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Induced Ovulation in Catfish *Heteropneustes fossilis* (Bloch) with Three Native GnRH Peptides

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The effect of three native GnRH peptides, viz; salmon GnRH (sGnRH), mammalian GnRH (mGnRH) and chicken GnRH II (cGnRH II), on spawning of the catfish *Heteropneustes fossilis* (Bloch) was compared. D-Lys^{6}sGnRH, was used as the control. The peptides were synthesized by the Merrifield solid phase method, purified by HPLC and reconstituted in saline. The fish were injected intramuscularly. cGnRH II was more potent than both sGnRH and mGnRH in inducing spawning in this species of catfish. There was no significant difference in the average diameter of the egg obtained using the three peptides.

Key words: Native GnRH peptides, induced spawning, mGnRH, sGnRH, cGnRH II, catfish, *Heteropneustes fossilis*

Gonadotropin Releasing Hormone (GnRH), a decapeptide synthesized and released from the hypothalamus, is a key molecule in vertebrate reproduction. It stimulates the release of gonadotropins from the anterior pituitary, which in turn regulate gonadal maturation and function. There is a remarkable homology in the sequence of amino acids among the five forms of GnRH molecule that are known to exist in vertebrates. Two or more forms often exist within the brain of a single species (King & Millar, 1990; Habibi *et al.*, 1992). Habibi *et al.* (1992) report that although both salmon GnRH (sGnRH) and chicken GnRH II (cGnRH II) stimulate gonadotropin (GtH) as well as growth hormone (GH) release from the goldfish pituitary, cGnRH II is more active in releasing GtH whereas sGnRH tends to have more potency in terms of growth hormone release. However, in masu salmon *Oncorhynchus masou masou*, sGnRH alone is involved in ovarian maturation through regulation of GtH synthesis and release while cGnRH II has little or no involvement in reproduction (Amano *et al.*, 1992).

The purpose of the present study was to compare the efficacy of three native GnRH peptides, namely, mammalian GnRH (mGnRH), sGnRH and cGnRH II on induced ovulation in catfish *Heteropneustes fossilis* (Bloch). An important food fish of India, this species is also known for its therapeutic properties (Jhingran, 1991). However, a major constraint in popularizing its culture is the unavailability of seed in sufficient quantities.

Materials and Methods

Fish *H. fossilis* (25-35 g body weight) were procured from the local fish market and were acclimated to laboratory conditions (24-26°C) for 3 weeks. They were then transferred to aquaria maintained at 30°C for photothermal (12 h day and 12 h night) treatment for a period of 4 weeks. sGnRH, mGnRH and cGnRH II were synthesized in the protein chemistry laboratory of the institute by the Merrifield solid phase method (Rivier *et al.*, 1986). The peptides were purified by HPLC. They were reconstituted in 0.9% saline to prepare stock

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solutions of 1 mg/ml, which were further diluted in saline to obtain the desired concentrations of 10, 50 and 100 mg/kg body weight for each of the peptides. Mature females selected on the basis of soft and distended bellies were transferred to experimental tanks (n=10/dose). They were injected intramuscularly; the volume of injection was maintained at 1µl/g body weight for all treatments. D-Lys^{6}sGnRH analogue was injected at a dose of 25 µg/kg body weight as a positive control. Diameter of the eggs was measured with a Nikon binocular microscope fitted with a micrometer.

Results and Discussion

All the three peptides studied could induce ovulation in *H. fossilis*. However, the fish did not spawn on their own and had to be hand stripped at all concentrations except at the highest concentration of cGnRH II. cGnRH II induced spawning in 90% of the fish at 100 mg/kg body weight without handstripping. It was more potent than sGnRH at all doses. sGnRH and mGnRH also induced spawning at all doses when gentle pressure was applied to release the eggs. However, mGnRH was the least effective among the three peptides (Table 1). More pressure had to be applied for release of eggs and their number was relatively less. Spawning was 100% in the control (D-Lys^6 sGnRH) and the fish did not require hand stripping at the dose used (25 mg/kg b. wt.). There was no significant difference in the average diameter of the egg between the three treatments and also, among the different doses of the same treatment. In the control, eggs were of uniform size and translucent while in the treatments with different GnRH, they were not uniform and dead eggs were also observed. In fish induced with cGnRH II, the number of eggs released was about 20 times more than in the other two treatments.

All the three native GnRH peptides can hasten final maturation and ovulation in *H. fossilis* as observed in the present study, indicating that catfish pituitary may have a broad specificity in its hormone releasing potency. cGnRH II was more effective in inducing spawning compared to sGnRH and mGnRH. A similar observation was made by Ngamvongchon *et al.* (1992 b) in Thai catfish *Clarias macrocephalus* (Gunther), where structure-function studies of five natural forms of GnRH and eight analogues on reproduction revealed cGnRH II to be significantly more effective at a dose of 300 mg/kg than the control for the induction of ovulation. cGnRH II was more active than sGnRH., mGnRH, cGnRH I and lamprey GnRH

Table 1. Comparison of the efficacy of three different GnRH peptides on the induced ovulation in the Indian catfish *Heteropnuestes fossilis* (Bloch)

No.	Treatment	Dose (µg/kg)	Wt. of fish (g)	Percentage of animals spawned (%)	Average dia. of egg (mm)
		10		60	0.966±0.05
1	sGnRH	50	25-30	60	0.986±0.05
		100		80	1.0±0.1
		10		40	0.986±0.II
2	mGnRH	50	25-30	60	0.966±0.04
		100		70	0.91±0.09
		10		80	0.97±0.06
3	cGnRH II	50	25-30	80	1.0±0.02
		100		90*	1.0±0.07
4	D-Lys^{6}sGnRH	25	25-30	100*	1.1±0.03

* Spawned without handstripping

(lGnRH) in releasing gonadotropins from perifused fragments of pituitary in goldfish (Habibi *et al.*, 1992). Two forms of GnRH (catfish GnRH –I and –II) purified from brain extracts of the catfish *C. macrocephalus* (Gunther) showed catfish GnRH-II (cfGnRH-II) to be identical to cGnRH-II (Ngamvongchon *et al.*, 1992a). Rebers *et al.* (1995) also report the presence of two forms of GnRH, namely, cGnRH-II and cfGnRH in the brain and pituitary extracts of African catfish, *Clarias gariepinus* (Burchell). Of these, cGnRH-II showed higher affinity to pituitary GnRH receptor. The greater potency of cGnRH II compared to sGnRH and mGnRH in *H. fossilis* observed in the present study may suggest the presence of a similar kind of GnRH in *H. fossilis*.

cGnRH II is highly conserved and the most widespread GnRH peptide occurring in amphibians, reptiles, birds and early evolved mammals (King & Millar, 1990). cGnRH II is present in all classes of fishes. It coexists with dogfishGnRH (dfGnRH) in chondrichthyes, with mGnRH in primitive osteichthyes and some primitive teleosts, with sGnRH in most teleosts and with cfGnRH in catfishes. It is also present in Perciformes, the recent teleosts (Montero & Dufour, 1996).

To date, most studies on induced spawning in fish using GnRH analogues have used analogues of mGnRH and sGnRH. The results are often variable. Potency of an analogue shows species specificity. In the present study, cGnRH II was seen to be a better agent for inducing spawning in *H. fossilis* than sGnRH and mGnRH. This is probably the first report of induced ovulation and spawning in the catfish *H. fossilis* with cGnRH-II. Since cGnRH II is present in most fish species and appears to be the most potent among other native peptides in comparative studies, induced spawning of fish using analogues of cGnRH II may have considerable potential in aquaculture and needs to be investigated.

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