INTRODUCTION

The role of endogenous opioid peptides in the control of sexual maturation and reproduction of mammals is already well established (Yen et al. 1985, Piva et al. 1986, Ferin 1987, Gopalan et al. 1991, Kalra 1993). They act at all levels of the hypothalamo-pituitary-gonadal axis (Blank et al. 1986, Cacicedo and Sanchez-Franco 1986, Leposavic et al. 1991, Smith and Gallo 1997, Kaminski et al. 2004) and are also involved in gonadal steroid feedback on gonadotropin secretion from the pituitary (Barb et al. 1986, Martini et al. 1989, Behrens et al. 1993). The action of opioids on many aspects of reproduction in mammals is mainly inhibitory (Yilmaz et al. 1996, Smith et al. 1997, Kawamura et al. 2003).

In fish, opioids are also involved in the regulation of LH secretion but data on this aspect of fish reproduction are still very scarce. Opioids modulate GnRH and dopamine secretion, the two main factors controlling LH release in fish (Martini et al. 1989, Cheng 1996, Sokolowska-Mikolajczyk et al. 2002a,b, 2005, Socha et al. 2003). As in mammals, opioids may also mediate the gonadal steroid feedback on LH secretion in fish (Sokolowska-Mikolajczyk et al. 2002a,b, 2010).

Opioid regulation of LH release in higher vertebrates involves mediation of numerous neurotransmitter systems and other hormones which are also involved in LH synthesis and/or secretion, such as ghrelin or leptin (Kalra and Simpkins 1981, Aurich et al. 2002, Dudás and Merchenthaler 2004). This may also be true for fish.

Ghrelin is a newly discovered pleiotropic hormone that is involved mainly in the regulation of body energy homeo-
ostasis. It has also been proved that ghrelin affects many other processes including the control of the hypothalamo-pituitary-gonadal axis in higher vertebrates. In primates, sheep, and rats ghrelin has an inhibitory impact on the reproductive system (Furuta et al. 2001, Vulliémoz et al. 2004, Fernandez-Fernandez et al. 2006, Iqbal et al. 2006). In fish, like in mammals, ghrelin controls food intake and regulates the release of pituitary hormones (GH and LH) (Unniappan et al. 2002, 2004, Sokolowska-Mikolajczyk et al. 2009). Limited data on the role of ghrelin in sexual maturation or reproduction of fish show that, unlike in mammals, ghrelin stimulates LH release, acting directly on the pituitary (Grey and Chang 2008, Sokolowska-Mikolajczyk et al. 2009) and/or at the level of hypothalamus (Unniappan and Peter 2004). It seems possible that at this level ghrelin interacts with other peptides and neurotransmitters, which are also involved in gonadotropin release, such as endogenous opioid peptides.

The concept of the endogenous opioid interaction with ghrelin in mammals is based on the similarity of the receptors for both hormones: ghrelin receptors belong to a small subset of 7TM (seven transmembrane domain) receptors. Some other members of this subset are the motilin receptor and, to a certain degree, the somatostatin and opioid receptors (Holst et al. 2003). There are also anatomical examples of such interaction. It has recently been observed that ghrelin immunoreactive hypothalamic neurons innervate other peptidergic systems such as proopiomelanocortin (POMC) neurons (Cowley et al. 2003), which synthesize β-endorphin. Guan et al. (2008) suggest that POMC-producing neurons are modulated via synaptic communication with ghrelin-containing neurons and that these neurons may have a positive feedback effect on POMC-containing neurons through direct synaptic contacts.

Also physiological examples exist of the interactions between these two regulating systems: ghrelin produces excitatory effects on the neurons of the arcuate nucleus (Riediger et al. 2003), where endogenous opioids containing neurons are located (Bloom et al. 1979). There are reports showing a positive influence of ghrelin on opioid peptide release and/or activity (Kamegai et al. 2000). Pierzchała-Koziec and Zubel (2008) found an interaction of opioids and ghrelin in response to the activation of the immune system in lambs and Pierzchała-Koziec et al. (2006) demonstrated that ghrelin is involved in regulation of opioids and leptin release in mice.

As regards the relationship between ghrelin and opioid peptides in the regulation of gonadotropin release in fish, we know of no data in the scientific literature. Therefore, the aim of the present in vitro study was to examine the effects of human ghrelin and opioid receptor antagonist naltrrexone on LH secretion from common carp pituitary cells.

**MATERIALS AND METHODS**

**Fish.** The experiment was conducted on sexually mature common carp, *Cyprinus carpio* L., in June 2005. Because one part of this experiment concerning the spontaneous and sGnRH-analogue-stimulated LH release under the influence of ghrelin has already been published (Sokołowska-Mikolajczyk et al. 2009), the control values are the same for both reports. Eight females and 10 males weighing an average of 1.95 ± 0.30 and 1.46 ± 0.06 kg, respectively, were netted from the outdoor ponds of the Fishery Station belonging to the Department of Ichthyobiology and Fisheries, University of Agriculture in Krakow, Poland. Gonad maturity was specified as a percentage of body weight (gonadosomatic index GSI).

Male gonads (GSI = 4.2 ± 0.8) were at the stage of spermiation, GSI for female gonad was 22.13 ± 2.64. Fish were transferred to flow-through basins (1.5 m³ volume). Fish were exposed to a simulated natural photoperiod (16L : 8D) and water temperature of 18 ± 2°C for three days. On the day of the in vitro experiment fish were anaesthetised with 2-phenoxy-ethanol (Merck, Germany) at 0.3 mL · L⁻¹ of water, killed by decapitation and the pituitary gland were collected and placed in sterile ice-cold medium (MEM-Eagle, Sigma-Aldrich, USA) buffered with 15 mM Heps (Sigma-Aldrich, USA) and 9 mM sodium bicarbonate (P.O.Ch., Poland).

**Culture technique.** The enzymatic dispersion of the pituitary glands (separate pools of female and male pituitaries) and the technique of cell culture were described in detail elsewhere (Mikołajczyk et al. 1990, Sokolowska-Mikolajczyk et al. 2009). In brief, collected glands (separately from males and females) were chopped into small pieces and subjected to dispersion for 6–8 h at 20°C in the medium containing 0.1% (w/v) collagenase H (Boehringer Mannheim, Germany) and 1% BSA (Sigma-Aldrich, USA). The cells were harvested by 10-min centrifugation (200 m·s⁻²) at 20°C and washed twice with pre-incubation medium containing 2% (v/v) serum substitute (Ultroser SF, Sepracor S.A., France) and 1% (v/v) antibiotic-antimycotic (Sigma-Aldrich, USA).

Cell viability test and cell counting were performed with a Thoma haemocytometer. Cells were resuspended in the pre-incubation medium and transferred into four 96-well microplates (Nunc A/S Denmark) coated with Poly-L-lysine (Sigma-Aldrich, USA) (two plates for cells of female pituitary pool and two for male pituitary pool). Each well contained approximately 5 × 10⁴ cells in 250 μL of medium. Then the plates were sealed and incubated for 48 h at 22°C.

On the third day of culture the pre-incubation medium was replaced with medium containing ghrelin (Human, 1–18, Peptides International-Louisville, USA) at a concentration of 10⁻⁷ or 10⁻⁶ M, the opioid antagonist naltrrexone (Sigma-Aldrich, USA) at a concentration of 10⁻⁶ M or the combination of ghrelin (both concentrations) and naltrrexone. Control wells were filled up with medium without any supplementation. Each treatment group consisted of five wells containing cells from either the pool of male or female pituitaries. Two identical microplates were prepared. One of these was incubated for 10 h and the other for 24 h at 22°C. At the end of the incubation periods the plates were centrifuged (200 m·s⁻²) for 10 min at 20°C, and the media were collected and frozen at −20°C until LH determination by ELISA (Kah et al. 1989). Sensitivity of the performed ELISA...
was in the range 0.6–100 ng·mL⁻¹ with the intra- and inter-assay coefficients of variance at 5% and 9%, respectively.

**Statistics.** Mean basal LH concentrations (LH secretion in the presence of the control medium only) were 3.147 ng·mL⁻¹ for females and 2.146 ng·mL⁻¹ for males. LH levels measured in the medium samples were recalculated and presented as a percentage of basal secretion (secretion in the presence of the control medium). Then data were analysed using the nonparametric two-tailed Mann–Whitney U-test. The differences between the means were determined as significant for \( P < 0.05 \).

The experiment was conducted according to the guidelines given by the Institutional Ethical Committee.

**RESULTS**

**Effects of ghrelin on LH secretion**

**Pituitary cells from females.** Cells treated with ghrelin at 10⁻⁶ M for 10 h released 72.2 percentage points more LH than cells incubated with control medium. Lower concentration of ghrelin (10⁻⁷ M) had no significant effect on LH secretion to the medium, though LH concentrations increased by 62.9 percentage points in comparison to control (Fig. 1). After 24-h incubation there were no significant differences in LH secretion between cells incubated in the presence of ghrelin and in control medium (Fig. 2).

**Pituitary cells from males.** The incubation of male cells for 10 or 24 h in the presence of ghrelin (both concentrations) did not cause any significant changes in LH secretion in comparison to the control, after either 10 or 24 h of culture (Figs. 3, 4).

**Effects of naltrexone on LH secretion**

**Pituitary cells from females.** Ten hours of cell culture in the presence of naltrexone did not change the LH release into the medium (Fig. 1). A significant 52.2-percentage-point increase of LH secretion in comparison to control incubations was found after 24-h incubation (Fig. 2).

**Pituitary cells from males.** Pituitary cells did not respond to naltrexone presence in the medium and the changes in LH secretion after 10- or 24-h incubation were not significant in relation to the control (Figs. 3, 4).

**Effects of ghrelin and naltrexone on LH secretion**

**Pituitary cells from females.** Ten hours of cell incubation in the presence of the combination of ghrelin (both concentrations) and naltrexone showed a significant increase of LH secretion in comparison to the control (by 141.8 and 126.7 percentage points, respectively). After 24 h of incubation the differences between ghrelin- and naltrexone-treated cells and control incubations were still significant, since LH levels in the presence of ghrelin (10⁻⁷ M) + naltrexone and ghrelin (10⁻⁶ M) + naltrexone were 132.6 and 105.2 percentage points higher, respectively (Figs. 1, 2). The combination of ghrelin and naltrexone stimulated LH release also in comparison to the appropriate concentration of ghrelin alone by 122.2 and 110.57 percentage points, respectively.

![Fig. 1. Percentage of basal LH secretion from female pituitary cells incubated for 10 h with control medium, human ghrelin at 10⁻⁷ or 10⁻⁶ M, naltrexone (10⁻⁶ M) or the combination of naltrexone with ghrelin at 10⁻⁷ or 10⁻⁶ M; Bars represent means and SEM are given as vertical error bars (n = 5); *\((P < 0.05)\), **\((P < 0.01)\) vs. control](image1)

![Fig. 2. Percentage of basal LH secretion from female pituitary cells incubated for 24 h with control medium, human ghrelin at 10⁻⁷ or 10⁻⁶ M, naltrexone (10⁻⁶ M) or the combination of naltrexone with ghrelin at 10⁻⁷ or 10⁻⁶ M; Bars represent means and SEM are given as vertical error bars (n = 5); *\((P < 0.05)\), **\((P < 0.01)\) vs. control; ##\((P < 0.01)\) vs. ghrelin 10⁻⁷ M](image2)

![Fig. 3. Percentage of basal LH secretion from male pituitary cells incubated for 10 h with control medium, human ghrelin at 10⁻⁷ or 10⁻⁶ M, naltrexone (10⁻⁶ M) or the combination of naltrexone with ghrelin at 10⁻⁷ or 10⁻⁶ M; Bars represent means and SEM are given as vertical error bars (n = 5); *\((P < 0.05)\), **\((P < 0.01)\) vs. control; ##\((P < 0.01)\) vs. ghrelin 10⁻⁷ M; \(\bigstar(P < 0.05)\) vs. ghrelin 10⁻⁶ M](image3)
Fig. 4. Percentage of basal LH secretion from male pituitary cells incubated for 24 h with control medium, human ghrelin at 10^{-7} or 10^{-6} M, naltrexone (10^{-6} M) or the combination of naltrexone with ghrelin at 10^{-7} or 10^{-6} M; Bars represent means and SEM are given as vertical error bars (n = 5). **(P < 0.01) vs. control; # (P < 0.05) vs. ghrelin 10^{-7} M

Pituitary cells from males. The combination of both tested agents resulted in the highest LH secretion after 10 h of incubation (Figs. 3, 4). Indeed, LH levels increased by 105.7 and 137.5 percentage points and by 100.8 and 117.3 percentage points in comparison with the control and the respective ghrelin concentration. After a 24-h incubation period, only the lower ghrelin concentration combined with naltrexone induced a significant increase in LH release (79.2 and 80.3 percentage points, compared to control and ghrelin, respectively).

DISCUSSION

Gonadotropin secretion in fish is regulated by many factors acting at all levels of the hypothalamo-pituitary-gonadal axis. The most important factors that received the most attention are gonadotropin-releasing hormone and dopamine, which inhibits gonadotropin release (Chang and Peter 1983, 1984, Peter et al. 1986, 1991). Other factors like norepinephrine, g-amino butyric acid, melatonin, neuropeptide Y, cholecystokinin (Van der Kraak et al. 1998), opioids (Cheng unpublished) Rosenblum and Peter 1989, Sokolowska-Mikolajczyk et al. 2002a, b, 2005), ghrelin (Unniappan et al. 2002, Sokolowska-Mikolajczyk et al. 2009) and leptin (Peyon et al. 2001, Weil et al. 2003) are also involved in the regulation of gonadotropin secretion. They can act directly at the level of the pituitary gonadotrophs, but also by the modulation of GnRH and dopamine secretion from the hypothalamus. Usually they act at both levels (Trudeau 1997). The in vivo experiments conducted on goldfish and common carp demonstrated the involvement of opioids or ghrelin in gonadotropin release, but they failed to identify the exact site of their action (Rosenblum and Peter 1989, Sokolowska-Mikolajczyk et al. 2002a, b, Unniappan and Peter 2005). The in vitro approach demonstrated that both peptides act directly on gonadotrophins of goldfish and common carp (Sokolowska-Mikolajczyk et al. 2002a, b, Socha et al. 2003, Unniappan and Peter 2004, Sokolowska-Mikolajczyk et al. 2005, 2009). The present work investigated the interaction of opioids with ghrelin, because ghrelin is implicated in the regulation of opioid release in mice (Pierzchała-Koziec et al. 2006). It has recently been found that ghrelin immunoreactive hypothalamic neurons innervate other peptidergic systems such as proopiomelanocortin neurons (Cowley et al. 2003).

In the presently reported experiment female carp pituitary cells in the presence of ghrelin alone at a concentration of 10^{-6} M significantly (P < 0.05) increased LH levels in comparison to the control medium by 72.2 percentage points after 10 h of incubation. A ten times lower concentration of ghrelin also increased LH secretion (by 62.9 percentage points), but this difference was not significant (Fig. 1). Male pituitary cells incubated and treated in the same way as female-derived cells did not respond to ghrelin (Figs. 3, 4). These in vitro effects of ghrelin on LH secretion in female and male carp have already been described by Sokolowska-Mikolajczyk et al. (2009). Unniappan and Peter (2004) also observed stimulatory influence of ghrelin in goldfish pituitary cell incubation system, but with a 100 times lower dose than in our experiment. In the same publication the authors showed that also in vivo ghrelin caused LH to rise in goldfish. In rats, ghrelin predominantly inhibits in vivo LH secretion (Furuta et al. 2001, Kawamura et al. 2003, Vulliémoz et al. 2004), but in vitro data are not so consistent (Fernandez-Fernandez et al. 2005) and show that the response is related to the steroid levels in the investigated animals. In our experiment, differential response to ghrelin depending on the sex of the fish-donor of pituitary cells may indicate that the steroid levels, which vary by sex, are responsible for a particular response or lack thereof.

In the presently reported in vitro experiment naltrexone alone at 10^{-6} M did not change LH secretion from female carp pituitary cells at 10 h of culture, but after 24 h LH secretion under the influence of naltrexone was higher (P < 0.01) than in the control incubations. In the case of male-derived cells incubated and treated in the same way as female cells, no response to naltrexone after either 10 or 24 h of incubation was noted (Figs. 3, 4). The choice of naltrexone dose used in the experiment was based on the results of Socha et al. (2003) who showed, in the in vitro carp pituitary cell culture, the dose-dependent increase of LH levels under 10^{-8}, 10^{-6}, 10^{-4}, or 10^{-2} M of naltrexone with a concentration of 10^{-6} M being the minimal effective one. However the lack of the reaction to naltrexone in the in vitro experiment with carp has already been recorded in another experiment with female and male carp pituitary cell incubations performed during the late gonadal recrudescence (Sokolowska-Mikolajczyk et al. 2005). Also in vivo results on common carp (Sokolowska-Mikolajczyk et al. 2002a, b) confirm that opioid influence on gonadotropin release is affected by the actual sex steroid milieu. It may be true also for ghrelin in fish, but there are not yet enough data to support such a statement.

Carp pituitary cells treated with the combination of ghrelin (10^{-7} or 10^{-6} M) and naltrexone released the high-

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* Cheng K.W. 1996. Molecular studies of proopiomelanocortin in goldfish brain. M.Phil. Thesis. The University of Hong Kong, Hong Kong.
est amount of LH into the cultured medium (Figs. 1–4). In female cells after 10 h of incubation, the amounts of LH were significantly higher than in the control incubations by 148.1 or 126.7 percentage points, respectively (Fig. 1). In 24-h incubations LH concentrations were still higher than in the control (132.6 and 105.2 percentage points) and also compared to ghrelin alone (by 122.2 or 110.6 percentage points, respectively) (Fig. 2). A similar phenomenon was observed in male cells: 10-h incubation caused a significant increase of LH secretion into the culture media in comparison to the control (by 105.7 and 137.5 percentage points), to ghrelin (100.8 and 117.3 percentage points) and naltrexone alone (87.1 and 118.9 percentage points, respectively) (Fig. 3). After 24-h culture only ghrelin at a dose of 10^-7 M with naltrexone significantly increased LH secretion by 79.2 percentage points in relation to the control and by 80.34 percentage points in relation to ghrelin alone (Fig. 4). To summarize the results of the present investigation, if ghrelin and naltrexone act alone on the carp pituitary cells the effect is sex dependent—there is a stimulation of LH secretion in the case of female cells but no response of male cells. If both hormones are applied together a significant increase of LH secretion to the medium is observed for cells of female and male origin, indicating a possible potentiation of naltrexone on ghrelin LH-releasing action.

It is interesting that even if there is no response to naltrexone or to ghrelin alone, like in male cells, the stimulation of LH is so strong when both hormones are present in the medium (Figs. 3, 4). These effects are not easy to explain because of insufficient amount of data, which comes from the in vitro study only. It is presumed that both hormones may act through the same receptor type localised on gonadotrophic cells and the sum of ligand concentrations stimulates this receptor type more strongly than a single dose of each of the hormones. It is also possible that one hormone can up-regulate the number of other hormone receptors. This explanation is only speculation and to be closer to the real situation many more in vivo and in vitro studies should be done. They would explain the relationships between opioids and ghrelin in LH secretion control at all levels of the hypothalamo-pituitary-gonadal axis. In this context the problem of opioid and ghrelin participation in gonadal steroid feedback on gonadotropin release in fish could be clarified, because to date there has been no convincing data explaining the mediation of which hormone and at which level is responsible for this feedback. Evidence on opioid involvement in this process in fish is scarce and no data on ghrelin exist to our knowledge. As opioids and ghrelin can act through the same receptor type in mammals (Pierzchala-kozic 2004) and probably also in fish (differential response to ghrelin depending on the sex of donor fish cells in the present findings), we cannot rule out the possibility that gonadal steroids affect the hypothalamus and pituitary indirectly through several factors (in this case ghrelin and opioids) which interact between each other. However, without further research on this subject such a statement is still a matter of conjecture.

REFERENCES

Aurich C., Gerlach T., Aurich J.E., Hoppen H.-O., Lange J., Parvizi N. 2002. Dopaminergic and opioidergic regulation of gonadotropin and prolactin release in stallions. Reproduction in Domestic Animals 37 (6): 335–340. DOI: 10.1046/j.1439-0531.2002.00370.x.

Barb C.R., Kraeling R.R., Rampacke G.B., Whisnant C.S. 1986. Influence of stage of the estrous cycle on endogenous opioid modulation of luteinizing hormone, prolactin, and cortisol secretion in the gilt. Biology of Reproduction 35 (5): 1162–1167. DOI: 10.1095/biolreprod.35.5:1162.

Behrens C., Aurich J.E., Klug E., Naumann H., Hoppen H.-O. 1993. Inhibition of gonadotrophin release in mares during the luteal phase of the oestrous cycle by endogenous opioids. Journal of Reproduction and Fertility 98 (2): 509–514. DOI: 10.1530/jrf.0.0980509.

Blank M.S., Fabri A., Catt K.J., Dufau M.L. 1986. Inhibition of luteinizing hormone release by morphine and endogenous opiates in cultured pituitary cells. Endocrinology 118 (5): 2097–2101. DOI: 10.1210/endo-118-5-2097.

Bloom F., Battenberg E., Rossier J., Ling N., Guillemine R. 1979. Neurons containing b-endorphin in rat brain exist separately from those containing enkephalin: immunocytochemical studies. Proceedings of the National Academy of Sciences USA 75 (3): 1591–1595. DOI: 10.1073/pnas.75.3:1591.

Cacicedo L., Sánchez Franco F. 1986. Direct action of opioid peptides and Naloxone on gonadotropin secretion by cultured rat anterior pituitary cells. Life Sciences 38 (7): 617–625. DOI: 10.1016/0024-3205(86)90055-X.

Chang J.P., Peter R.E. 1983. Effects of dopamine on gonadotropin release in female goldfish, Carassius auratus. Neuroendocrinology 36 (5): 351-357. DOI: 10.1159/000123480.

Chang J.P., Peter R.E. 1984. Influences of norepinephrine and p-adrenergic mechanisms on gonadotropin secretion in female goldfish, Carassius auratus. General and Comparative Endocrinology 55 (1): 89–95. DOI: 10.1016/0016-6480(84)90132-1.

Cowley M.A., Smith R.G., Pronchuk N., Grove K.L., Strasburger C.J., Bloom F., Battenberg E., Rossier J., Ling N., Guillemin R. 1986. Inhibition of luteinizing hormone release by morphine and endogenous opiates in cultured pituitary cells. Endocrinology 118 (5): 2097–2101. DOI: 10.1210/endo-118-5-2097.

Dudas B., Merchenthaler I. 2004. Close anatomical associations between b-endorphin and luteinizing hormone-releasing hormone neuronal system in the human diencephalon. Neuroscience 124 (1): 221–229. DOI: 10.1016/j.neuroscience.2003.11.021.

Ferin M. 1987. A role for the endogenous opioid peptides in the regulation of gonadotropin secretion in the primate. Hormone Research 28 (2–4): 119–125. DOI: 10.1016/S0896-6273(03)00063-1.

Fernandez-Fernandez R., Martini A.C., Navarro V.M., Castellano J.M., Dieguez C., Aguilar E., Pinilla L., Tenasempere M. 2006. Novel signals for the integration of energy balance and reproduction. Molecular and Cellular Endocrinology 254–255: 127–132. DOI: 10.1016/j.mce.2006.04.026.
Fernandez-Fernandez R., Tena-Sempere M., Navarro V.M., Barreiro M.L., Castellano J.M., Aguilar E., Pinilla L. 2005. Effects of ghrelin upon gonadotropin-releasing hormone and gonadotropin secretation in adult female rats: in vivo and in vitro studies. Neuroendocrinology 82 (5–6): 245–255. DOI: 10.1159/000092753

Furuta M., Funabashi T., Kimura F. 2001. Intracerebroventricular administration of ghrelin rapidly suppresses pulsatile luteinizing hormone secretion in ovariectomized rats. Biochemical and Biophysical Research Communications 288 (4): 780–785. DOI: 10.1006/bbrc.2001.5854.

Gopalan C., Currie I.S., Gilmore D.P. 1991. Effects of opiates on hypothalamic amine turnover and LH levels in castrated rats. Biogenic Amines 8 (2): 141–147.

Grey C.L., Chang J.P. 2008. Ghrelin-induced growth hormone and gonadotropin releases from goldfish pituitary cells utilize both calcium entry and the PKC pathway. P. 55–5. In: Sixth International Symposium on Fish Endocrinology, 22–27 June 2008, Calgary Canada.

Guan J.-L., Okuda H., Takenoya F., Kintaka Y., Yagi M., Wang L., Seki M., Hori Y., Kageyama H., Shioida S. 2008. Synaptic relationships between proopiomelanocortin- and ghrelin-containing neurons in the rat arcuate nucleus. Regulatory Peptides 145 (1–3): 128–132. DOI: 10.1016/j.regpep.2007.09.028.

Holst B., Cygankiewicz A., Jensen T.H., Ankersen M., Schwartz T.W. 2003. High constitutive signaling of the ghrelin receptor—identification of a potent inverse agonist. Molecular Endocrinology 17 (11): 2201–2210. DOI: 10.1210/me.2003-0069.

Iqbal J., Kurose Y., Canny B., Clarke L.J. 2006. Effects of central infusion of ghrelin on food intake and plasma levels of growth hormone, luteinizing hormone, prolactin, and cortisol secretion in sheep. Endocrinology 147 (1): 510–519. DOI: 10.1210/edrv-2005-1048.

Kah O., Pontet A., Nunez Rodriguez J., Calas A., Breton B. 1989. Development of an enzyme-linked immunosorbent assay for goldfish gonadotropin. Biology of Reproduction 41 (1): 68–73. DOI: 10.1095/biolreprod41.1.68.

Kalra S.P. 1993. Mandatory neuropeptide-steroid signaling for the preovulatory luteinizing hormone-releasing hormone discharge. Endocrine Reviews 14 (5): 507–538. DOI: 10.1210/edrv-14-5-507.

Kalra S.P., Simpkins J.W. 1981. Evidence for noradrenergic mediation of opioid effects on luteinizing hormone secretion. Endocrinology 109 (3): 776–782. DOI: 10.1210/endo-109-3-776.

Kamegai J., Tamura H., Shimizu T., Ishii S., Sugihara H., Wakabayashi I. 2000. Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. Endocrinology 141 (12): 4797–4800. DOI: 10.1210/en.141.12.4797.

Kaminski T., Siawrys C., Bogacka I., Okrasa S., Przala J. 2004. The influence of opioid peptides on steroidogenesis in porcine granulosa cells. Reproduction in Domestic Animals 39 (1): 25–32. DOI: 10.1046/j.1439-0531.2003.00471.x.

Kawamura K., Sato N., Fukuda J., Kodama H., Kumejai J., Tanikawa H., Nakamura A., Honda Y., Sato T., Tanaka T. 2003. Ghrelin inhibits the development of mouse preimplantation embryos in vitro. Endocrinology 144 (6): 2623–2633. DOI: 10.1210/en.2003-0033.

Leposavic G., Cover P.O., Buckingham J.C. 1991. In vivo and in vitro studies on the opioidergic control of the secretion of gonadotrophin-releasing hormone and luteinizing hormone in sexually immature and adult male rats. Neuroendocrinology 53 (6): 579–588. DOI: 10.1159/000125777.

Martini L., Dondi D., Limonta P., Maggi R., Piva F. 1989. Modulation by sex steroids of brain opioid receptors: Implications for the control of gonadotropins and prolactin secretion. Journal of Steroid Biochemistry 33 (4B): 673–681. DOI: 10.1016/0022-4731(89)90477-9.

Mikołajczyk T., Weil C., Epler P., Breton B. 1990. Calcium ions as a mediator in GnRH action on gonadotropin release in the common carp (Cyprinus carpio L.). Reproduction Nutrition Development 30 (4): 483–492. DOI: 10.1051/md:19900403.

Peter R.E., Chang J.P., Nahorniak C.S., Omeljaniuk R.J., Sokolowska M., Shih S.H., Billard R. 1986. Interactions of catecholamines and GnRH in regulation of gonadotropin secretion in teleost fish. Recent Progress in Hormone Research 42: 513–548.

Peter R.E., Trudeau V.L., Slokey B.D. 1991. Brain regulation of reproduction in teleost. Bulletin of the Institute of Zoology, Academia Sinica. Monograph 16: 89–118.

Peyon P., Zanuy S., Carrillo M. 2000. Action of leptin on in vitro luteinizing hormone release in the European sea bass (Dicentrarchus labrax). Biology of Reproduction 65 (5): 1573–1578. DOI: 10.1095/biolreprod.65.5.1573.

Pierzchala-Kozicc K. 2004. The role of opioids in the modulation of ghrelin action. Symposium “Advances of Experimental Endocrinology”. Polish Journal of Endocrinology 5: 657.

Pierzchala-Kozicc K., Zujbel J. 2008. Expression of ghrelin and opioid mRNAs in the hypothalmo-pituitary-adrenal axis after immune system activation. Endocrine Abstracts 16: 477.

Pierzchala-Kozicc K., Zujbel J., Kanitz E., Misiak M. 2006. Ghrelin modulates the in vitro release of opioids and leptin from the hypothalamus of fed and fasted mice. Abstracts Frontiers in Neuroendocrinology 27 (1): 34–35. DOI: 10.1016/j.yfne.2006.03.070.

Piva F., Limonta P., Maggi R., Martini L. 1986. Stimulatory and inhibitory effects of the opioids on gonadotropin secretions. Neuroendocrinology 42 (6): 504–512. DOI: 10.1159/000124495.

Riediger T., Traeber M., Schmid H.A., Scheel C., Lutz T.A., Scharrer E. 2003. Site-specific effects of ghrelin on the neuronal activity in the hypothalamic arcuate nucleus. Neuroscience Letters 341 (2): 151–155. DOI: 10.1016/S0304-3908(02)01381-2.

Rosenblum P.M., Peter R.E. 1989. Evidence for the involvement of endogenous opioids in the regulation of gonadotropin secretion in male goldfish, Carassius auratus. General and Comparative Endocrinology 73 (1): 21–27. DOI: 10.1016/0016-6480(89)90051-8.

Smith M.J., Gallo R.V. 1997. Further studies on the suppression of luteinizing hormone release due to activation of...
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medial preoptic-anterior hypothalamic area µ-opioid receptors. Brain Research Bulletin 42 (1): 1–7. DOI: 10.1016/S0361-9230(96)00224-9.

Smith R.G., Van der Ploeg L.H.T., Howard A.D., Feighner S.D., Cheng K., Hickey G.J., Wyvrratt M.J.jr., Fisher M.H., Nargund R.P., Patchett A.A. 1997. Peptidomimetic regulation of growth hormone secretion. Endocrine Reviews 18 (5): 621–645. DOI: 10.1210/er.18.5.621.

Socha M., Sokolowska-Mikołajczyk M., Mikolajczyk T., Chyb J., Epler P. 2003. Naltrexone stimulates LH secretion in vitro from the common carp (Cyprinus carpio L.) pituitary gland. Archives of Polish Fisheries 11 (1): 5–16.

Sokolowska-Mikołajczyk M., Socha M., Mikolajczyk T., Chyb J., Epler P. 2002a. Seasonal short-term effects of naltrexone on LH secretion in male carp (Cyprinus carpio L.). Comparative Biochemistry and Physiology C 131 (3): 379–385. DOI: 10.1016/S1532-0456(02)00027-3.

Sokolowska-Mikołajczyk M., Socha M., Mikolajczyk T., Chyb J., Epler P. 2002b. The effects of morphine and naltrexone on the in vitro LH secretion from male and female carp pituitary gland. Comparative Biochemistry and Physiology C 141 (4): 325–331. DOI: 10.1016/j.cbpc.2005.09.001.

Sokolowska-Mikołajczyk M., Socha M., Szczerbik P., Epler P. 2009. The effects of ghrelin on the in vitro spontaneous and sGnRH-A stimulated luteinizing hormone (LH) release from the pituitary cells of common carp (Cyprinus carpio L.). Comparative Biochemistry and Physiology A 153 (4): 386–390. DOI: 10.1016/j.cbpa.2009.03.012.

Sokolowska-Mikołajczyk M., Socha M., Epler P. 2010. Steroid effects on the in vitro LH secretion from pituitary cells of sexually immature carp (Cyprinus carpio L.) under the influence of naltrexone and morphine. Czech Journal of Animal Science 55 (6): 340–347.

Trudeau V.L. 1997. Neuroendocrine regulation of gonadotrophin II release and gonadal growth in the goldfish, Carassius auratus. Reviews of Reproduction 2 (1): 55–66. DOI: 10.1530/ror.0.0020055.

Unniappan S., Peter R.E. 2004. In vitro and in vivo effects of ghrelin on luteinizing hormone and growth hormone release in goldfish. American Journal of Physiology: Regulatory, Integrative and Comparative Physiology 286 (6): R1093–R1101. DOI: 10.1152/ajpregu.00669.2003.

Unniappan S., Peter R.E. 2005. Structure, distribution and physiological functions of ghrelin in fish. Comparative Biochemistry and Physiology A 140 (4): 396–408. DOI: 10.1016/j.cbpa.2005.02.011.

Unniappan S., Lin X., Cervini L., Rivier J., Kaiya H., Kangawa K., Peter R.E. 2002. Goldfish ghrelin: molecular characterization of the complementary deoxyribonucleic acid, partial gene structure and evidence for its stimulatory role in food intake. Endocrinology 143 (10): 4143–4146. DOI: 10.1210/en.2002-220644.

Unniappan S., Canosa L.F., Peter R.E. 2004. Orexigenic actions of ghrelin in goldfish: feeding-induced changes in brain and gut mRNA expression and serum levels, and responses to central and peripheral injections. Neuroendocrinology 79 (2): 100–108. DOI: 10.1159/000076634.

Van Der Kraak G., Chang J.P., Janz D.M. 1998. Reproduction. Pp. 465–488. In: Evans D.H. (ed.) The physiology of fishes. CRC Press LLC, New York.

Vulliémoz N.R., Xiao E., Xia-Zhang L., Germond M., Rivier J., Ferin M. 2004. Decrease in luteinizing hormone pulse frequency during a five-hour peripheral ghrelin infusion in the ovarioctomized rhesus monkey. Journal of Clinical Endocrinology and Metabolism. 89 (11): 5718–5723. DOI: 10.1210/jc.2004-1244.

Weil C., Le Bail P.Y., Sabin N., Le Gac F. 2003. In vitro action of leptin on FSH and LH production in rainbow trout (Onchorhynchus mykiss) at different stages of the sexual cycle. General and Comparative Endocrinology 130 (1): 2–12. DOI: 10.1016/S0016-6480(02)00504-X.

Yen S.S., Quigley M.E., Reid R.L., Ropert J.L., Cetel N.S. 1985. Neuroendocrinology of opioid peptides and their role in the control of gonadotropin and prolactin secretion. American Journal of Obstetrics Gynecology 152 (4): 485–493.

Yilmaz B., Gilmore D.P., Wilson C.A. 1996. Inhibition of the pre-ovulatory LH surge in the rat by central noradrenergic mediation: Involvement of an anaesthetic (urethane) and opioid receptor agonists. Biogenic Amines 12 (5): 423–435.