XIII International
Congress of
Comparative
Endocrinology

Yokohama, Japan
16-21 November 199

The interrelationship of PMY, GABA and GnRH in the sea lamprey, *Petromyzon marinus*

J.K. Mac INTYRE, C. CHASE, S.A. TOBET * and S.A. SOWER

Department of Biochemistry
University of New Hampshire, Durham, New Hampshire (USA)
** Shriver Center and Harvard Medical School,*
Cambridge, Massachusetts (USA)

SUMMARY

The present study has determined that both PMY and GABA interact with the reproductive neuroendocrine axis of the sea lamprey. Lamprey GnRH-I and -III levels within the brain were elevated and circulating estradiol levels were suppressed in response to exogenous treatment of lamprey PMY in the female sea lamprey. Using an immunocytochemical procedure, GABA neurons were visualized in close proximity to developing GnRH neurons in the preoptic area/rostral hypothalamus, suggesting GABA may be influencing GnRH neuronal development similar to other vertebrates.

INTRODUCTION

Gonadotropin-releasing hormone (GnRH) is the major hypothalamic peptide which exerts regulatory control over reproductive events in all classes of vertebrates. In the sea lamprey, *Petromyzon marinus*, two forms of GnRH lamprey GnRH-I and -III, have been identified and their structures determined (cited in Sower, 1998). The ability of lamprey GnRH-I and -III to influence major events involved in sexual maturation and reproduction of the sea lamprey, such as steroidogenesis and spermiation and/or ovulation, has provided evidence for the importance of these two forms of GnRH in reproductive events of the lamprey (Sower, 1998).

Many factors have been identified in vertebrates which are able to modulate reproductive events through the influence on the hypothalamic-pituitary-gonadal axis. Two such modulators have been found to establish relationships with GnRH neurons from the earliest stages of their development. Thus neuropeptide Y(NPY) and γ-aminobutyric acid (GABA) have been shown to influence reproductive processes in vertebrates, and have been found in and around GnRH neurons during their migration in several different species. NPY is a 36 amino acid peptide that has been shown to act at the level of the hypothalamus and pituitary to alter GnRH and gonadotropin (GTH) release respectively (reviewed in Larhammar, 1996). Immunocytochemical studies have determined, that in both teleosts and mammals, NPY containing cells can be identified in close proximity to GnRH containing cells (Larhammar, 1996). Whether NPY exerts a stimulatory or inhibitory effect at either of these levels has proven to be highly dependent on the hormonal milieu. In teleosts, NPY is able to stimulate GnRH and GTH release from the hypothalamus and pituitary and potentiate GnRH induced GTH release when conducive steroidal conditions exist (Larhammar, 1996).

Recently, a NPY-like peptide, known as peptide methionine-tyrosine (PMY) was isolated first from the intestine and then from the brain of the sea lamprey (Conlon et al., 1991; 1994). PMY is structurally more similar to NPY than other NPY-family members as it has the same amino acid residues at key positions identified in all other vertebrate forms of NPY (Conlon et al., 1991). Preliminary studies indicated that PMY was able to suppress estradiol levels in female sea lamprey (Conlon et al., 1994). The significance of PMY in the reproductive cycle of the sea lamprey remains to be determined.

The inhibitory neurotransmitter γ-aminobutryic acid, GABA, is distributed somewhat ubiquitously throughout the central nervous system. GABA and agents which alter GABAergic function significantly affect reproductive processes through their action at the hypothalamus and pituitary. In mammals, GABA containing neurons have been visualized along the GnRH neuronal migratory pathway, and in some cells GnRH and GABA were coexpressed(Tobet et al., 1996a). We recently delineated the development of GnRH containing neurons in the prolarval and larval sea lamprey (Tobet et al., 1996). Further examination of factors, such as GABA, which may potentially influence development of GnRH containing cells is necessary in order to expand our understanding of the development of the reproductive system in the sea lamprey.

The objective of this study is two-fold. First, this study examined the effects of PMY on brain lamprey GnRH-I and -III and plasma estradiol levels in the adult female sea lamprey during the final reproductive season. Secondly, we have begun exploring the developmental relationship between GABA and GnRH in the larval sea lamprey.

MATERIALS AND METHODS

Adult landlocked or sea run female sea lampreys were obtained from Hammond Bay Biological Station, Michigan, USA or the Cocheco River, New Hampshire, respectively, during May 1995 and 1996 and transported to the Anadromous Fish and Aquatic Invertebrate Research (AFAIR) Laboratory, University of New Hampshire, Durham, N.H. The lampreys were maintained in an artificial stream, supplied with flow-through reservoir water, under natural photoperiod and at ambient temperature.

Adult landlocked female sea lamprey received a single intraperitoneal injection of saline (control) or saline containing synthetic PMY. In 1995, each treatment group, consisting of ten lamprey, was injected with either 0.6% saline or a does of 0.05, 0.10 or 0.15 μg PMY/g lamprey and four lamprey were injected with 0.15 μg PMY/g lamprey. Four hours after injection, blood, brain and gonad tissue samples were taken. Plasma was assayed for 17β-estradiol levels by radioimmunoassay. Brains were stored at -80°C until extracted, eluted by HPLC and assayed for lamprey GnRH-I and -III by RIA.

Embryonic sea lamprey were produced by stripping several hundred eggs from mature females and fertilized with sperm from mature males at the AFAIR

laboratory. The developing lamprey were staged according to Piavis (1961). Two groups of ten lamprey were collected every ten days and each group was fixed in either acrolein or Bouins-Hollande sublimate (BHS). Whole heads of individual lamprey, 10-40 days old, were processed and immunocytochemical procedures for GnRH and GABA experiments were adapted from procedures previously described (Tobet et al., 1996b).

RESULTS AND CONCLUSIONS

This study is the first to examine the influence of lamprey PMY on the hypothalamus of the sea lamprey. In both the 1995 and 1996 reproductive seasons lamprey GnRH-I and -III levels were elevated in response to all doses of PMY (fig.1). The ability of PMY to elevate brain lamprey GnRH-I and -III content is consistent with the function of NPY observed in other vertebrates.

In both 1995 and 1996, brain lamprey GnRH-III content was significantly higher than brain lamprey GnRH-I content for all PMY treatment groups including controls. In the sea lamprey the evidence to date has indicated that lamprey GnRH-I and -III both act as neurohormones involved in the regulation of reproductive processes (Sower, 1998). Recent work in our laboratory has shown however, that the brain content of lamprey GnRH-III remains significantly higher than that of lamprey GnRH-I for the majority of the reproductive season (MacIntyre and Sower, unpublished). This would account for the significantly higher lamprey GnRH-III levels observed in this experiment.

Administration of PMY resulted in a decrease in circulating plasma estradiol levels. Significant decreases in plasma estradiol levels were observed with doses of 0.10 and 0.15 µg PMY/g lamprey in 1995 and at a dose of 0.05 µg PMY/g lamprey in 1996. These results are in agreement with those reported by Conlon et. al (1994) in which a dose of 0.15 µg PMY/g lamprey also resulted in a decrease in plasma estradiol. It is undetermined whether PMY altered estradiol concentration through direct action at the ovaries or if PMY affected pituitary function. A common trend existed in the 1995 and 1996 seasons in which estrogenic responsiveness was observed at the doses in which there were no significant elevations in GnRH.

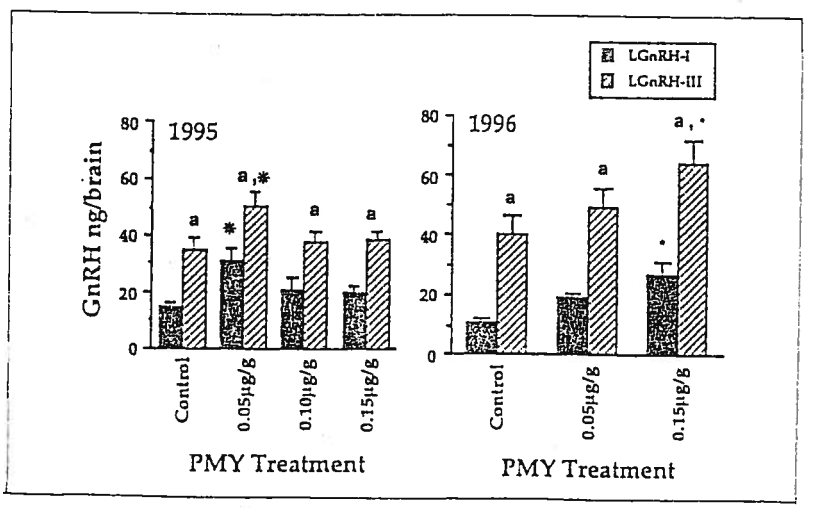

Figure 1. Mean brain lamprey GnRH-I and -III levels (ng/brain) in female sea lamprey injected with saline (control), 0.05, 0.10 and 0.15 ug PMY/g lamprey in 1995 (upper graph) and injected with saline (control), 0.05 and 0.15 ug PMY/g lamprey in 1996 (lower graph). Bars depict +/- SEM. Significant differences within group are indicated with a * and between groups with a 'a'($P < 0.05$).

Cells containing GABA immunoreactivity (GABA-ir) were distributed throughout the developing lamprey brain within 10 days of fertilization. As development progressed the populations of GABA neurons became more distinct. Twenty days after fertilization three distinct populations of cells containing GABA-ir were visualized. These populations were located in the olfactory bulb/telencephalon region, within the diencephalon, specifically the preoptic area/rostral hypothalamus, and in the midbrain. In the preoptic area/rostral hypothalamus, cells were distributed in the periventricular region. This pattern of GABA distribution was observed until the oldest age examined, 50 days after fertilization.

The development of GnRH containing neurons within the brain of larval lamprey has been examined previously (Tobet et al., 1996b). In the sea lamprey, unlike other vertebrates, GnRH neurons do not appear to originate in the olfactory placode but rather GnRH cells seem to originate in the cell dense proliferative zone of the diencephalon (Tobet et. al 1996b). Cells containing GABA-ir were found in the same general region of the diencephalon in which GnRH containing cells appear to originate. Immunoreactive GnRH was found in the same distribution within the preoptic area/rostral hypothalamus as previously reported. Cells containing GnRH-ir were again not observed in the olfactory placode. Cells containing GABA-ir in the hypothalamus of sea lamprey at day 10-20 appeared to be in the same general region in which GnRH cells arose between 20 and 30 days after fertilization. Examination of closely matched sections suggested that cells containing GABA-ir and GnRH-ir in the rostral hypothalamus and preoptic area were closely opposed, but segregated from the earliest appearance of GnRH. The close proximity of cells containing GnRH-ir and GABA-ir during the development of the sea lamprey suggests that GABA may play a significant role in the establishment and development of GnRH neurons.

In conclusion, this study determined that PMY appears to play a significant role in the regulation of reproductive processes of the adult female sea lamprey. In addition, the close proximity of GnRH and GABA containing cells suggests a possible developmental relationship between these two factors.

REFERENCES

CONLON, J.M., BJORNHOLM, B., JORGENSEN, F.S., YOUSON, J.H. and SCHWARTZ, T.W. Primary structure and conformational analysis of peptide methionine-tyrosine, a peptide related to neuropeptide Y and peptide YY isolated from the lamprey intestine. Eur.J. Biochem. 199: 293-298. 1991.

CONLON, J.M., BALUSUBRAMANIAM, A. and SOWER, S. Purification of a neuropeptide Y-related peptide from the brain of the sea lamprey and its effects on steroidogenesis. Reg. Peptides. 50:167-175. 1994.

LARHAMMAR, D. Evolution of neuropeptide Y, peptide YY and pancreatic polypeptide. Reg. Peptides. 62:1-11. 1996.

PIAVIS, G.W. Embryological stages in the sea lamprey and effects of temperature on development. Fishery Bulletine Fish and Wildl. Serv. 61: 111-143. 1961.

SOWER, S.A. Brain and pituitary hormones of lampreys, recent findings and their evolutionary significance. American Zoologist. In Press. 1998.

TOBET, S.A., CHICKERING, T.W. KING, J.C., STOPA, E.G., KIM, K., KUO-LeBLANK, V. and SCHWARTING, G.A. Expression of γ-aminobutyric acid and gonadotropin-releasing hormone during neuronal migration through the olfactory system. Endocrinology.137:5415-5420. 1996a.

TOBET, S.A.,. CHICKERING, T.W. and SOWER, S.A. Relationship of gonadotropin-releasing hormone (GnRH) neurons to the olfactory system in developing lamprey, *Petromyzon marinus*. J. Comp. Neurol. 376:97-111. 1996b.