E staining, slides were immersed in hematoxylin for 15 min, treated briefly with HCl solution, rinsed in tap water for 10 min and subsequently immersed in eosin for additional 10 min.

2.3. Vital stain

Cell viability after 12 days in culture was determined using the fluorescent exclusion dye, propidium iodide (Sigma), which penetrates dying cells and labels their nuclei while remaining impermeable to cells with an intact membrane. Cultures were placed for 1 h in Ringer’s saline followed by incubation with Ring-er’s saline containing 5 µg/ml propidium iodide for 1 h, washed again with Ringer’s saline, and then imaged on a standard inverted fluorescence microscope using a rhodamine filter set (488 nm/515 nm). Background fluorescence was subtracted using empty membranes stained with PI.

2.4. Electrophysiology

For recording, slices were gently separated from the membrane and transferred to a chamber attached to the stage of an upright microscope (Axioskop FS, Zeiss, Oberkochen, Germany) continuously superfused with Ringer’s saline at room temperature. Endocrine cells were viewed with a ×60, 0.9 numerical aperture, water immersion objective lens (Olympus, Munich, Germany). Whole cell recordings were made under infrared differential interference contrast (IR-DIC) microscopic control using the patch-clamp technique (Hamill et al., 1981). Patch pipettes, pulled from borosilicate glass capillaries (Hilgenberg, Maisfield, Germany) on a Narishige PP83 puller, had resistances of 2.5–4.5 MΩ. Membrane currents were recorded using either the conventional, whole cell, patch-clamp technique or the nystatin-perforated, patch-clamp technique. The standard pipette solution contained (in mM): 135 potassium gluconate, 2 MgCl2, 1 CaCl2, 11 EGTA, 3 ATP (magnesium salt), and 10 HEPS (potassium salt), pH 7.25. For the patch-pipette solution for nystatin-perforated patch recordings, a stock solution containing 10 mg/ml nystatin (Sigma–Aldrich Corp.) in acidified methanol was prepared and added to the pipette solution to a final concentration of 400 µg/ml. Only cells from the anterior pituitary were recorded. An Axoclamp-2A amplifier (Axon Instruments, Union City, CA) in Bridge mode or an Axopatch-200B amplifier in fast current clamp mode was used to record membrane voltages. Care was taken to maintain membrane access resistance as low as possible (usually 5–7 MΩ and always <10 MΩ). Command current protocols were generated and data were acquired on-line with a Digidata 1320A interface (Axon Instruments). Data were low pass filtered at 10 kHz (–3 dB, four-pole Bessel or one-pole Butterworth built-in filter) and sampled at a digitalization frequency of 20 kHz. Voltages were not corrected for liquid junction potential. Data were analyzed using PClamp 9 (Axon Instruments) and Microcal Origin 6.0 software.

2.5. Long-term effects of GnRH on LH and FSH secretion

For examination of the effect of continuous exposure to GnRH on LH and FSH levels, slice-cultures were prepared from 9 fish. From each fish, two slices were placed on a single membrane. On day 3 in culture, the medium was changed before [D-Ala6,Pro2,Neu5]–mammalian GnRH (sGnRHa) was added to the medium to a final concentration of 10−7 M (n = 6 and n = 3 for the GnRH and control groups, respectively). The medium was replaced and sGnRHa was added every 48 h for 6 days, and medium samples were taken every 24 h. Levels of
gonadotropins in medium were measured by a specific ELISA developed for tilapia (Aizen et al., 2007b). Sensitivity of the measurements was 15.84 pg/ml for LH and 0.24 pg/ml for FSH. Interassay covariance (CV) was 14.8% and 12.5% and intraassay CV was 7.2% and 8% for LH and FSH, respectively.

2.6. Immunohistochemistry

Slices in day 10–14 in culture were fixed in 10% formalin, and embedded in paraffin, and 6-μm sections were mounted on slides. Immunohistochemical staining for tFSH, tLH and tGH was performed using the high polymer method (Histofine simple stain MAX PO; Nichirei Co., Tokyo, Japan), as previously described (Aizen et al., 2007a). Homologous antibodies against recombinant tilapia LHβ (Kasuto and Levavi-Sivan, 2005), FSHβ (Aizen et al., 2007a) or GH (Melamed et al., 1995) were used.

2.7. Statistics

Numerical data in the text are presented as the mean ± SEM. Significance of differences between group means was determined by one-way ANOVA followed by Newman–Keuls test with p < 0.05 using the Graph-Pad Prism software (GraphPad, San Diego, CA).

3. Results

3.1. Viability and cytoarchitecture in the slice cultures

Organotypic slice cultures survived for at least 3 weeks in vitro without any obvious morphological degeneration. The vitality of six hypothalamo-pituitary slice cultures was assessed using the fluorescent exclusion dye propidium iodide (PI), which is taken up only by dead cells (Macklis and Madison, 1990). On day 12 in culture, most cells in the stained slices did not exhibit fluorescence signal, indicating that the slices were still highly viable (Fig. 1). By day 30 in culture, fluorescence signal was observed throughout the slices (data not shown). In order to examine the effect of culture conditions on the organization of cells in the slices, we performed immunohistochemical staining for tFSH, tLH and tGH. Although slice cultures did become more elongated as compared to acute slices, the general arrangement of FSH, LH and GH cells remained largely unaffected (Fig. 2).

3.2. Electrical characteristics of the slice cultures

The electrical membrane properties of anterior pituitary cells in organotypic slice cultures were studied on day 12 in culture. Although no attempt was made to verify the hormonal content of the recorded cells, recordings were made from the general area known to contain mostly LH cells. Recordings using the perforated patch-clamp technique revealed a resting potential of −59.8 ± 1.6 mV (n = 7). Voltage-gated currents were elicited by applying voltage steps from −90 mV to +20 mV (holding potential −70 mV, pulse duration 300 ms, n = 5). Fig. 3 depicts current records from one cell, and an average current–voltage curve from 5 cells.

Cells occasionally exhibited spontaneous spikes and spikelets at rest. Two out of 7 cells generated continuous membrane oscillations at a frequency of 0.56 ± 0.1 Hz (Fig. 4A and C), with spikes and spikelets appearing at the peak of the depolarizing waves. Hyperpolarization to −75 mV decreased the amplitude but did not affect the frequency of the oscillations (Fig. 4B). While spikes were no longer visible, spikelets continued to appear on at the depolarizing peak of the potential waves.

3.3. GnRH-induced gonadotropin secretion

In order to examine long-term responsiveness of the slice cultures to GnRH, we measured daily secretion of LH and FSH in response to GnRH administered every 48 h, for 6 days, at a final concentration of 10−7 M. No significant change was recorded in the control group throughout the experiment (Fig. 5). GnRH significantly increased daily LH secretion in comparison with the control group in days 1, 4 and 5 (Fig. 5A). While LH secretion on day 1 rose to a ratio to basal level of 2.22, the highest increases were observed in days 4 and 5 of exposure to GnRH (ratio to basal level of 3.57 and 2.94, respectively). Only on day 6, LH secretion was reduced in the slice cultures receiving GnRH, to a secretion level lower than those in the control group. FSH levels were higher in slices administered with GnRH in comparison with the control group on days 1 and 3–6 (Fig. 5B). Here, the largest increase appeared at day 1, with FSH secretion at a ratio to basal level of 2.61.

4. Discussion

Several approaches to the culturing of pituitary cells are currently available. These include a variety of dissociated (cell) tissue culture and perfusion of pituitary fragments. While conventional dissociated pituitary cell cultures and perfusion of pituitary fragments have successfully maintained the ability to respond to hypothalamic peptidergic neurons in vitro, neither of these methods permit long-term culture of the pituitary cells. This is particularly important due to the special organization of the endocrine cells in the fish pituitary (Levavi-Sivan et al., 2010). The present study shows that organotypic hypothalamo-pituitary slice cultures conserve important neuroendocrine characteristics, such as cell organization, electrical activity and hormone release, for up to 3 weeks in culture.

Since organotypic slice cultures can be kept vital for a sustained period of time, while retaining, to a large degree, the characteristic cytoarchitecture of the original tissue (Gahwiler, 1988), it is a highly effective technique for the examination of long-term effects on the organization of cells in the slices.
of GnRH on gonadotropin secretion. Since cell-to-cell interactions via gap-junctions in the pituitary may influence secretory activity (Fletcher et al., 1975; Soji and Herbert, 1989), not only in mammals but also in fish (Levavi-Sivan et al., 2005), the preservation of the original organization of the cells in the preparation is an important advantage for the elucidation of the dynamics of secretion from the pituitary. This is especially important in the fish compared to mammals, as the different secretory cell types in the adenohypophysis are segregated into distinct sections (see Fig. 2 A). As Fig. 2B–E demonstrates, while the slices were somewhat flattened and elongated, the relative organization of LH, FSH and GH-secreting cells remained largely unaffected: LH-secreting cells were observed throughout the rostral pars distalis, FSH-secreting cells were located in the dorsal region of the PPD along the projections of the neurohypophysis, and GH-secreting cells were observed in large abundance close to the border with the neurohypophysis. This organization of the cells corresponds to that previously observed for tilapia (Yaron et al., 2003; Aizen et al., 2007a; Golan and Levavi-Sivan, 2013).

As gap-junctions between pituitary cells have been shown in mammals to facilitate intercellular communication, not only within small clusters of cells, but between cells in an extended three-dimensional networks (Hodson et al., 2012), we aimed to produce a preparation that will preserve the three-dimensional intercellular relationships in the pituitary, rather than conserving its organization in only two dimensions. While slices cultured in roller-tubes become flattened to a single layer of cells, the interface method for slice cultures, in which slices are placed directly on semiporous membranes and thus are in contact with the medium on one side and with oxygen on the other side, produces cultures that remain several cell-layers thick (Gähwiler et al., 1997). As can be seen in Fig. 1, flattening of the slice cultures to a single layer was not necessary for proper visualization of the cultures and for electrophysiological recordings. In general, organotypic cultures of rat pituitaries retain features of their in vivo counterparts, including cytoarchitecture (Wray et al., 1988; Maurer and Wray, 1997), spontaneous electrical activity (Gahwiler and Herrling, 1981), voltage-dependent calcium conductance (Mouginot et al., 1997), rhythmic neurosecretion (Shinohara et al., 1993; Tominaga et al., 1994), and rhythmic mRNA fluctuations (Carter and Murphy, 1989).

The involvement of ion channels, calcium influx and changes in membrane potential in secretion from pituitary gonadotrophs have been extensively documented in mammals (Stojilkovic et al., 2010). While in the fish, the relation between changes in membrane potential and hormonal secretion has not yet been elucidated, several types of currents have been detected in cultures of dispersed cells (Van Goor et al., 1996), and electrical properties of cells have been recorded in acute slices (Levavi-Sivan et al., 2005). Previous studies have shown that organotypic cultures of the pituitary in mammals maintained the same membrane properties as acute slices (Guerineau et al., 1997). In the present study, we similarly aimed at developing a preparation that will not alter the general electrical characteristics of pituitary cells. Anterior

Fig. 2. Immunohistochemistry of tilapia pituitary. (A) H&E stain of acute slices confirmed the sagittal angle of the acute section. (B–E) Hypothalamo-pituitary slices in day 12 in culture immunostained with normal rabbit serum (B), anti-GH (C), anti-rFSHβ (D), and anti-rLHβ (E).

Fig. 3. Perforated-patch recordings from pituitary cells in slices on day 12 in culture. (A) Membrane current was studied using 300-ms voltage steps of incremental amplitude. (B) Peak current–voltage relationship based on voltage-clamp recordings from 5 cells.
pituitary cells on day 12 in culture had a membrane potential of about −60 mV and displayed spontaneous spikes and spikelets. These results are in accordance with previous reports in acute hypothalamo-pituitary slices from the same species (Levavi-Sivan et al., 2005). Two of the examined cells presented continuous membrane voltage oscillations at a frequency of 0.56 Hz. Oscillations in membrane potential have been observed in mammalian gonadotropes in response to GnRH and were shown to be reflected in oscillations in intracellular calcium concentration (Tse and Hille, 1992; Hille et al., 1995). It has been suggested that the importance of the oscillations in membrane potential is in the activation of voltage-dependent calcium channels, enabling the replenishment of calcium in intracellular stores (Tse and Hille, 1993). An essential part of this mechanism is the opening of SK calcium-activated K⁺ channels (Tse and Hille, 1992; Stojilkovic et al., 1994). This current facilitates the hyperpolarization of the cell from a relatively high resting potential of −35 mV to about −85 mV, thus bringing voltage-gated Na⁺ and Ca²⁺ channels out of steady-state inactivation (Tse and Hille, 1992; Hille et al., 1995). It was recently shown that in the Atlantic cod there are differences in the properties of fshb- and lhb-expressing pituitary cells. The electrophysiological responses to GnRH differ between the two gobadotropins, presumably due to differences in ion channel repertoire. In particular, BK channels are predominantly expressed in fshb-expressing cells, whereas lhb-expressing cells mainly express SK2 channels. Both cell types were found to respond to GnRH stimulation with elevated [Ca²⁺], and increased excitability (Hodne et al., 2013). Moreover, different Ca²⁺ responses to GnRH were shown by FSH and LH cells from whole brain-pituitary preparations of the medaka (Karigo et al., 2014).

In goldfish, SK calcium-activated K⁺ current has not been observed, and resting potential was −60 mV. It was suggested that since a large proportion of voltage sensitive Na⁺ and Ca²⁺ channels are available for activation at this resting potential, transient hyperpolarizations due to the SK calcium-activated K⁺ current may not be required to bring them out of steady-state inactivation (Van Goor et al., 1996). The resting potential reported here – as well as that reported in acute slices (Levavi-Sivan et al., 2005) – is in accord with the studies in goldfish. Furthermore, the frequency of the oscillations we observed is significantly higher than that reported in mammals (Tse and Hille, 1992). Thus, the mechanism underlying the spontaneous oscillations we report here is likely to be different than that reported in mammals in response to GnRH. The electrophysiological properties of pituitary cells were investigated, using organotypic cultures of rat pituitaries, with both intracellular and patch-clamp recordings after 2–7 weeks in vitro. Cellular resting membrane potentials were −50 mV, and spontaneous or depolarization-induced action potentials were found in ~50% of cells. Records of voltage-dependent outward membrane currents showed that cells expressed functional voltage-gated channels (Guérineau et al., 1997).

While hyperpolarization of the cells to a potential of −75 mV decreased the amplitude of the oscillations, it did not affect their frequency, which may indicate that the oscillations are network-driven. This is further strengthened by the fact that while hyperpolarization abolished the appearance of spikes at the depolarizing end of the oscillations, spikelets were still visible. It has been suggested that such spikelets in the fish pituitary reflect spikes in neighboring cells, in a network connected via gap junctions (Levavi-Sivan et al., 2005). Anterior pituitary cells have been shown to be coupled in mammals (Morand et al., 1996; Le Tissier et al., 2012) and tilapia (Levavi-Sivan et al., 2005), and the expression of Cx43, Cx26 and Cx36 has been established in mammals (Meda et al., 1993; Yamamoto et al., 1993; Belluardo et al., 2000). Organotypical hypothalamo-pituitary slice cultures provide a preparation for further examination of cell-to-cell communication in the fish pituitary.

The ability of pituitary cells in the organotypic culture to secrete LH and FSH in response to GnRH administration was maintained even after 2 weeks in culture. Thus, it serves as an ideal preparation for the examination of long-term effects of GnRH on the dynamics of hormonal secretion. Due to the technical difficulties discussed above, experiments examining the effect of continuous administration of GnRH on LH secretion from pituitary fragments have been limited to 24 h (Avitan et al., 2007). Furthermore, because of the inability, until recently (Aizen et al., 2007), to detect FSH levels in teleosts, the effect of exogenous GnRH on FSH secretion in vitro has not yet been examined. Thus, this is the first report on the long-term effects of GnRH on FSH and LH secretion from fish gonadotropins in an experimental environment that retains the cytoarchitecture of the pituitary. Continuous exposure of fish to GnRH, usually in the form of implants, is commonly practiced in fish aquaculture (Mylonas et al., 2010). GnRH acts via...
G-protein-coupled receptors (GPCRs) on gonadotropes to stimulate the secretion and synthesis of FSH and LH. For many GPCRs, the intracellular C-terminal tail plays a key role in agonist-induced phosphorylation and desensitization of the hormonal secretion (Pierce and Lefkowitz, 2001). It has been reported that mammalian GnRH receptors (GnRHRs) do not possess a C-terminal tail and show desensitization of LH secretion in response to continuous GnRH stimulation (Levavi-Sivan et al., 2010), whereas fish GnRHRs (including tilapia) do have a C-terminal tail (Blomenrohr et al., 1999) and are more resistant to sustained exposure to GnRH. This might explain the observed lack of agonist desensitization in the tilapia pituitary after several days of exposure to GnRH.

On day 12 in culture, slices were placed in medium that contains 100 nM sGnRHa. Slice cultures in the control group exhibited low-magnitude fluctuations in gonadotropin levels throughout the experiment, most likely as a result of responses to endogenous GnRH in the hypothalamo-pituitary slices. Nevertheless, significant effects of continuous exposure to exogenous sGnRHa on LH and FSH secretion were clearly observed. Secretion of both LH and FSH significantly increased at day 1, decreased at day 2, and then increased again in response to GnRH. However the pattern of the response to LH and FSH was slightly different: while the secretion of LH was similar throughout the experiment, FSH levels increased in response to GnRH but the overall response decreased from one pulse to the other. The elimination of sGnRHa-induced secretion of both LH and FSH after 48 h was probably the result of desensitization to GnRH. A reduction in GnRH-induced LH secretion to basal levels has been reported in tilapia after 17–19 h. This reduction was not due to depletion of LH stores within the pituitary (Avitan et al., 2007). Gonadotrophs in tilapia possess the GnRH type 3 receptor (Levavi-Sivan and Avitan, 2005). The tGnRHR3, unlike mammalian GnRH receptors, has a C-tail which is essential for rapid internalization and desensitization of G-protein-coupled receptors (Sealfon et al., 1997). Accordingly, tGnRHR3 undergoes internalization within 30 min of exposure to sGnRHa – much earlier than the reduction in LH secretion (Avitan et al., 2007). Thus, additional mechanisms are responsible for the dynamics of the desensitization of gonadotropin release in tilapia in response to continuous GnRH administration.

Both LH and FSH were increased in the first 24 h in response to GnRH, as was shown before (Avitan et al., 2007). Although GnRH-induced secretion of both LH and FSH was reduced after 48 h, continuously administered GnRH was able to induce both gonadotropins secretion in subsequent days. These results may corroborate the results obtained in vivo in tilapia: during a reproductive cycle, which lasts for 12 days, we found two parallel peaks of both FSH and LH. The first one, on day two was probably related to the vitellogenic phase, while the second peak, occurred on the 11th day, toward spawning (Aizen et al., 2007b). While the possibility of such priming mechanism is appealing in light of the relatively long LH surge in the spawning cycle in tilapia (Aizen et al., 2007b), further studies using organotypical slice culture are required to determine a pattern of long-term GnRH-induced gonadotropin secretion and to elucidate the mechanisms involved behind these patterns.

In addition to facilitating the study of long-term effect of exogenous hypothalamic factors on the pituitary, a preparation that retains a functional connection between the hypothalamus and the pituitary opens the door for a variety of manipulations of the interaction between the hypothalamus and the pituitary. This provides a way to gain insight into secretion patterns of hypothalamic factors in the fish, in the absence of a portal system. The integrity of the hypothalamic–pituitary connection in the acute slices was confirmed electrophysiologically (Bloch et al., 2004).

In summary, we have produced an organotypic hypothalamo-pituitary slice culture, which preserves the cytoarchitecture of the pituitary while maintaining viability for several weeks. The slices retain important characteristics of the original tissue, such as electrical activity and hormonal secretion, as well as a connection between the hypothalamus and the pituitary. Thus, slice cultures can be a useful tool for the investigation of long-term secretion mechanisms in the pituitary.

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References


