Bull. Inst. Zool., Academia Sinica **23**(1): 131-136 (1984)

IMMUNOREACTIVE LUTEINIZING HORMONE-RELEASING HORMONE IN SOME FRESHWATER FISHES OF TAIWAN

WALTER CHIA-MO WAN, REN-SUN PAN, DAN-YANG LIN AND MON-CHOU FANN

Institute of Zoology, Academia Sinica, Taipei, Taiwan 115, Republic of China

(Received March 15, 1983)

Walter Chia-Mo Wan, Ren-Sun Pan, Dan-Yang Lin and Mon-Chou Fann (1983) Immunoreactive luteinizing hormone-releasing hormone in some freshwater fishes of Taiwan. *Bull. Inst. Zool. Academia Sinica* **23**(1): 131-136. Seven species of living freshwater fishes of Taiwan were used for this study. The pituitaries (P) and the area of the hypothalamus (H) of all fishes, but the brains of pond loachs (*Misgurnus anguillicaucatus*) and eels (*Anguilla japonica*), were removed following decapitation. The tissues from the same species and the same sex were pooled, dehydrated and homogenized in cool acetone. The acid extracts (E) were subjected for luteinizing hormone releasing hormone (LHRH) measurements by a double antibody radioimmunoassay (RIA). Immunoreactive luteinizing hormone-releasing hormone-like hormone (Ir-LHRH) has been found in H (and the brains of pond loach and eel), for their dose-response curves parallel to the inhibition curves of the reference materials, statistically. Except the PE of female of white spotted catifishes (*Clarias batrachus*) and both sexes of golden carps (*Cyprinus carpio*) which showed similar phenomenone as in HE, in the other species only interference to the labelled LHRH bindings was observed. The measurements of ir-LHRH are (1) *C. batrachus* ♀ HE: 1130 pg/gland, ♀ PE: 368 pg/gland; ♂ HE: 425; (2) gold fish (*Carassius auratus*) ♀ HE: 718; ♂ HE: 1166; (3) golden carp ♀ HE: 256, ♀ PE: 192; ♂ HE: 333, ♂ PE: 87; (4) *Oreochromis* sp. ♀ HE: 42, (5) snakehead (*Channa maculatus*) ♀ HE: 106. The ir-LHRH in the brain E of *M. anguilicaudatus* is 125, in that of *A. japonic* is 181. It is possible that the presence of ir-LHRH in the P is from the contamination of H tissue, for the H tissue is interdigitated with the P anatomically; and ir-LHRH may also possible be transformed into other functional-like but immunoactive-unlike molecules in the process of transportation from H to P.

The hypothalamic hypophysiotrophic peptides have been studied in many species, and the significances of their physiological importance have also been proved repeatedly. The chemical structures of luteinizing hormone-releasing hormone (LHRH) in fish is not identical to that of ovine or porcine origin (Barnett *et al* 1982), but evidences provided by others indicated that normal function of pituitary depends on its stalk in connection with the hypothalamus (Johansen, 1967; Ball *et al.* 1972). Crim, Dickhoff and Gorbman (1978) reviewed the piscine hypothalamic hypophysiotrophic peptides and indicated that many investigators have shown the pressence of immunoreactive luteinizing hormone-releasing hormone (ir-LHRH) in the brain of many Osteichytes and the release of gonadotropin (GTH) in response to hypothalamic

extract (HE) or synthetic LHRH. The Synthetic LHRH induced GTH release has been confirmed *in vivo* (Peter, 1980) and *in vitro* (Crim and Evans, 1980). Nevertheless, Deery (1974) could not detect LHRH in HE of goldfish and dogfish by a radioimmunoassay (RIA) system.

In this report, we use a RIA system with a sensitivity of 10 pg (Wang *et al.* 1983) to measure ir-LHRH in HE and pituitaries of the aquaculture fishes that are available in Taiwan.

MATERIALS AND METHODS

Six species of living freshwater fishes which cultivated in northern part of Taiwan, were purchased from local market, they were kept in running water for about eleven hours before reaching to our laboratory. While pond loach (*Misgurnus anguillicaudatus*) were caught and kept in an aquarium for about three months.

Three females and 3 males of white spotted catfish (*Claria batrachus*), 4 females and 1 male of gold fish (*Carassius auratus*), 3 females and 4 males of golden carp (*Cyrinus carpio*), 6 females of tilapia (*Oreochromis* sp.), 3 pond loach and 3 eel (*Anguilla japonica*) were decapitated right after arrival to the laboratory. The hypothalamus and the pituitaries of the fishes were removed, and put right to precooled acetone for dehydration, while the brains of pond loachs and eels, were similiarly treated. The tissue weights were then estimated by weighing the dehydrated tissue before homogenizing with a glass homogenizer thoroughly in acetone. The samples were boiled in a 100°C water bath to dryness, and extracted by 1.0 ml of 0.1 N HCl and homogenized once again. After centrifugation (3000 rpm 30 min, 4°C) the supernatant were neutralized by 0.1 N NaOH and centrifuged once again. The supernatant were subjected to radioimmunoassay (RIA) measurement.

Synthetic LHRH (Sigma Co.) were used to radioiodination and standard references. The method of radioiodination was followed the description of Koch *et al.* (1976) with some modifications. Four μg of LHRH were dissolved in 40 μl doubled distilled water and mixed with 50 μl of neutralization buffer (0.5 M PB, pH 6.9) in a small glass tube, 0.5 mCi of Na ^{125}I (New England Nuclear) was then added to the mixture. Following the addition of Chloramin T (100 μg in 200 μl of 0.05 M PB, pH 6.9), the mixture was shaked for 90 sec and $Na_2S_2O_5$ (125 μg in 100 μl of 0.05 M PB, pH 6.9) was then added to stop the reaction. Two hundred μl of transfer solution (0.03 M KI-16% sucrose in 0.05 M) was put into the reaction tube and the mixture was transfered to the top of a pre-packed Sephadex G-10 column 1.0×20.0 cm. Rinse solution (0.03 M KI-8% sucrose in 0.05 M PB) was used to wash the reaction tube and also put into the column for purification.

The anti-sera for RIA, both anti-LHRH antiserum and anti-rabbit gamma globulin, are gifts from Dr. P. S. K. Wang (Yang-Ming Medical College, Taipei). The characteristics of antiserum has been described and not cross reacted with the other hypothalamic peptides, the sensitivity is 10-12 pg (Wang *et al.* 1982). The procedure for RIA were followed the descriptions of Koch *et al.* (1976), only the volume was reduced to 1/10 (Follet *et al.*, 1972, Liao and Wan, 1983). The coefficients of variance of within and between assays were 4.0% and 8.2%, respectively.

RESULTS AND DISCUSSIONS

Many investigators indicated the existence of LHRH in the brains of many fishes but the results were varied (see review by Crim *et al.*, 1978). Deery (1974), used a RIA procedure with a sensitivety of 80 pg, reported that ir-LHRH is absent in HE of gold fish and dogfish. In our system, with a sensitivity of 10 pg, the dose-radioactive binding curves of the HE (or brain extract of pond loachs and eels) of the Taiwan freshwater fishes indicated demonstrated a paralellism to the inhibition curve of synthetic LHRH (Table 1 and Fig. 1). The paralellism of the curves is tested by Student's *t*-test to find out whether the linear regression

TABLE I.

Radioimmunoassayble LHRH in the pituitaries and hypothalami of some freshwater fishes in Taiwan[1].

Species	Sex	BW (g)	GSI[2]	LHRH concentration						numbers of fishes
				Hypothalamus			Pituitary gland			
				parallelism[3]	pg/gland	pg/mg[4]	parallelism	pg/gland	pg/mg[4]	
Clarias batrachus	♂	560.0±23.0	0.12- 0.31	P	425[5] (247-603)	142 (97-187)	NP	?	?	3
	♀	543.3±18.5	1.37- 9.71	P	1130	297	P	368 (156-589)	90 (38-142)	3
Carassius auratus	♂	70.0	2.14	P	1166	436	NP	?	?	1
	♀	80.0±13.5	7.44-11.12	P	718 (360-1078)	365 (153-567)	NP	?	?	4
Cyprinus carpio	♂	190.0±40.2	0.33- 0.90	P	333 (227-440)	202 (128-266)	P	87 (92-112)	28 (20-36)	4
	♀	160.0±100.0	2.40- 4.31	P	256 (183-328)	346 (262-469)	P	192 (182-202)	202 (192-213)	2
Oreochromis sp.	♀	545.0±46.8	0.14- 0.56	P	42 (18-67)	10 (4-15)	NP	?	?	6
Channa maculatus	—	336.6±13.3	0.31- 3.37	P	106 (92-120)	40 (35-45)	NP	?	?	3
Misgurnus anguillicaudatus	—	11.9± 1.3	0.47- 0.59	P	125[6] (112-139)	9[6] (8-10)				3
Anguilla japonica	—	430.0±30.5	0.15- 0.57	P	181[6] (138-225)	14[6] (13-16)				3

(1) measured by synthetic LHRH RIA system.
(2) GSI=(weight of gonad/body weight)×100.
(3) parallelism to the respective standard curves by *t* test at the level of $P<0.05$; NP: Nonparallel; P: parallel.
(4) The wt of hypothalami were obtained after 8-10 min acetone dehydration.
(5) Mean values of repeated measurements from pooled samples and its range in parentheses with numbers of fish indicated as the last column of this table. ?=indicates the presence of inhibiting effect on antigen-antibody reaction, but not suitable for quantitive estimation
(6) Whole brain.

coefficient, differ, significantly, or not from the commonly curves. The cross-reactivity is, standard agreed, to indicate that substance(s) is in the extract with a similar immunoactivity with the synthetic decapeptide, that is, ir-LHRH-like substance. We still adopte the abbreviation as ir-LHRH. It is interesting to note that much less ir-LHRH is detected in snakehead, pond loach and eel while their gonads are too small to identify grossly for their sex, although the gonad-somatic index for sexual maturation has different criteria for different species.

In the pituitary extract of female catifish and both sexes of golden carp, the dose-radioactive binding curves paralell to the standard curves of the synthetic LHRH (Table 1 and Fig. 2). In the other species, the pituitary extracts interfere the binding of LHRH with its antiserum, but no paralellism was observed (Table 1). It is known that the hypothalamic tissue is interdigitated with pituitary in many species (Zambrano *et al.*, 1972), thus in dissecting pituitaries the contamination of hypothalamic tissue can not be completely avoided. It is possible this contamination contributes the ir-LHRH that detected

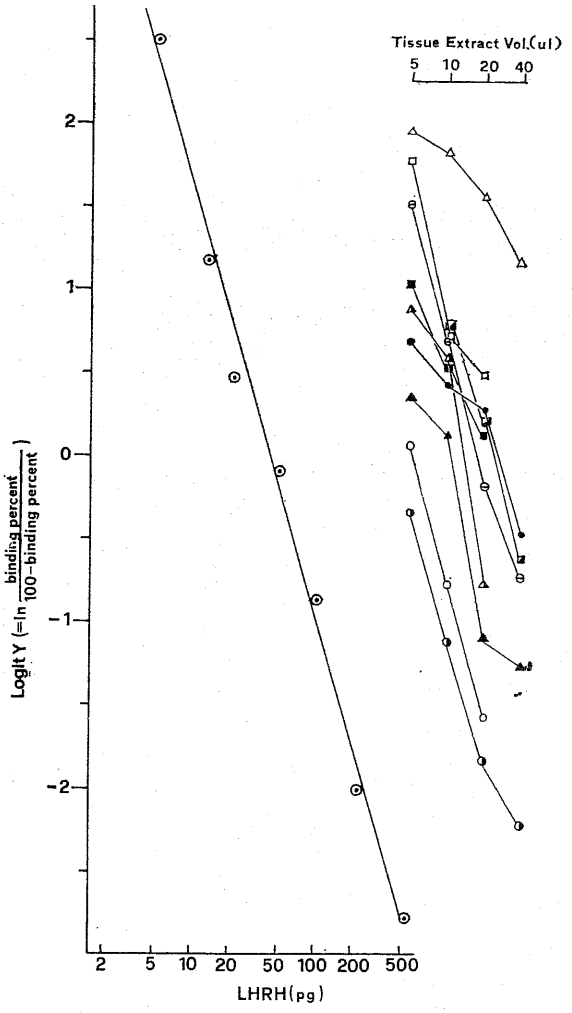

Fig. 1. Immunoreactive LHRH in the hypothalamic extract of △ (♂) ○ (♀) *C. batrachus*, ◭ (♂) ◑ (♀) *C. auratus*, ▲ (♂) ● (♀) *C. carpio* ⊖ *oreochromis* sp. □ *C. maculatus* and brain extract of ◪ *M. anguillicaudatus* and ■ *A. japonica*. ⊙—⊙ a standard inhibition curve of synthetic LHRH.

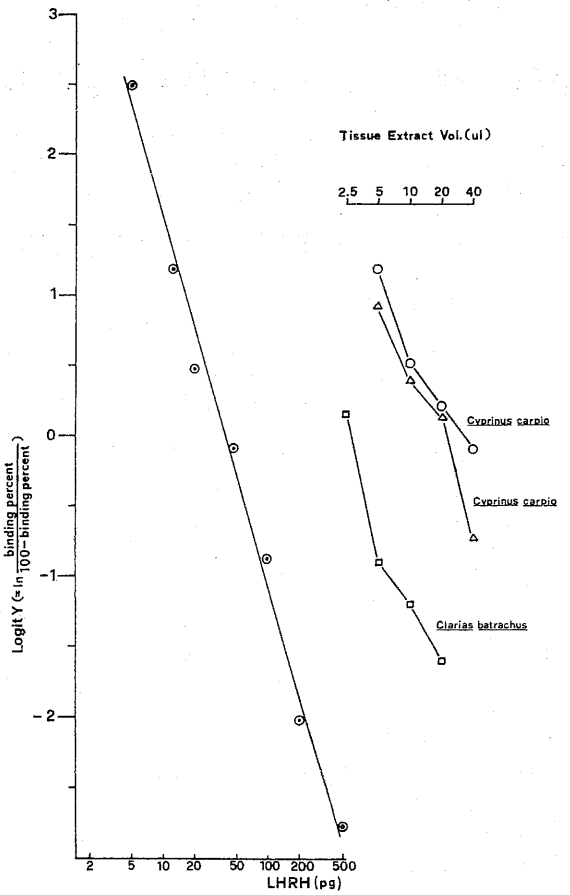

Fig. 2. Immunoreactive LHRH in the pituitary extract △ (♀), ○ (♂) *C. carpio* □ (♀) *C. batrachus*. ⊙—⊙ a standard inhibition curve of synthetic LHRH.

in the pituitary extract.

Schreibman *et al.* (1979) demonstrated the presence of LHRH in and between the gonadotrophs of pars intermedia in teleosts, and in mammals, it was also demonstrated that LHRH receptor on the membrane of gonadotrophs in the pituitary thus the existance of LHRH molecule seems ascertained. The chemical structure of LHRH in fish has been identified, which is not identical with the synthetic LHRH (Barnett *et al.* 1982), it is very possible that the difference in the structure of LHRH of fish will have different immunoactivity, in term of immunoreaction with anti-synthetic LHRH antiserum. This antiserum may not very suitable to measure LHRH in some fish, especially that in the gonadotrophs of some fish pituitaries.

In our experiments except female catifish and both sexes of golden carp, the pituitary extracts of other species demonstrated the fact that a certain material is present in the pituitary only with the ability to interfere the binding of labelled synthetic LHRH with its antibody, but no competitivity. It may suggest that the hypothalamic LHRH of fish had been modified to other structure in or during entering the gonadotrophic cells for physiological means.

REFERENCES

BALL, J. N., B. K. BAKER, M. OLIVEREAU and R. E. PETER (1972) Investigations on hypothalamic control of adenohypophysial functions in teleost fish. *Gen. Comp. Endocr. Suppl.* **3**: 11-21.

BARNETT. F. H., J. JOHN, S. REICHLIN and I. M. D. JACKSON (1982) Three LHRH like substances in a teleost fish brain, none identical with the mammalian LHRH decapeptide. *Biochem. Biophys. Res. Commun.* **105**: 209-216.

CRIM, J. W., W. W. DICKHOFF and A. GORBMAN (1978) Comparative endocrinology of piscine hypothalamic hypophysiotropic peptides: distribution and activity. *Amer. Zool.* **18**: 411-424.

CRIM, L. W. and D. M. EVANS (1980) LHRH stimulated gonadotropin release from the rainbow trout pituitary gland: an *in vitro* assay for detection of teleost gonadotropin releasing factor (s). *Gen. Comp. Endocrinol.* **40**: 283-290.

DEERY, D. J. (1974) Determination by radioimmunoassay of the luteinizing hormone-releasing hormone content of the hypothalamus of the rat and some lower vertebrates. *Gen. Comp. Endocrinol.* **24**: 280-285.

FOLLETT, B. K., C. G. SCANES and F. J. CUNNINGHAM (1972) A radioimmunoassay for avian luteinizing hormone. *J. Endocrinol.* **52**: 359.

JOHANSEN, P. H., (1967) The Role of the pituitary in the resistance of the goldfish (*Carassius auratus* L.) to a high temperature. *Can. J. Zool.* **45**: 329-345.

KOCH, Y., M. WILCHEK, M. FRIDKIN, P. CHOBSIENG, U. ZOR and H. R. LINDNER (1973) Production and characterization of an antiserum to synthetic gonadotropin-releasing hormone. *Biochem. Biophys. Res. Commun.* **55**: 616-622.

LIAO, C. F. and W. C. M. WAN (1983) Serum gonadotropic changes in thyroidectomized rats following orchidectomy or LRH Challenge. *Bull. Inst. Zool. A*cademia Sinica. **22**(1): 49-56.

PETER, R. E. (1980) Serum gonadotropin levels in mature male goldfish in response to luteinizing hormone (LHRH) and des-Gly^{10}-(D-Ala^{6})-LHRH ethylamide. *Can. J. Zool.* **58**: 1101-1104.

SCHREIBMAN, M. P., L. R. HALPERN, H. J. TH. GOVS and H. MARGOLIS-KAZAN (1979) Identification of luteinizing hormone-releasing hormone in the brain and pituitary gland of a fish by immunocytochemistry. *J. Exp. Zool.* **210**: 153-160.

WANG, P. S. K., W. H. YU and Y. Y. CHEN (1983) Radioimmunoassay for gonadotropin-releasing hormone: evaluation of method and applications. *Chinese J. Physiol.* **26**: (In press).

ZAMBRANO, D. (1972) Innervation of the teleost pituitary. *Gen. Comp. Endocr. Suppl.* **3**: 22-31.

臺灣淡水魚類之性釋素免疫反應測定

萬家茂　潘潤生　林端陽　范夢樵

本篇報告是用七種臺灣常見的淡水魚（泥鰍、錦鯉、金魚、吳郭魚、鰻魚、 鯰魚及鱧魚），其中泥鰍與鰻魚是取腦外，其餘五種均分別取出腦下腺及下視丘，置於冷丙酮，研磨、 脫水，再以酸萃取。此酸萃取液以放射免疫法（RIA）測量性釋素的含量。結果發現，在各種魚的下視丘（泥鰍及鰻魚的腦中）均可發現有性釋素的免疫活性， 其劑量反應曲線與標準物質之抑制曲線相平行。在腦下腺只有雌鯰魚及錦鯉（兩性）有相同的現象，其他的魚只能觀察到萃取液對標準性釋素的結合性有干擾， 但不平行。在腦下腺中可測到性釋素的存在，可能是受下視丘組織污染所造成的， 因就解剖學來看， 下視丘組織與腦下腺是相連的； 且性釋素在下視丘運送到腦下腺時， 可能會轉變成功能酷似而免疫性不同於性釋素的分子。