Reprod Nutr Dev (1990) 30, 463-469

Original article

Metabolic effects of C_{21} steroids in female *Epinephelus akaara* (Teleostei: Serranidae)

T B Ng [1]*, N Y S Woo [2]

[1] *Department of Biochemistry;*
[2] *Department of Biology, The Chinese University of Hong Kong, Shatin, NT, Hong Kong*

(Received 7 November 1989; accepted 19 March 1990)

Summary — Cortisol, 11-deoxycorticosterone, progesterone, 17α-hydroxy-progesterone, 17α-hydroxy-20β-dihydroprogesterone, or a combination of the last 2 steroids, were injected into different groups of immature female *Epinephelus*. None of the steroids tested had significant effects on serum electrolyte level, and hepatosomatic and gonadosomatic indices. Serum glucose concentration was elevated after treatment with cortisol, 11-deoxycorticosterone, 17α-hydroxy-20β-dihydroprogesterone or a combination of 17α-hydroxy-20β-dihydroprogesterone and 17α-hydroxyprogesterone. Muscle protein concentration was lowered after treatment with cortisol, 17α-hydroxy-20β-dihydroprogesterone or a combination of 17α-hydroxyprogesterone and 17α-hydroxy-20β-dihydroprogesterone. Liver protein was significantly elevated after treatment with progesterone but lowered after cortisol treatment. The results suggest that oocyte maturation is an energy consuming process, and that steroid hormones regulating these processes, including 17α-hydroxy-20β-dihydroprogesterone and 11-deoxycorticosterone adjust metabolism to provide energy for these processes.

metabolism / corticosteroid / progestagen / teleost

Résumé — Effets métaboliques des stéroïdes C_{21} chez la femelle d'*Epinephelus akaara* (Teleostei : Serranidae). *Du cortisol, de la 11-deoxycorticostérone, de la progestérone, de la 17α-hydroxyprogestérone, de la 17α-hydroxy-20β-dihydroprogestérone ou une combinaison des 2 derniers stéroïdes ont été injectés à différents groupes de femelles immatures d'*Epinephelus. *Aucun des stéroïdes n'a eu d'effets significatifs sur le niveau des électrolytes sanguins, ni sur les index hépatosomatiques et gonadosomatiques. La concentration du glucose sanguin est augmentée après traitement par le cortisol, la 11-déxycorticostérone, la 17α-hydroxy-20β-dihydroprogestérone ou une combinaison de la 17α-hydroxy-20β-dihydroprogestérone et de la 17α-hydroxyprogestérone. La concentration des protéines musculaires est diminuée après le traitement par le cortisol, la 17α-hydroxy-20β-dihydroprogestérone ou une combinaison de la 17α-hydroxyprogestérone et de la 17α-hydroxyprogestérone. Les protéines du foie sont significativement augmentées après le traitement par la progestérone, mais diminuées après le traitement par le cortisol. Ces résultats suggèrent que le développement de l'ovocyte est un processus consommateur d'énergie sous contrôle des hormones stéroïdes, 17α-hydroxy-20β-dihydroprogestérone et 11-déoxycorticostérone, qui ajustent le métabolisme en vue de fournir l'énergie nécessaire à ces processus.*

métabolisme / corticostéroïde / progestagène / poisson téléostéen

* Correspondence and reprints

INTRODUCTION

Oocyte maturation and ovulation in teleosts are dependent on pituitary gonadotropin. The action of gonadotropin on oocyte maturation is thought to be mediated through the production of maturational (C_{21}) steroids (Kanatani and Nagahama, 1980; Scott *et al,* 1983). Progesterone-related maturational steroids, especially 17α-hydroxyprogesterone and 17α-hydroxy-20β-dihydroprogesterone have been detected in the blood of mature female teleosts (Idler *et al,* 1959; Campbell *et al,* 1980) and have been shown to play a role in oocyte maturation and ovulation (Jalabert, 1976; Jalabert *et al,* 1977; Goetz and Theofan, 1979; Duffey and Goetz, 1980; Iwamatsu, 1980). 11-Deoxycorticosterone is capable of inducing oocyte maturation in species like *Brachydanio rerio* (Van Ree *et al,* 1977), *Perca flavescens* (Goetz and Theofan, 1979; *Salmo gairdneri* (Jalabert, 1976) and *Heteropneustes fossilis* (Sundararaj, 1979). Cortisol level has also been demonstrated to increase with sexual maturation (Wingfield and Grimm, 1977; Cook *et al,* 1980). The increase in corticosteroids in some species at the end of the sexual cycle has been associated with spawning migration, which requires energy (McBride *et al,* 1986). Cortisol was effective in inducing final maturation in catfish (Goswami and Sundararaj, 1974) and Japanese medaka (Hirose, 1972) oocytes, although more recent literature has indicated that 17α-hydroxy-20β-dihydroprogesterone is probably the maturation inducing steroid (Sundararaj *et al,* 1985; Sakai *et al,* 1987). Many changes in lipid, carbohydrate and protein metabolism occur in parallel with sexual maturation (Ng *et al,* 1984, 1986) but the metabolic requirements for oocyte maturation remains poorly understood. The metabolic effects of estradiol-17β, testosterone, progesterone and cortisol have been examined (Chan and Woo, 1978; Wiegand and Peter, 1980; Ng *et al,* 1984).

In view of the fact that the metabolic effects of 11-deoxycorticosterone, 17α-hydroxyprogesterone and 17α-hydroxy-20β-dihydroprogesterone have not previously been examined in teleosts, we decided to undertake a study to investigate the effects of the 3 steroids and in addition those of cortisol and progesterone which have not been studied before in immature grouper. The same experimental protocol employed in studying the metabolic effects of estradiol-17β and testosterone in immature grouper (Ng *et al,* 1984) was utilized in the present investigation. Immature fish were chosen because the metabolic effects of estradiol on the immature grouper generally resembled metabolic changes associated with normal vitellogenesis (Ng *et al,* 1984). The presence of low plasma levels of gonadal steroids in immature fish was another reason for the choice.

MATERIALS AND METHODS

Immature female red grouper *(Epinephelus akaara),* with a gonadosomatic index (*GSI*: gonad weight x 100%/body weight) of 0.25%, a body weight of 140–180 g and a body length of 10–15 cm, were obtained from local fish farms and allowed to acclimate to laboratory aquaria at 32 ‰ salinity for 1 wk prior to experimentation. *Epinephelus akaara* is a protogynous hermaphrodite (Chan and Yeung, 1983) and we have examined the ovaries of immature female specimens in a previous study (Tam *et al,* 1983). We have found that the ovaries of immature female groupers (*GSI* : 0.21%) contain germ cells at various stages of development (23.7% in the primary oocyte stage, 45.4% in the nucleolar oocyte stage and 30.4% in the perinucleolar stage). The fish used in the present study corresponded to those of our 1983 study (Tam *et al,* 1983) in terms of ovarian development. Steroids (all from Sigma Chemical Company, St Louis, USA) were made up in peanut

oil and injected intraperitoneally into female fish every other day for a period of 9 days (*ie*, the fish received a total of 5 injections). A dose of 500 µg steroid per kg body weight per injection and 7 fish per group were used for each of the following steroids: cortisol, progesterone, 17α-hydroxyprogesterone, 17α-hydroxy-20β-dihydroprogesterone and 11-deoxycorticosterone. One group received a combination of 17α-hydroxyprogesterone and 17α-hydroxy-20β-dihydroprogesterone (500 µg of each steroid per kg per injection). Peanut oil was injected intraperitoneally into one group which served as the control group. The dose of steroids used in this study was the same as that previously employed for a study on the effects of estradiol-17β and testosterone (Ng *et al*, 1984). The two sex steroids exerted profound metabolic effects at that dosage. Twenty-four hours after the last injection, body weights were recorded, then the fish were bled and killed, and the gonadosomatic and hepatosomatic indices measured. Blood samples were allowed to clot at room temperature for 45 min, then centrifuged to obtain serum. Portions of liver and epaxial muscle samples were collected and together with the serum samples, were stored at –20° C until analyzed.

Serum sodium, potassium and calcium concentrations were determined, after appropriate dilution, by means of a flame photometer (ISA Biologie). Serum chloride concentrations were determined using a Corning chloride meter. Serum glucose concentrations were determined by using a coupled glucose oxidase–peroxidase reaction (Sigma). Serum protein was determined according to the method as described by Hartree (1972), lipids by the method of Woodman and Price (1972) and cholesterol by using a kit (#351) from Sigma Chemical Company. Liver and muscle protein was determined after alkaline digestion of the tissues.

Results were presented as means ± SEM. Statistical difference between groups was assessed using one-way analysis of variance followed by Duncan's multiple range test.

RESULTS

The steroids tested lacked any effect on hepatosomatic and gonadostomatic indices (table I). Hematocrit was significantly lowered only in the group receiving 11-deoxycorticosterone (table II). No effect on

Table I. Effects of various steroid hormones on hepatosomatic index (HSI), gonadosomatic index *(GSI)*, muscle and liver protein contents.

	HSI (%)	*GSI (%)*	*Muscle protein (g/100 g)*	*Liver protein (g/100 g)*
Control	1.42 ± 0.11	0.35 ± 0.05	12.9 ± 0.45	12.6 ± 0.76
Cortisol	1.47 ± 0.18	0.35 ± 0.05	9.81 ± 0.96*	8.32 ± 1.40
Progesterone	1.13 ± 0.11	0.30 ± 0.03	12.0 ± 0.45	16.2 ± 0.6*
17α–OHP	1.33 ± 0.11	0.38 ± 0.07	12.1 ± 0.51	14.5 ± 1.61
17α,20β–$(OH)_2$P	1.32 ± 0.06	0.33 ± 0.03	8.73 ± 1.17*	15.1 ± 0.88
17α–OHP + 17α,20β–$(OH)_2$P	1.17 ± 0.06	0.47 ± 0.05	9.18 ± 0.57*	13.1 ± 1.96
11–DOC	1.61 ± 0.21	0.30 ± 0.03	12.1 ± 1.20	14.7 ± 0.80

A dose of 500 µg steroid per kg body weight per injection was used. Values are expressed as means ± SEM. (n = 7)
* Difference from control statistically significant (95% confidence level). 17α–OHP = 17α–hydroxyprogesterone. 17α,20β–$(OH)_2$P : 17α-hydroxy-20β-dihydroprogesterone. 11–DOC = 11-deoxycorticosterone.

serum Na^+, K^+, Ca^{2+} and Cl^- was detected (table III). Serum protein concentration was lowered only in the groups receiving 17α-hydroxyprogesterone and 11-deoxycorticosterone. Serum glucose concentration was significantly elevated after administration of cortisol, 11-deoxycorticosterone, 17α-hydroxy-20β-dihydroprogesterone and a combination of 17α-hydroxyprogesterone and 17α-hydroxy-20β-dihydroprogesterone. Serum concentrations of cholesterol and total lipid were lowered after 11-deoxycorticosterone administration (table III). Muscle protein content was significantly depressed after treatment with cortisol, 17α-hydroxy-20β-dihydroprogesterone, and a combination of 17α-hydroxyprogesterone and 17α-hydroxy-20β-dihydroprogesterone. Hepatic protein content was lowered after cortisol treatment but elevated after treatment with progesterone (table I).

Table II. Effects of various steroid hormones on hematocrit, Na^+, K^+, Ca^{2+} and Cl^-. Table I footnote also applies here.

	Hct (%)	*Na^+ (mM)*	*K^+ (mM)*	*Ca^{2+} (mM)*	*Cl^- (mM)*
Control	29.7 ± 1.6	172.6 ± 2.5	3.12 ± 0.18	1.79 ± 0.03	161.4 ± 6.2
Cortisol	26.3 ± 0.9	169.0 ± 2.6	3.14 ± 0.22	1.96 ± 0.07	153.0 ± 6.6
Progesterone	27.5 ± 1.2	161.8 ± 6.1	3.05 ± 0.18	2.85 ± 0.60	151.6 ± 4.4
17α–OHP	26.4 ± 1.5	168.0 ± 5.4	3.43 ± 0.17	1.80 ± 0.02	151.1 ± 4.2
17α,20β–$(OH)_2$P	28.7 ± 1.7	176.4 ± 9.4	3.58 ± 0.19	1.76 ± 0.08	162.2 ± 6.4
17α–OHP + 17α,20β–$(OH)_2$P	28.3 ± 2.4	188.2 ± 7.4	3.38 ± 0.13	1.72 ± 0.12	159.4 ± 7.5
11–DOC	25.7 ± 0.6*	167.4 ± 2.8	3.43 ± 0.08	1.67 ± 0.06	150.0 ± 1.9

Table III. Effects of various steroid hormones on serum concentrations of protein, glucose, total lipid and cholesterol. Table I footnote also applies here.

	Protein (mg/ml)	*Glucose (mg/100 ml)*	*Total lipid (g/100 ml)*	*Cholesterol (g/100 ml)*
Control	10.6 ± 0.83	28.1 ± 1.2	0.40 ± 0.02	0.20 ± 0.02
Cortisol	10.3 ± 0.34	32.7 ± 0.86*	0.44 ± 0.04	0.23 ± 0.03
Progesterone	11.3 ± 1.12	27.4 ± 1.06	0.37 ± 0.02	0.18 ± 0.03
17α–OHP	7.7 ± 0.78*	29.4 ± 1.38	0.37 ± 0.03	0.21 ± 0.03
17α,20β–$(OH)_2$P	12.1 ± 1.58	32.1 ± 1.30*	0.35 ± 0.01	0.22 ± 0.04
17α–OHP + 17α,20β–$(OH)_2$P	8.4 ± 1.07	37.5 ± 2.43*	0.40 ± 0.03	0.26 ± 0.02
11–DOC	7.6 ± 1.06*	38.3 ± 4.26*	0.34 ± 0.01	0.14 ± 0.01*

DISCUSSION

None of the steroids tested augmented the hepatosomatic index or serum Ca^{2+} concentration. Estradiol-17β increases the 2 aforementioned parameters in the immature red grouper due to its effect on vitellogenin synthesis (Tam *et al,* 1983). The results therefore suggest that cortisol, 11-deoxycorticosterone and progestagens have no effect on hepatic vitellogenin production. The gonadosomatic index remained unchanged after treatment with any of the aforementioned steroids. Ovarian growth occurs during vitellogenesis in the presence of gonadotropins which stimulate vitellogenin incorporation into oocytes (Idler and Ng, 1979; Ng and Idler, 1979), and may occur during oocyte maturation because of oocyte hydration (Wallace and Selman, 1978). The serum concentrations of Na^+, K^+, and Cl^- were not altered by any of the steroids tested, not even by 11-deoxycorticosterone which may be a mineralocorticoid. The latter result is somewhat unexpected. However, its mineralocorticoid effects might have been masked by hemodilution. The lower serum concentrations of protein, cholesterol and total lipid after treatment with 11-deoxycorticosterone were probably due to hemodilution as witnessed in a lower hematocrit. The elevated serum glucose concentration was, however, a genuine effect which was probably due to the structural resemblance between 11-deoxycorticosterone and cortisol and therefore the binding of 11-deoxycorticosterone to cortisol receptors. Cortisol also elicited an augmented serum glucose concentration, consistent with the finding in other teleost species, *eg* the eel *Anguilla japonica* (Chan and Woo, 1978). Progesterone and 17α-hydroxyprogesterone did not affect serum glucose concentration, but 17α-hydroxy-20β-dihydroprogesterone or a combination of 17α-hydroxyprogesterone and 17α-hydroxy-20β-dihydroprogesterone caused serum glucose level to increase above the control value. It has been demonstrated that testosterone administered to immature grouper using the same protocol did not cause hyperglycemia (Ng *et al,* 1984). The generally higher serum glucose concentration in mature sea bream (Ng *et al,* 1986) and grouper (Ng *et al,* 1984) than in immature fish lends support to the role of glucose as an energy source during sexual maturation. The progestagens tested did not exert an effect on serum concentrations of total lipid and cholesterol. Wiegand and Peter (1980) have noted that progesterone lacked any effect on plasma triglyceride level in goldfish. On the other hand, estradiol and testosterone elicited hyperlipidemia in immature grouper (Ng *et al,* 1984).

The lower muscle protein content observed after treatment with cortisol, 17α-hydroxy-20β-dihydroprogesterone, and a combination of 17α-hydroxyprogesterone and 17α--hydroxy-20β-dihydroprogesterone implies increased muscle protein degradation which ultimately led to enhanced gluconeogenesis. A role of cortisol in stimulating proteolysis and gluconeogenesis has been reported for various fish species (*eg Salmo gairdneri:* Freeman and Idler 1973; *Anguilla japonica:* Chan and Woo, 1978). This effect was not noted after treatment with estradiol or testosterone (Ng *et al,* 1984). Decreased hepatic protein content was observed after cortisol treatment (Chan and Woo, 1978). Increased liver protein was noted after treatment with progesterone. However, it was not due to vitellogenin synthesis.

To recapitulate, the present investigation revealed that, unlike estradiol-17β, which exerts profound effects on protein, lipid, carbohydrate and calcium metabolism (Ng *et al,* 1984), progesterone and

17α-hydroxyprogesterone had little metabolic effects while 17α-hydroxy-20β-dihydroprogesterone, cortisol and 11-deoxycorticosterone increased serum glucose concentration. The hyperglycemic effect of estradiol-17β noted previously (Ng *et al,* 1984) and a similar effect of 17α-hydroxy-20β-dihydroprogesterone and 11-deoxycorticosterone observed in the present investigation suggest that vitellogenesis and oocyte maturation are energy-consuming processes and that steroid hormones regulating these processes adjust metabolism to provide energy for these processes.

REFERENCES

Campbell CM, Fostier A, Jalabert B, Truscott B (1980) Identification and quantification of steroids in the serum of rainbow trout during spermiation and oocyte maturation. *J Endocrinol* 85, 371-378

Chan DKO, Woo NYS (1978) Effect of cortisol on the metabolism of the eel, *Anguilla japonica. Gen Comp Endocrinol* 35, 205-215

Chan STH, Yeung WSB (1983) Sex control and sex reversal in fish under natural conditions. *In: Fish Physiology* (Hoar WS, Randall DJ, Donaldson EM, eds) Academic Press, New York, vol 9B, pp 171-222

Cook AF, Stacey NE, Peter RE (1980) Periovulatory changes in serum cortisol levels in the goldfish, *Carassius auratus. Gen Comp Endocrinol* 40, 507-510

Duffey RJ, Goetz FW (1980) The *in vitro* effects of 17α-hydroxy-20β-dihydroprogesterone on germinal vesicle breakdown in brook trout *(Salvelinus fontinalis)* oocytes. *Gen Comp Endocrinol* 41, 563-565

Freeman HC, Idler DR (1973) Effects of corticosteroids on liver transaminases in two salmonids, the rainbow trout *(Salmo gairdnerii)* and the brook trout *(Salvelinus fontinalis). Gen Comp Endocrinol* 20, 69-75

Goetz FW, Theofan G (1979) *In vitro* stimulation of germinal vesicle breakdown and ovulation of yellow perch *(Perca flavescens)* oocytes. Effects of 17α-hydroxy-20β-dihydroprogesterone and prostaglandins. *Gen Comp Endocrinol* 37, 273-285

Goswami SV, Sundararaj BT (1974) Effects of C_{18}, C_{19} and C_{21} steroids on *in vitro* maturation of oocytes of the catfish, *Heteropneustes fossilis* (Bloch). *Gen Comp Endocrinol* 23, 282-285

Hartree EF (1972) Determination of protein: a modification of the Lowry method that gives a linear photometric response. *Anal Biochem* 48, 422-427

Hirose K (1972) Biological study on ovulation *in vitro* of fish. IV. Induction of *in vitro* ovulation in *Oryzias latipes* oocyte using steroids. *Bull Jpn Soc Sci Fish* 38, 457-461

Idler DR, Ronald AP, Schmidt PJ (1959) Biochemical studies on sockeye salmon during spawning migration. VII. Steroid hormones in plasma. *Can J Biochem Physiol* 37, 1227-1238

Idler DR, Ng TB (1979) Studies on two types of gonadotropins from both salmon and carp pituitaries. *Gen Comp Endocrinol* 38, 412-440

Iwamatsu T (1980) Studies on oocyte maturation of the medaka, *Oryzias latipes.* VIII. Role of follicular constituents in gonadotropin- and steroid-induced maturation of oocytes *in vitro. J Exp Zool* 211, 231-239

Jalabert B (1976) *In vitro* oocyte maturation and ovulation in rainbow trout *(Salmo gairdneri),* northern pike *(Esox lucius)* and goldfish *(Carassius auratus). J Fish Res Board Can* 33, 974-988

Jalabert B, Breton B, Brzuska E, Fostier A, Wieniawski J (1977) A new tool for induced spawning; The use of 17α-hydroxy-20β-dihydroprogesterone to spawn carp at low temperature. *Aquaculture* 10, 353-364

Kanatani H, Nagahama Y (1980) Mediators of oocyte maturation. *Biomed Res* 1, 273-291

McBride JR, Fagerlund UHM, Dye HM, Bagshaw J (1986) Changes in structure of tissues and in plasma cortisol during the spawning migration of pink salmon *Oncorhynchus gorbuscha* (Walbaum). *J Fish Biol* 29, 153-166

Ng TB, Idler DR (1979) Studies on two types of gonadotropins from both American plaice and winter flounder pituitaries. *Gen Comp Endocrinol* 38, 410-420

Ng TB, Woo NYS, Tam PPL, Au CYW (1984) Changes in metabolism and hepatic ultrastructure induced by estradiol and testosterone in immature female *Epinephelus akaara* (Teleostei, Serranidae). *Cell Tissue Res* 236, 651-659

Ng TB, Tam PPL, Woo NYS (1986) Sexual maturation in the black sea bream, *Mylio macrocephalus* Teleostei, Sparidae: changes in pituitary gonadotropes, hepatocytes and related biochemical constituents in liver and serum. *Cell Tissue Res* 245, 207-213

Sakai N, Iwamatsu T, Yamauchi K, Nagahama Y (1987) Development of the steroidogenic capacity of medaka *(Oryzias latipes)* ovarian follicles during vitellogenesis and oocyte maturation. *Gen Comp Endocrinol* 66, 333-342

Scott AP, Sumpter JP, Hardiman PA (1983) Hormone changes during ovulation in the rainbow trout *(Salmo gairdneri). Gen Comp Endocrinol* 49, 128-134

Sundararaj BI (1979) Some aspects of oocyte maturation in catfish. *J Steroid Biochem* 11, 701-707

Sundararaj BI, Goswami SV, Lamba VJ (1985) Oocyte maturation in teleost fishes. *In: Current Trends in Comparative Endocrinology* (Lofts B, Holmes WN, eds) Hong Kong University Press, Hong Kong, pp 369-372

Tam PPL, Ng TB, Woo NYS (1983) Effects of œstradiol-17β and testosterone on the histology of pituitary, liver, ovary and skin of previtellogenic *Epinephelus akaara* (Teleostei, Serranidae). *Cell Tissue Res* 231, 579-592

Van Ree GE, Lok D, Bosman G (1977) *In vitro* induction of nuclear breakdown in oocytes of the zebrafish *Brachydanio rerio* (Ham Buch). Effects of the composition of the medium and of protein and steroid hormones. *Proc K Ned Akad Wet Ser C* 80, 353-371

Wallace RA, Selman K (1978) Oogenesis in *Fundulus heteroclitus.* I. Preliminary observation on oocyte maturation *in vivo* and *in vitro. Dev Biol* 62, 354-369

Wiegand MD, Peter RE (1980) Effect of sex steroids on plasma lipids in the goldfish, *Carassius auratus. Can J Zool* 58, 967-972

Wingfield JC, Grimm AS (1977) Seasonal changes in plasma cortisol, testosterone and œstradiol-17β in the plaice, *Pleuronectes platessa* L. *Gen Comp Endocrinol* 31, 1-11

Woodman DD, Price CP (1972) Estimation of serum total lipids. *Clin Chim Acta* 38, 39-43

Wright RS, Hunt SMV (1982) A radioimmunoassay for 17α, 20β-dihydroxy-4-pregnen-3-one: its use in measuring changes in serum levels at ovulation in Atlantic salmon *(Salmo salar),* coho salmon *(Oncorhynchus kisutch),* and rainbow trout *(Salmo gairdneri). Gen Comp Endocrinol* 47, 475-482