GENERAL AND COMPARATIVE ENDOCRINOLOGY 86, 147–151 (1992)

Influence of Dihydrotestosterone on Sex Determination in Channel Catfish and Blue Catfish: Period of Developmental Sensitivity

KENNETH B. DAVIS,* CHERYL A. GOUDIE,† BILL A. SIMCO,*
TERRENCE R. TIERSCH,*,[1] AND GARY J. CARMICHAEL†,[2]

Ecological Research Center, Department of Biology, Memphis State University, Memphis, Tennessee 38152; and †United States Department of Agriculture, Agricultural Research Service, Catfish Genetics Research Unit, P.O. Box 38, Stoneville, Mississippi 38776

Accepted July 23, 1991

Treatment of channel catfish with 0.2, 20, or 200 mg/liter of dihydrotestosterone (DHT) in the water during the egg stage or during egg and sac-fry stages did not alter the expected 1:1 sex ratio of the progeny. Feeding DHT at 200 mg/kg of feed for the first 21 days after yolk sac absorption resulted in 80% females; this proportion was increased by combining feeding with treatment of 200 mg DHT/liter in the sac-fry stage (90%) or in the egg and sac-fry stage (97%). In contrast, treatment of blue catfish sac-fry with 200 mg DHT/liter, with or without the combination of feeding DHT at 200 mg/kg food, resulted in 100% female populations. Neither clomiphene citrate, an estrogen-receptor blocking agent, nor clofibrate, an inhibitor of hepatic synthesis of cholesterol, affected the sex ratio of channel catfish, and neither of these compounds altered the feminizing effect of 200 mg DHT/kg when fed in combination with DHT. The nonaromatizable androgen DHT is not as effective as many other androgens in producing paradoxical female populations of channel catfish. However, feminization of blue catfish by treatment of sac-fry indicates that this species is more susceptible to hormonal manipulation and that the period of sex determination may occur earlier in development than in channel catfish.

A variety of models for sex determination have been described in teleosts which include species with male or female homogamety and species with environmental or behavioral sex determination (for review see Chan and Yeung, 1983). Experimental reversal of phenotypic sex can be achieved by the administration of sex hormones during critical periods of gonadal differentiation (Chan and Yeung, 1983). In general, estrogens produce all-female populations and androgens produce all-male populations (Hunter and Donaldson, 1983). Paradoxical feminization by high doses of androgens has been described in cichlids (Hackmann and Reinboth, 1974; Nakamura, 1975) and in rainbow trout (*Oncorhynchus mykiss*) (Solar *et al.*, 1984).

Functional feminization of channel catfish (*Ictalurus punctatus*) results from adding various estrogens or androgens to the diet during the first 21 days of feeding (Goudie *et al.*, 1983; Davis *et al.*, 1990). The effect of a potent feminizing androgen, ethynyltestosterone, was reduced when the treatment period was decreased. Interestingly, the nonaromatizable androgens DHT and 11-ketotestosterone also produced populations of channel catfish with high percentages of females (Davis *et al.*, 1990). No hormonal treatment that directs sex deter-

[1] Current address: United States Department of Agriculture, Agricultural Research Service, Catfish Genetics Research Unit, P.O. Box 38, Stoneville, MS 38776.

[2] Current address: United States Department of the Interior, U.S. Fish and Wildlife Service, Region 2, Division of Fisheries, P.O. Box 1306, Albuquerque, NM 87103.

0016-6480/92 $1.50

mination in a masculine direction has been found for this species.

Paradoxical feminization was thought to be due to the aromatic conversion of androgens to estrogenic substances (Fishman, 1982), but feminization of channel catfish with natural and synthetic nonaromatizable compounds (Davis *et al.*, 1990) indicated that aromatase activity was not the primary cause of feminization with the protocol used. Timing and dosage of hormonal administration are known to be critical for sex manipulation. Embryonic determination of the direction of gonadal organogenesis in channel catfish may be earlier for testes than ovaries and may require treatment prior to feeding to induce masculinization. Baker *et al.* (1988) produced predominately male populations of chinook salmon (*O. tshawytscha*) by two immersions of eyed eggs or newly hatched fry in 200 μg/liter 17-methyltestosterone for 2 hr 1 week apart. An alternative approach in inducing masculinization could involve the blockage of estrogen receptors or interference with endogenous steroid synthesis to allow testicular development.

This study evaluated the influence of a nonaromatizable androgen (DHT), an estrogen blocking agent (clomiphene citrate), and an inhibitor of cholesterol synthesis (clofibrate) alone and in combination on sex determination during early development of channel catfish and blue catfish (*Ictalurus furcatus*). The hormone-sensitive periods during embryological development were compared between channel catfish and blue catfish.

MATERIALS AND METHODS

Spawns from channel catfish were collected early in the morning and fertilized eggs were separated into six treatment groups and one control group of about 80 ml egg volume each (approximately 1740 eggs). Groups in separate compartments were treated daily at different developmental stages: as fertilized eggs (E), as sac-fry (SF), as eggs and sac-fry (E + SF), as swim-up fry (F21), as sac-fry and swim-up fry (SF + F21), and as eggs and sac-fry and swim-up fry (E + SF + F21). Groups treated as eggs (E, E + SF, and E + SF + F21) were separated by hand into small masses so that the hormone reached all the eggs. Groups were treated daily by turning off the water and adding the hormone solution. Eggs and sac-fry were treated with 0.2, 20, or 200 mg of DHT/liter of tank water. The hormone was dissolved in ethanol and diluted with water, and the proper volume was added to the hatching trough. After 1 hr, the water was turned on and the hormone was progressively diluted out of the tank. Eggs were treated daily from the first day after spawning until hatching; sac-fry treatments were begun at hatching and continued until feeding began. Swim-up fry were fed DHT at concentrations of 0.2, 20, or 200 mg of hormone/kg of commercial fish food. The fish were fed to satiation three times a day for the first 21 days of feeding. A similar experiment of a smaller scope was conducted on blue catfish, in which only sac-fry and swim-up fry were treated with DHT.

Clomiphene citrate at 20 or 200 mg/kg food or clofibrate at 525 or 1050 mg/kg food, alone or in combination with 200 mg DHT/kg food, was fed to channel catfish swim-up fry for the first 18 days of feeding.

Treatment groups were reared separately until the fish were 10–15 cm long when sex was determined by dissection (Goudie *et al.*, 1983) of up to 300 of the remaining fish. Sex ratios of all groups were evaluated individually by χ^2 analysis with Yates correction term (Strickberger, 1985) based on an expected 1:1 sex ratio. A probability of 0.05 was considered significant.

RESULTS AND DISCUSSION

Mortality was similar in all treatments and only the highest concentration of DHT consistently altered sex ratios and only in the female direction. Functional XY female channel catfish from hormone-treated groups have been identified by progeny testing (Davis *et al.*, 1990), therefore the altered sex ratios are not due to differential mortality of the sexes. Treatments were not effective at lower dietary concentrations nor when applied only in the water to eggs or to eggs and sac-fry (Fig. 1). Channel catfish treated only as sac-fry with 200 mg/liter had a small (58%), but significantly increased, percentage of females. Feeding 200 mg DHT/kg of food produced 80% females; this effectiveness increased if the

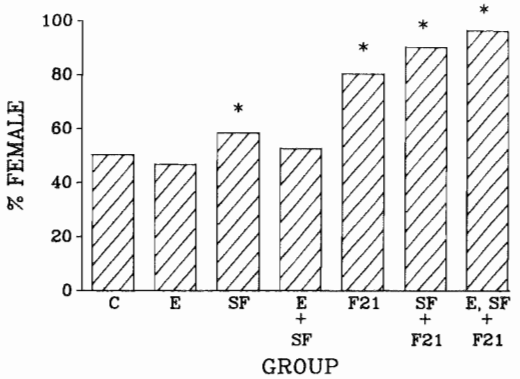

FIG. 1. Percentage of female channel catfish resulting from exposure as fertilized eggs (E) or sac-fry (SF) to 200 mg/liter dihydrotestosterone (DHT) in the water, as swim-up fry (F21) fed DHT at 200 mg/kg food for the first 21 days, or combinations of these treatments and controls (C). Treatments that had sex ratios significantly different ($P < 0.05$) from 1:1 by χ^2 analysis are indicated by an asterisk. Similar experiments with 0.2 or 20 mg/liter did not alter the sex ratio of the progeny. The number of fish used to determine the ratios in all groups was between 200 and 300.

fish were also treated as sac-fry (90%) and as eggs and sac-fry (96%). The increased percentage of females in groups treated in multiple stages could have been due to a cumulative effect of the hormone.

An earlier paper suggested that the feminizing effect of androgens may be due to the aromatization of the androgen used (Goudie *et al.*, 1983); however, DHT is a nonaromatizable androgen that apparently cannot be converted to an estrogen (Crim *et al.*, 1981; Callard, 1984). DHT is a less potent feminizing androgen in channel catfish than many androgens we have investigated. The concentrations used here did not produce 100% female populations, although they were much higher than concentrations of other androgens previously reported to be 100% effective (Davis *et al.*, 1990). The reason for the difference in potency between DHT and the other hormones is not clear; however, the paradoxical feminization by androgens is probably not due to aromatization to estrogens.

Immersion of eggs in hormone solutions alone or combined with subsequent feeding of hormone-treated diets has successfully affected gonadal differentiation in Atlantic salmon and rainbow trout (Donaldson and Hunter, 1982; Solar *et al.*, 1984). However, the onset of gonadal differentiation in channel catfish does not occur until after feeding begins. Neither DHT nor two aromatizable androgens, methyltestosterone and ethynyltestosterone, affected the sex ratio when fish were immersed twice for 2 hr during the sac-fry stage, although all three androgens feminized catfish when fed throughout the first 21 days (Davis *et al.*, 1990). The present study encompassed the concentrations used by Davis *et al.* (1990) and extended the treatment period back to the egg stage. If the period of testis differentiation occurs after that of the ovary, the period of sensitivity could be later than the 21 days of feeding evaluated in this study. Additionally, the developmental cue for testis differentiation may not be androgen dependent at any treatment period.

The control population of blue catfish was 65% female and was significantly different from a 1:1 sex ratio, as well as from the experimental groups. A sex ratio skewed toward females has been noticed in other populations of blue catfish (C. A. Goudie, unpublished observations). Blue catfish populations were 100% feminized by DHT regardless of the developmental stage of treatment (Fig. 2). Blue catfish have an earlier period of sensitivity to the feminizing effect of DHT and are more responsive to this hormone than channel catfish.

Clomiphene citrate had no effect on the sex ratio of developing fish and did not change the effect of DHT (Table 1). This drug induced a surge of gonadotropin in female fish (Breton *et al.*, 1975; Billard and Peter, 1977) and competitively inhibited mammalian estrogen receptors (Kato *et al.*, 1968; Kahwanago *et al.*, 1970; Hsuch *et al.*,

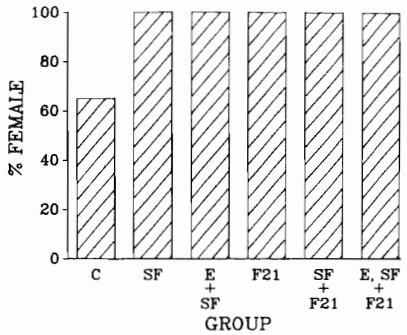

FIG. 2. Percentage of female blue catfish resulting from exposure as fertilized eggs (E) or sac-fry (SF) to 200 mg/liter dihydrotestosterone (DHT) in the water, as swim-up fry (F21) fed DHT at 200 mg/kg food, or combinations of these treatments. The treatment was feminizing and was 100% effective in all experimental groups. The control group (C) had significantly fewer females than any experimental group and was significantly different ($P < 0.05$) from a 1:1 sex ratio. One hundred fish were used in all experimental groups and 200 fish were used in the control group.

1978). While receptor types may be different for activation of ovarian development in young catfish, those receptors either were not blocked by clomiphene citrate or blockage of estrogen receptors did not influence ovarian development in catfish.

Clofibrate also had no effect on the sex ratio of channel catfish and did not alter the feminizing effect of DHT (Table 1). Clofibrate inhibits the hepatic synthesis of cholesterol in mammals (Levy, 1980). Biosynthetic pathways that are dependent on cholesterol as a precursor, such as for androgens and estrogens, may not be important in normal sex determination. This explanation is speculative, however, since steroid production was not measured.

A gene located on the Y chromosome in mammals, referred to in mice as Tdy (testis-determining gene on the Y), is responsible for testicular development. A prime candidate for Tdy has been mapped and cloned (Page *et al.*, 1987; Sinclair *et al.*, 1990). Recently, insertion of a gene from the sex-determining region of the mouse Y chromosome into XX mice induced testis differentiation (Koopmann *et al.*, 1991), but nothing is known about the normal control of expression of this gene. The pattern of sex inheritance of channel catfish can also be explained on the basis of a single gene (Davis *et al.*, 1990). Paradoxical feminization of channel catfish may be due to exogenous steroids overriding the normal mechanism of testis differentiation by directly stimulating ovarian development or by their inhibition of the expression of a gene that is testis determining.

TABLE 1

PERCENTAGE OF FEMALE CHANNEL CATFISH RESULTING FROM ORAL TREATMENT WITH CLOMIPHENE CITRATE OR CLOFIBRATE ALONE OR IN COMBINATION WITH 200 mg/kg DIHYDROTESTOSTERONE (DHT)

Treatment	No. of fish (*n*)	Concentration (mg/kg food)	Females (%)
Control	50	0	54
Clomiphene citrate	100	20	44
	100	200	44
Clofibrate	50	525	44
	50	1050	46
Dihydrotestosterone	50	200	82*
DHT + clomiphene citrate	100	200 + 20	73*
	50	200 + 200	86*
DHT + clofibrate	101	200 + 525	83*
	63	200 + 1050	71*

Note. Hormone-treated food was given during the first 18 days of feeding.

* Significantly different from 50% by χ^2 analysis ($P < 0.05$).

ACKNOWLEDGMENTS

We thank Terry Bates, Myrtis Ford, Robert Tate, and Mary Anna Davis for help in the laboratory and C. Biggers, M. Beck, J. Grizzle, M. S. Fitzpatrick, C. Lessman, N. Parker, B. Wolters and T. Wong for reviewing the manuscript. Supported in part by funds from USDA Grant 88-34123-3504 and USDI, FWS Contract 14-16-0009-89-929.

REFERENCES

Baker, I. J., Solar, I. I., and Donaldson, E. M. (1988). Masculinization of chinook salmon (*Oncorhynchus tshawytscha*) by immersion treatments using 17α-methyltestosterone around the time of hatching. *Aquaculture* **72,** 359–367.

Billard, R., and Peter, R. E. (1977). Gonadotropin release after implantation of antiestrogens in the pituitary and hypothalamus of goldfish, *Carassius auratus*. *Gen. Comp. Endocrinol.* **32,** 213–220.

Breton, B., Jalabert, B., and Fostier, A. (1975). Induction de decharges gonadotropes hypophysaires chez la carp (*Cyprinus carpio* L.) a l'aide du citrate de cisclomiphene. *Gen. Comp. Endocrinol.* **25,** 400–404.

Callard, G. (1984). Aromatization in brain and pituitary: An evolutionary perspective. *In* "Aromatization of Hormonal Steroids in the Neuroendocrine Structures" (F. Celotti, F. Naftolin, and L. Martini, Eds.), Vol. 13, pp. 70–102. Raven Press, New York.

Chan, S. T. H., and Yeung, W. S. B. (1983). Sex control and sex reversal in fish under natural conditions. *In* "Fish Physiology" (W. S. Hoar, D. J. Randall, and E. M. Donaldson, Eds.), Vol. 9B, pp. 171–222. Academic Press, New York.

Crim, L. W., Peter, R. E., and Billard, R. (1981). Onset of gonadotropic hormone accumulation in immature trout pituitary gland in response to estrogen or aromatizable androgen steroid hormones. *Gen. Comp. Endocrinol.* **44,** 374–381.

Davis, K. B., Simco, B. A., Goudie, C. A., Parker, N. C., Cauldwell, W., and Snellgrove, R. (1990). Hormonal sex manipulation and evidence for female homogamety in channel catfish. *Gen. Comp. Endocrinol.* **78,** 218–223.

Donaldson, E. M., and Hunter, G. A. (1982). Sex control in fish with particular reference to salmonids. *Canad. J. Fish. Aquat. Sci.* **39,** 99–110.

Fishman, J. (1982). Biochemical mechanism of aromatization. *Cancer Res.* **42,** 3277S–3280S.

Goudie, C. A., Redner, B. D., Simco, B. A., and Davis, K. B. (1983). Feminization of channel catfish by oral administration of steroid sex hormones. *Trans. Amer. Fish. Soc.* **112,** 670–672.

Hackmann, E., and Reinboth, R. (1974). Delimitation of the critical stage of hormone-influenced sex differentiation in *Hemihaplochromis multicolor* (Hilgendorf) (Cichlidae). *Gen. Comp. Endocrinol.* **24,** 42–53.

Hsuch, A. J. W., Erikson, G. F., and Yen, S. S. C. (1978). Sensitization of pituitary cells to luteinizing hormone releasing hormone by clomiphene citrate *in vitro*. *Nature* (*London*) **273,** 57–59.

Hunter, G. A., and Donaldson, E. M. (1983). Hormonal sex control and its application to fish culture. *In* "Fish Physiology" (W. S. Hoar, D. J. Randall, and E. M. Donaldson, Eds.), Vol. 9B, pp. 223–303. Academic Press, New York.

Kahwanago, J., Heinrich, W. L., and Herrmann, W. I. (1970). Estradiol "receptors" in hypothalamus and anterior pituitary gland: Inhibition of estradiol binding by SH-group blocking agents and clomiphene citrate. *Endocrinology* **84,** 1319–1326.

Kato, J., Kobayashi, T., and Villee, C. A. (1968). Effect of clomiphene on the uptake of estradiol by the anterior hypothalamus and hypophysis. *Endocrinology* **82,** 1049–1052.

Koopman, P., Gubbay, J., Vivian, N., Goodfellow, P., and Lovell-Badge, R. (1991). Male development of chromosomally female mice transgenic for Sry. *Nature* **351,** 117–121.

Levy, R. I. (1980). Drugs used in the treatment of hyperlipoproteinemias. *In* "The Pharmacological Basis of Therapeutics" (A. G. Gilman, L. S. Goodman, and A. Gilman, Eds.), pp. 834–847. Macmillan, New York.

Nakamura, M. (1975). Dosage-dependent changes in the effect of oral administration of methyltestosterone on gonadal sex differentiation in *Tilapia mossambica*. *Bull. Fac. Fish. Hokkaido Univ.* **26,** 99–108.

Page, D. C., Mosher, R., Simpson, E. M., Fisher, E. M. C., Mardon, G., Pollack, J., McGillivray, B., de la Chapelle, A., and Brown, L. G. (1987). The sex-determining region of the human Y chromosome encodes a finger protein. *Cell* **51,** 1091–1104.

Sinclair, A. H., Berta, P., Palmer, M. S., Hawkins, J. R., Griffiths, B. L., Smith, M. J., Foster, J. W., Frischauf, A. M., Lovell-Badge, R., and Goodfellow, P. N. (1990). A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature* (*London*) **346,** 240–244.

Solar, I. I., Donaldson, E. M., and Hunter, G. A. (1984). Optimization of treatment regimes for controlled sex differentiation and sterilization in wild rainbow trout (*Salmo gairdneri* Richardson) by oral administration of 17α-methyltestosterone. *Aquaculture* **42,** 129–139.

Strickberger, M. W. (1985). "Genetics." Macmillan, New York.