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THE DIFFERENCE IN THE DEGREE OF STEROID-BINDING ACTIVITY IN FISHES

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Ching-Fong Chang and Mei-Ru Chen (1990) The difference in the degree of steroid-binding activity in fishes. *Bull. Inst. Zool., Academia Sinica* **29**(1): 41–47. The degree of steroid-binding activity in the plasma bound to testosterone, estradiol-17β, progesterone and cortisol varied in 38 species of fishes. The majority of species examined (65.7%) had a high degree of sex steroid-binding activity. High degree of progesterone-binding activity and cortisol-binding activity were observed in 10.5% and 2.6% of the species studied, respectively. The degree of steroid-binding activity in fishes differed from that of mammals. Most species of mammals have high degree of cortisol-binding activity but with low degree of other steroid-binding activity. No significant degree of steroid-binding activity was found in the hemolymph of 4 species of shrimps. No sexual difference and regular circardian rhythm in the degree of steroid-binding activity in common carp were observed.

Key words: Fish, Steroid, Steroid-binding protein.

Estradiol-17β, androgens, progestogens and corticosteroids are known to involve in reproductive processes in teleosts. Specific steroid-binding proteins may have some significance in altering free hormone levels and the concentration of hormone available to target tissues. The binding between protein and steroid is with high affinity and limited capacity. It has been shown that the steroid-binding protein retards steroid metabolism (Baird *et al.*, 1969). Pasmanik and Callard (1986) indicated that the interaction between steroid-binding protein and steroid is very fast and quite unstable, displaying a maximal binding and a half-time dissociation within minutes in goldfish. Sex steroid (testosterone and estradiol-17β)-binding protein has been found in the plasma of several teleost species including salmon (Freeman and Idler, 1971; Lazier *et al.*, 1985), carp (Corvol and Bardin, 1973), brown trout (Pottinger, 1988), rainbow trout (Fostier and Breton, 1975) and goldfish (Pasmanik and Callard, 1986). Sex steroid-binding protein in goldfish has been partially characterized (Pasmanik and Callard, 1986). However, there is little information available about the different degree of this binding protein in various species of fishes. The existence of progesterone- and cortisol-binding proteins in fishes is also less clear.

The objective of the present study was to determine the different degree of steroid-binding activity (estradiol-17β, testosterone, progesterone and cortisol) in several species of fishes. The degree of binding activity in fishes was also compared to that of shrimps and mammals.

MATERIALS AND METHODS

A. Sample collection

Thirty-eight species adult fishes were obtained from culture ponds or by fishing vessel operated in the coastal waters in Taiwan. Each species was represented by at least 3 specimens. Four species of adult shrimps ($n=6$) and adult mammals (human, pig, dog, rabbit and mouse $n=6$) were sampled. To investigate the difference between sexes, one-year-old female ($n=13$, mean body weight$=48.5\pm2.8$g) and male ($n=13$, mean body weight $=42.6\pm2.6$g) common carps reared in the same condition were obtained. To study the binding activity profiles of circadian rhythm, six female common carps (mean body weight$=197.0\pm2.0$g) kept under 12 hours light and 12 hours dark photoperiod (12L/12D) for 14 days were bled every 8 hours interval for 48 hours from the beginning of 1.5 hours after the onset of light photoperiod. Blood was collected by caudal puncture with a heparinized syringe and plasma was obtained by centrifugation. Hemolymph was collected from pericardiac puncture in shrimp. Hemolymph and plasma were stored at −20°C until analysis.

B. Quantification of the steroid-binding activity

Aliquotes of 100 μl of charcoal treated-plasma or hemolymph (diluted 1:5 with buffer) were incubated in duplicate with 100 μl buffer containing 15,000 dpm of tritiated steroids (Wingfield *et al.*, 1984). Tubes were incubated 16 hours at 4°C. Bound steroids were separated from free steroids by adding a charcoal solution (0.25% charcoal and 0.025% dextran in buffer) followed by incubating mixtures on ice for 15 min and centrifugating at 4°C for 5 min. Radioactivity of the bound ^{3}H-steroid contained in supernates was determined by adding 5 ml scintillation cocktail (NE 266, Nuclear Enterprises) and counting in Beckman liquid scintillation counter. [2, 4, 6, 7-^{3}H] estradiol-17β (85–110 Ci/m mol), [1, 2, 6, 7-^{3}H] testosterone (80–105 Ci/m mol), [1, 2, 6, 7-^{3}H] progesterone (80–110 Ci/m mol) and [1, 2, 6, 7-^{3}H] cortisol (80–105 Ci/m mol) were purchased from Amersham Co. (Arlington Height, IL). Non-specific binding controls and steroid antiserum controls were run parallel with each assay. Non-specific binding was obtained by adding 1 μg of unlabeled steroid to displace the binding of tritiated steroid. Specific binding percentage was calculated by substracting the non-specific binding from the total binding percentage and expressed as mean ± SEM (SEM=standard error of mean). We defined the degree of binding activity was high if the percent specific binding was greater than 5%. The significance of circadian rhythm on the degree of steroid-binding activity in common carp were conducted with Duncan's new multiple range test (Steel and Torrie, 1980).

RESULTS

The distribution of the degree of steroid-binding activity varied among fish species (Table 1). The degree of binding activity for testosterone, estradiol-17β, progesterone and cortisol ranged from 0% to 80.1%, 0.8% to 75.9%, 0% to 24.6%, 0% to 7.0%, respectively. Based on the average percent binding from all the fishes in 38 species, the order of binding activity to steroid is testosterone>estradiol-17β>progesterone>cortisol (Table 2). We found that 78.9%, 71.0%, 10.5% and 2.6% collected species had a high degree of estradiol-, testosterone-, progesterone- and cortisol-binding activities in plasma, respectively (Table 2). A majority of species (65.7%) have high degree of sex steroid-binding activity, 15.8% of species did not have any or only have very low

Table 1
The degree of steroid-binding activities in 38 species of fishes

Species	No.[a]	Steroid-binding proteins (%)			
		T[b]	E_2[c]	P_4[d]	C[e]
Anguilla japonica (Japanese eel)	7	80.1±1.6[f]	75.9±0.8	1.9±0.5	1.4±0.1
Hypophthalmichthys molitrix (big head)	6	59.2±1.5	27.7±4.3	3.3±0.2	0
Arius maculatus (spotted catfish)	3	59.6±2.9	53.6±0.8	3.4±0.2	1.7±0.1
Parasilurus asotus (mudfish)	4	58.4±1.9	34.1±1.9	3.8±0.4	0
Clarias fuscus (freshwater catfish)	4	58.2±1.2	45.7±2.9	4.7±0.6	0.5±0.7
Ctenopharyngodon idellus (grass carp)	5	53.8±4.1	0.2±3.0	0.3±0.3	0
Synodintis nigriventris (africa catfish)	3	50.7±4.8	31.3±1.8	0	0
Chanos chanos (milk fish)	4	49.4±2.1	20.7±3.6	24.6±3.4	0
Carassius auratus (golden carp)	4	49.8±1.3	27.1±0.7	3.5±0.3	0
Acrossocheilus paradoxus (Taiwan seven stripes barbel)	4	39.8±5.4	38.5±5.9	3.0±0.9	0
Megalobrama amblycephala	5	38.4±3.3	14.7±1.8	1.8±0.2	0
Varicorhinus barbatulus	4	37.7±5.7	34.7±0.7	1.8±0.4	0.9±0.1
Cirrhina molitorella (mud carp)	4	37.3±3.5	8.0±1.8	9.0±2.6	0.4±0.2
Misgurnus anguillicaudatus (pond loach)	10	33.5±5.1	35.3±11.8	0	0.2±0.2
Salmo gairdneri (rainbow trout)	7	30.0±5.3	25.6±4.9	7.1±2.4	0
Zacco pachycephalus	4	29.3±6.0	11.6±4.8	0.6±0.6	0.3±0.2
Cyprinus carpio (common carp)	8	23.5±3.3	8.4±1.5	0.2±1.2	0.3±0.1
Galeus sauteri (small shark)	4	21.8±1.3	25.7±1.1	9.1±1.2	0
Trichiurus lepturus (hair tail)	5	14.7±3.5	9.1±1.9	1.3±0.4	0.2±0.1
Muraexesox cinereus (pink eel)	5	13.3±1.1	1.8±0.6	1.4±0.3	0.8±0.3
Therapon jarbus (three-stripped tigerfish)	6	11.3±1.3	10.2±3.3	2.2±0.4	1.2±0.9
Sphyrna zygaena (Linnaeus) (smooth-hammer head)	5	11.3±3.4	9.1±1.4	4.7±0.8	0.2±0.1
Plecoglossus altivelis (ayu)	6	8.8±2.2	6.7±2.6	1.2±0.4	4.2±1.1
Psammoperca waigiensis (glass-eye perch)	4	6.2±0.9	15.2±1.5	2.9±0.1	0
Lateolabrax japonicus (Japanese sea perch)	7	5.4±1.1	10.3±0.9	1.8±0.2	0.6±0.1
Epinephelus salmonides (grouper)	6	5.3±0.7	2.9±0.4	2.4±0.5	0.8±0.1
Mylio berda (yellow-finned seabream)	6	5.0±0.5	18.5±0.9	1.5±0.4	0.9±0.0
Girella melanichthys (small scale black fish)	3	4.8±1.5	7.7±0.4	3.3±1.0	0.3±0.2
Saurida undosquamis (Richardson) (brush-toothed)	3	4.2±1.1	5.4±1.1	2.7±0.9	7.0±4.5
Argyrosomus argentatus (white croaker)	6	3.8±0.9	4.9±0.2	0.6±0.3	0.5±0.1
Monopterus albas (Zuiew) (rice-field eel)	5	0.6±0.3	4.7±1.6	1.0±0.3	0.2±0.1
Tilapia zilli (tilapia)	10	0.7±0.2	2.6±0.5	1.7±0.7	0.7±0.3
Acanthopagrus schlegeli (black porgy)	9	1.2±0.4	5.2±1.0	0.3±0.1	0
Micropterus salmoides (largemouth bass)	3	0.7±0.2	0.8±0.1	0.6±0.1	0.1±0.1
Decaoterus maruadsi (amber fish)	6	1.1±0.2	6.7±0.9	0.2±0.1	0.7±0.3
Psenopsis anomala (butter fish)	4	0.2±0.2	5.0±2.1	4.4±1.3	0
Priacanthus macracanthus (red bulleye)	5	0	4.1±2.3	0.8±0.3	0.9±0.3
Atrobucca nibe (black croaker)	4	4.3±1.5	1.4±0.0	2.5±0.5	0

a. No.=number of fishes examined
b. T=testosterone
c. E_2=estradiol-17β
d. P_4=progesterone
e. C=cortisol
f. mean±SEM

Table 2
Summary of steroid-binding activities from 38 species of fishes

	Steroid-binding protein			
	E_2[d]	T[e]	P_4[f]	C[g]
Mean binding activity (%)[a]	17.7	24 0	3.0	0.7
No. of species[b]	30	27	4	1
Percentage[c]	78.9	71.0	10.5	2.6

a. Mean binding activity was calculated from 38 species.
b. No. of species having high degree (⩾5%) of steroid-binding activity.
c. Percentage of species having high degree (⩾5%) of steroid-binding activity.
d. E_2=estradiol-17β
e. T=testosterone
f. P_4=progesterone
g. C=cortisol

Table 3
Summary of steroid-binding activities from 38 species of fishes

Steroid binding proteins	No. of species[a]	Percentage[b]
E_2[c]+T[d]	25	65.7
T+P_4[e]	4	10.5
E_2+P_4	4	10.5
T+E_2+P_4	4	10.5
Low levels (or none)	6	15.8

a. Fish having high degree (⩾5%) or low degree (<5%) steroid-binding activity.
b. Percentage of species having high or low degree of steroid-binding activity.
c. T=testosterone
d. E_2=estradiol-17β
e. P_4=progesterone

Table 4
The degree of steroid-binding activities in shrimps

Species	Steroid-binding protein (%)			
	T[a]	E_2[b]	P_4[c]	C[d]
Metapenaeus monoceros (sand shrimp)	1.1±0.1[e]	0	0.2±0.1	0.2±0.1
Penaeus monodon (grass prawn)	0	0	3.3±0.4	0
Panaeus japonicus (Japanese tiger shrimp)	0.3±0.2	1.3±0.2	0.7±0.5	0.2±0.1
Macrobrachium rosenbergii (giant freshwater shrimp)	1.1±0.2	26.±0.6	2.7±0.3	2.8±0.3

a. T=testosterone b. E_2=estradiol-17β c. P_4=progesterone d. C=cortisol e. mean±SEM

Table 5
The degree of steroid-binding activities in mammals

Species[a]	Steroid-binding protein (%)			
	T[b]	E_2[c]	P_4[d]	C[e]
Human	2.1±0.6[e]	0.9±0.2	0.7±0.2	14.9±1.1
Dog	0	0	3.7±1.2	33.7±3.6
Rabbit	1.6±0.6	1.1±0.3	0	37.7±17.5
Pig	1.2±0.2	3.5±0.6	4.8±0.6	1.8±0.4
Mouse	0.1±0.1	0.8±0.1	0.1±0.1	2.4±0.4

a. Human, dog, rabbit and mouse are all males (n=6). There are 3 males and 3 females in pig.
b. T=testosterone c. E_2=estradiol-17β d. P_4=progesterone e. C=cortisol f. mean±SEM

Table 6
The steroid-binding activities in female and male common carps

	Steroid-binding protein (%)			
	E_2[a]	T[b]	P[c]	C[d]
Female (n=13)	5.8±0.5	43.5±1.6	3.2±0.5	1.6±0.1
Male (n=13)	5.7±0.5	41.4±0.9	3.9±0.4	1.6±0.1

a. E_2=estradiol-17β b. T=testosterone c. P_4=progesterone d. C=cortisol e. mean±SEM

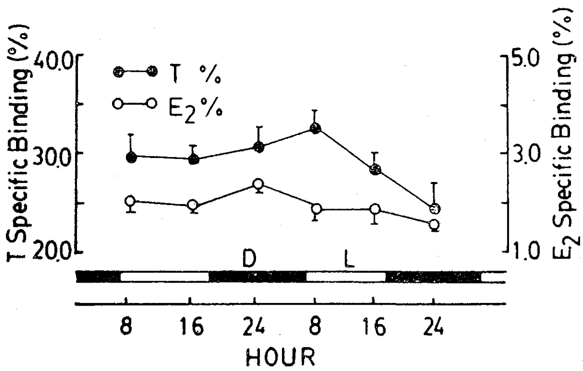

Fig. 1. Changes in the degree of testosterone (T)- and estradiol (E_2)-binding activities during different photoperiod (12L/12D) in common carp.

degree) steroid-binding activity (Table 3). In contrast, none of the species of shrimps had significant degree of steroid-binding activity (Table 4). Plasma in human, pig, dog, rabbit and mouse did not have high degrees of binding activities for testosterone, estradiol-17β and progesterone (Table 5). However, human, dog and rabbit had a high degree of cortisol-binding activity in plasma (Table 5). Female and male common carps had similar degrees of steroid-binding activity (Table 6). No significantly circadian rhythm in the degree of steroid-binding activity in common carps was observed (Fig. 1).

DISCUSSION

From the results of 37 teleosts, 1 elasmobranch (*Sphyrna zugaena*) and 4 shrimps, the degree of steroid-binding activity in fishes differed from those of shrimps and mammals. The data show that a majority of fishes have a high degree of sex steroid-binding activity in the plasma. Globulin is one of the proteins responded to high affinity binding for sex steroids (Pasmanik and Callard, 1986). No significant degree of any steroid-binding activity was observed in the hemolymph of shrimp. The cortisol-binding protein is one of major binding proteins of the mammalian plasma which is agreed to the findings of Seal and Doe (1963).

These findings of sex steroid-binding activity in a majority of fishes agree with those of other studies including those conducted using Atlantic salmon (Freeman and Idler, 1971; Lazier *et al.*, 1985), carp (Corvol and Bardin, 1973), rainbow trout (Fostier and Breton, 1975), brown trout (Pottinger, 1988) and goldfish (Pasmanik and Callard, 1986). High affinity sex steroid-binding protein were also found in other animals other than fishes such as newt (Ozon *et al.*, 1971), turtle, *Chrysemys picta* (Salhanick and Callard, 1980), hedgehog (Saboureau *et al.*, 1982), urodele amphibian, *Taricha granulosa* (Moore *et al.*, 1983), avian species (Wingfield *et al.*, 1984), and water shake, *Nerodia* (Riley *et al.*, 1988). Testosterone- and estradiol-binding proteins have been considered the identical protein in goldfish (Pasmanik and Callard, 1986) and rainbow trout (Fostier and Breton, 1975). The ligand binding ratio to testosterone and estradiol-17β varies from species to species in our studies. Also, 30 out of 38 species have high degree of estradiol-binding activity as compared to 27 out of 38 species have high degree testosterone-binding activity. Different affinities of the binding proteins for testosterone and estradiol-17β in different species are suggested.

Our results show that some of the teleost plasmas contain high degrees of progesterone- and cortisol-binding activities. Four species existing high degree of progesterone-bining activity also have high degrees of testosterone- and estradiol-binding activities in plasma but don't have cortisol-binding activity. Freeman and Idler (1971) observed an intermediate binding affinity and high capacity for cortisol in the plasma of salmon is similar to the albumin system in human. The degree of binding for steroid to albumin should be less significant after the treatment of charcoal solution because of the low binding affinity (Wingfield *et al.*, 1984). High binding affinities for progesterone and cortisol have been found in other animals other than teleosts. Idler and Freeman (1969) indicated that progesterone, estradiol-17β and testosterone were bound to a high degree to serum binding protein in elasmobranch. Martin (1975) also showed that estradiol-17β progesterone, testosterone and corticosterone were bound to the same protein in elasmobranch. A plasma protein bound with progesterone, and another bound with estradiol-17β and testosterone were shown in reptile (Martin and Xavier, 1981). The characterizations of progesterone- and cortisol-binding proteins in fishes need further study.

Due to the technical problem in sampling and difficulty in identification of gonad during the non-spawning season, the data of sexes in each fish were not obtained completely. However, no sexual differences and circadian rhythm in the degree of steroid-binding activity were observed in common carp plasma. Therefore, the data intended to suggest that the different degree of binding activity in various species are species specific, but not due to the sampling of different sexes and photoperiod. However, the possibility of the changes of steroid-binding activity in various species caused by the different sexes, season or body weight should also not be completely excluded.

In conclusion, a majority of species of fishes (65.7%) have a high degree of sex steroid-binding activity, 10.5% of the fish species have a high degree of progesterone-binding activity, only 2.6% of the fish species have a high degree of cortisol-binding activity. No significant degrees of steroid-binding activities were detected in the hemolymph of shrimps. The biochemical and physiological characteristics of the various steroid-binding proteins in the plasma of different fishes are under investigation.

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類固醇激素結合蛋白質在魚類之分布特性

張清風　　陳玫如

本報告研究 38 種淡水與海水魚類之類固醇激素結合蛋白質之結合力分布情形。其中 65.7%的魚種之血液中具有高濃度之性類固醇激素（雌二醇與睪固酮）結合蛋白質之結合力，10.5%的魚種之血液中具有高濃度之黃體激素結合蛋白質之結合力，而只有 2.6%的魚種之血液中具有高濃度之可體松激素結合蛋白質之結合力。而且 4 種蝦類中均無法測得任何類固醇激素結合蛋白質之結合力，此等分布特性與人類、豬、狗、兎子、小白鼠之大多具有高濃度可體松激素結合蛋白質之結果不同。且以鯉魚爲例，類固醇激素結合蛋白質之結果不同。且以鯉魚爲例，類固醇激素結合蛋白質之結合力之高低並無性別及光照週期之差異。