GREAT LAKES FISHERY COMMISSION

Research Completion Report[1]

Induced Sterility and Sex Inversion in Lampreys

by

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RESEARCH COMPLETION REPORT

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Executive Summary

A gonadal androgen receptor assay for lampreys was developed to provide a method for screening potential sex-inverting and sterilizing compounds. Such compounds could augment existing methods to control reproduction in sea lampreys through production of sterile or all male individuals.

The following are the technical objectives of the project:

1) Determine the presence and characteristics of androgen binding sites in gonads of juvenile and adult lampreys.
2) Determine which steroid(s) bind to gonadal androgen binding sites with the highest affinity.

During the two years of this study, we have accomplished the following:

- demonstrated the presence of specific binding sites for sex steroids in the ovaries and testes of lampreys
- established an assay to measure these binding sites
- provided a means for testing candidate compounds for sterilization and sex inversion
- defined the characteristics of the binding sites in relation to other steroid receptors
- tested the capacity of other steroids to compete for the binding site, thus identifying the best candidates for sterilization and sex inversion
- examined juvenile lampreys for androgen binding sites

We have demonstrated for the first time that androgen binding sites exist in the gonads of a member of the Petromyzontidae--the adult Pacific lamprey. Adult lamprey gonadal cytosol had specific binding for both a synthetic androgen, mibolerone, and a naturally occurring androgen, testosterone. The binding characteristics (namely, high affinity, low capacity, and steroid specificity) of these sites are similar to those of steroid receptors. Both ovarian and testicular cytosol had a high affinity, low capacity binding site for testosterone, and many more sites present in testicular cytosol than in ovarian cytosol. Furthermore, the binding of testosterone in testicular cytosol is highly steroid-specific; only 17α-methyltestosterone (MT) displaced ^{3}H-testosterone at excess concentrations similar to those of radioinert testosterone. These results suggest that MT may be the best candidate for sex inversion/hormonal sterilization studies.

Introduction and Rationale

One method utilized to control reproduction in sea lampreys (*Petromyzon marinus*) in the Great Lakes is the release of sterile males into spawning streams to compete with fertile males for access to females on the breeding grounds. To date, the most effective sterilizing technique has been the treatment of adult males with bisazir, a chemosterilant that requires special handling. The release of sterile male sea lampreys on spawning grounds to compete for access to female sea lampreys has successfully reduced the number of offspring produced by over 80% (Hanson and Manion 1980). A program is currently underway to test the effectiveness of this treatment in 27 tributaries to Lake Superior which requires the capture and treatment of 22,000 male sea lampreys (Bergstedt and Seelye 1992). The theoretical maximum reduction in larval production is 70%, given the ratio of sterile to normal males of 2.2:1.

For other species of fish, different methods for inducing sterility have been established, e.g., hormonal sterilization. However, this technique has not been developed to any extent for lampreys. Both bisazir treatment and hormonal sterilization have disadvantages. Bisazir treatment requires the collection of thousands of adult males and injection of each individual before release onto the spawning grounds. Hormonal sterilization entails the treatment of animals at earlier life stages, and requires the culture of individuals until maturation (unless it is proven safe to release sterile individuals into the environment). The major disadvantage of hormonal sterilization of lampreys can be overcome because large numbers of eggs and ammocoetes can be easily collected. Therefore, there is potential to produce very large numbers of sterile individuals for release onto the breeding grounds. Should methods become available for shortening the period necessary to culture lampreys, then hormonal sterilization may become even more cost effective.

The production of sterile or monosex populations of fish has become an important tool for aquaculture, especially with salmonids, tilapias, and carps (Donaldson and Benfey 1987). Sterility may be induced through the induction of triploidy (Thorgaard and Allen 1987) or through treatment with high doses of steroids, such as 17α-methyltestosterone, which are also used to masculinize fish (Hunter and Donaldson 1983). Induced triploidy has not been attempted in lampreys and treatment with steroids has had little success in altering gonadal differentiation (Hardisty and Taylor 1965; M. Docker, pers. comm.). It is possible that the hormone treatments occurred outside the time of sensitivity or that the steroids used were ineffective. The traditional mechanism by which steroids act is through a series of events initiated by binding of steroids to receptors in the cytosol of target cells and culminating in the steroid-receptor complex acting as DNA-binding transcription factor that modifies gene expression (O'Malley and Tsai 1992). The mechanism by which steroids alter gonadal differentiation has not been fully explained, but we have found steroid treatment to result in modification of sex-specific gonadal protein production in salmonids (Fitzpatrick

and Schreck 1991), which is consistent with a model of altered gene expression. Therefore, if certain androgens, for example, are capable of masculinizing or sterilizing the gonads of lampreys, then androgen receptors should be present during the critical periods of sensitivity. Identification of receptors and characterization of binding at various life stages may provide clues to when the animals would be sensitive to steroid treatment and which steroids are the most potent binding agents to those receptors.

Lamprey gonads remain sexually undifferentiated for a long period of development in comparison to other fishes such as salmon or tilapia. This has frustrated efforts to develop a hormone treatment because of the many potential combinations of compounds and treatment timing. Hormonal sterilization may entail treatment of ammocoetes at various life stages ranging from embryonic development through sexual differentiation and may require steroids that are different from those used commonly for other fish species. The proposed studies focused on the establishment of an assay to screen potential candidate steroids, thereby identifying the choice of compounds with which to treat ammocoetes. In addition, the assay could be used to establish the period of sensitivity to steroid treatment for sterilization or sex inversion, thereby pinpointing the most effective treatment periods, by correlating the number of receptors at various life stages.

The feasibility of accomplishing hormonal sterilization can be enhanced by gaining an understanding of the basic principles of steroid action on the gonads. To reach this understanding, we set out to achieve the following objectives:

1) Determine the presence and characteristics of androgen binding sites in gonads of juvenile and adult lampreys.
2) Determine which steroid(s) bind to gonadal androgen binding sites with the highest affinity.

Materials and Methods

Animal and Tissue Collection. Adult Pacific lamprey (*Lampetra tridentata*) were collected at the fish passage facility at Willamette Falls (near West Linn, OR) on 2 June 1993 and 20 April 1994 through the assistance of Craig Foster and a crew from Oregon Department of Fish and Wildlife. A total of 34 adults in 1993 and 70 adults in 1994 were transported on ice to the Smith Farm Fish Genetics and Performance Laboratory at Corvallis, OR. Gonads were removed into ice cold TEMS buffer (10 mM Tris-HCl, 1 mM EDTA, 20 mM Sodium molybdate, 12 mM Monothioglycerol, and 10% (v/v) glycerol; pH = 7.4). After all dissections were completed, gonads were weighed and homogenized in 2-3 volumes (w/v) of TEMS buffer with a motor driven piston. The homogenates were centrifuged at 1750 x g for 20 min at 4°C to pellet the nuclei and cellular debris. To remove endogenous steroids, the supernatants were incubated on ice with 0.5 volumes of 5% Charcoal-0.5% Dextran in TEMS, and centrifuged at 1750 x g for 20 min at 4°C. The nuclei were washed extensively with buffer and then extracted with extraction buffer (50 mM Tris-HCl, 1 mM EDTA, 12 mM Monothioglycerol, 0.7 M

KCl and 30% (v/v) glycerol; pH = 7.5) for 1 hr at 4°C. The supernatant and extracted nuclei fraction were centrifuged at 100,000 x g at 4°C for 60 min to prepare cytosolic and nuclear fractions which were stored at -80 C (nuclear fraction was incubated with 5% Charcoal-0.5% Dextran in TEMS for 10 min and then centrifuged at 1750 x g for 20 min at 4°C to remove endogenous steroids before storage). Protein content in each sample was determined by the method of Bradford (1976).

Outmigrating juvenile lampreys were collected at Leaburg smolt trap on the McKenzie River (a tributary of the Willamette River) and frozen by Oregon Department of Fish and Wildlife personnel. The gonads were dissected from these animals and processed as described above for adult gonads.

Binding Studies. A time course study was conducted to establish the time required to reach equilibrium for the receptor-steroid binding reaction. In brief, 0.150 ml of ovarian cytosol was incubated with 0.050 ml of 2 nM ^{3}H-mibolerone or ^{3}H-testosterone and 0.050 ml of either TEMS buffer (Total Binding tubes) or 1 μM radioinert mibolerone or testosterone (Non-specific Binding tubes) at 4°C. At 1, 2, 3, 4, 6, 12, and 24 hrs, the incubation was stopped by placing the tubes on ice and then adding 0.5 ml of 2.5% Charcoal-0.25% Dextran in TEMS, incubating for 10 min on ice, and finally centrifuging at 1750 x g for 20 min at 4°C to remove unbound steroid. Aliquots (0.5 ml) of the supernatants were decanted into scintillation vials containing 5 ml of scintillation cocktail and then counted for radioactivity. The difference between Total Binding and Non-specific Binding radioactivity was calculated and designated Specific Binding. To determine the optimal dilution of samples, the effect of diluting cytosol with buffer on binding over time was investigated through incubation of cytosol with ^{3}H-mibolerone for 12 (ovarian) or 24 hrs (ovarian and testicular). Specific Binding was calculated as before.

Once the time required for equilibrium binding was established, then the cytosol was measured for specific binding of ^{3}H-mibolerone, ^{3}H-testosterone, ^{3}H-11-ketotestosterone, and ^{3}H-estradiol. In brief, 0.150 ml of ovarian or testicular cytosol was incubated with a single concentration of ^{3}H-steroid in 0.05 ml and 0.05 ml of either TEMS buffer or 500-fold excess radioinert steroid at 4°C overnight. The bound steroid was separated as described above, measured for radioactivity, and Specific Binding calculated as before.

Saturation binding studies were conducted to determine the binding affinity and number of receptors in cytosolic fractions. In brief, 0.150 ml of ovarian cytosol was incubated for 2 hr at 4°C with 0.05 ml of ^{3}H-mibolerone or ^{3}H-testosterone at doses between 0.05 nM and 20 nM and 0.05 ml of either TEMS buffer or 500-fold excess radioinert mibolerone or testosterone. After incubation, the procedure for separating bound and unbound steroid was the same as that described above. The calculated specific binding data were analyzed by nonlinear regression using the equation $Y = (A*X)/(B+X)$ where X = the

incubation concentration of ^{3}H-steroid, Y = the concentration of ^{3}H-steroid specifically bound, A = number of binding sites (*Bmax*), and B = dissociation constant (*Kd*) (GraphPad Prism, San Diego, CA).

The steroid specificity of binding was determined in testicular cytosol through incubation of cytosolic extracts overnight at 4°C with 5 nM ^{3}H-testosterone alone (Total Binding) or with excess concentration (from 1- to 500-fold excess) of competing steroids. Maximum specific binding was calculated as the difference between Total Binding and Non-specific Binding (500-fold excess testosterone). Displacement of binding by other steroids was expressed as the difference between Total Binding and binding in the presence of competitor, divided by the maximum specific binding.

Results

A total of 104 lampreys were sampled for gonadal androgen binding. The mean weights and lengths (± SE) for lampreys collected were 62.3 ± 1.2 cm and 463.0 ± 24.1 g for females (n=23), 58.7 ± 1.4 cm and 372.7 ± 32.0 g for males (n=11) in 1993; 57.0 ± 0.7 cm and 359.5 ± 12.3 g for females (n=33), 54.1 ± 0.8 cm and 316.3 ± 10.8 g for males (n=37) in 1994. Each female had a single gonad full of readily discernible eggs; each male had small paired testes.

Time Course. The results of the time course studies indicated that ^{3}H-mibolerone binding reached equilibrium between 12 and 24 hr at 4°C in ovarian cytosol and that a dilution of 1:1 was best for maximizing Specific Binding in both ovarian and testicular cytosols (Fig. 1). Based on these results, all further incubations with ^{3}H-mibolerone were conducted overnight at 4°C. Similar

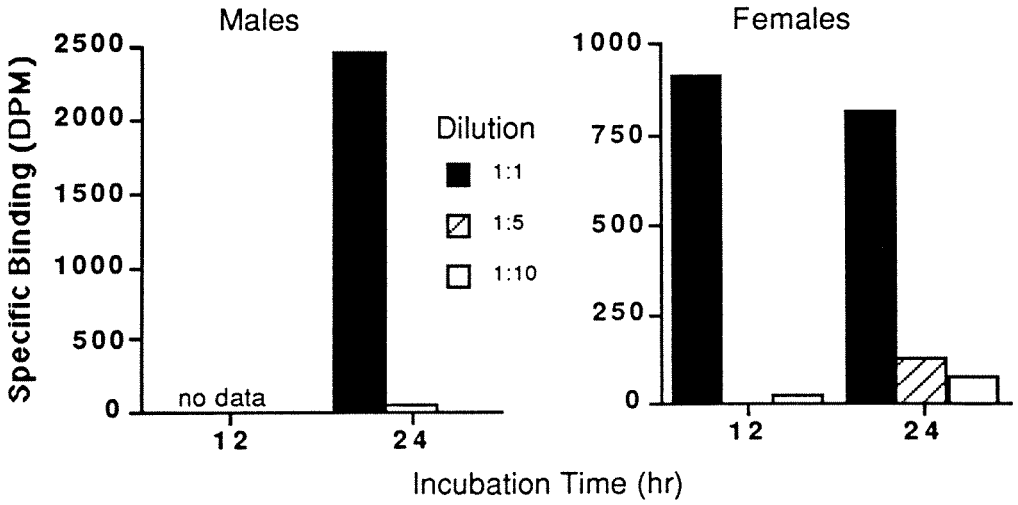

Fig. 1. Specific binding of [^{3}H]mibolerone in testicular and ovarian cytosol of Pacific lamprey. Cytosol which had been diluted 1:1, 1:5 (females only), or 1:10 with buffer was incubated for the indicated time at 4 C in the absence (Total Binding) or presence (Non-specific Binding) of 500-fold excess radioinert mibolerone. Specific binding was calculated as the difference between Total and Non-specific Binding. Each bar represents the mean of triplicate determinations.

results were achieved with incubation of ovarian cytosol with ^{3}H-testosterone. Equilibrium was reached between 12 and 24 hr at 4°C (Fig. 2) and all further incubations with ^{3}H-testosterone were conducted overnight at 4°C.

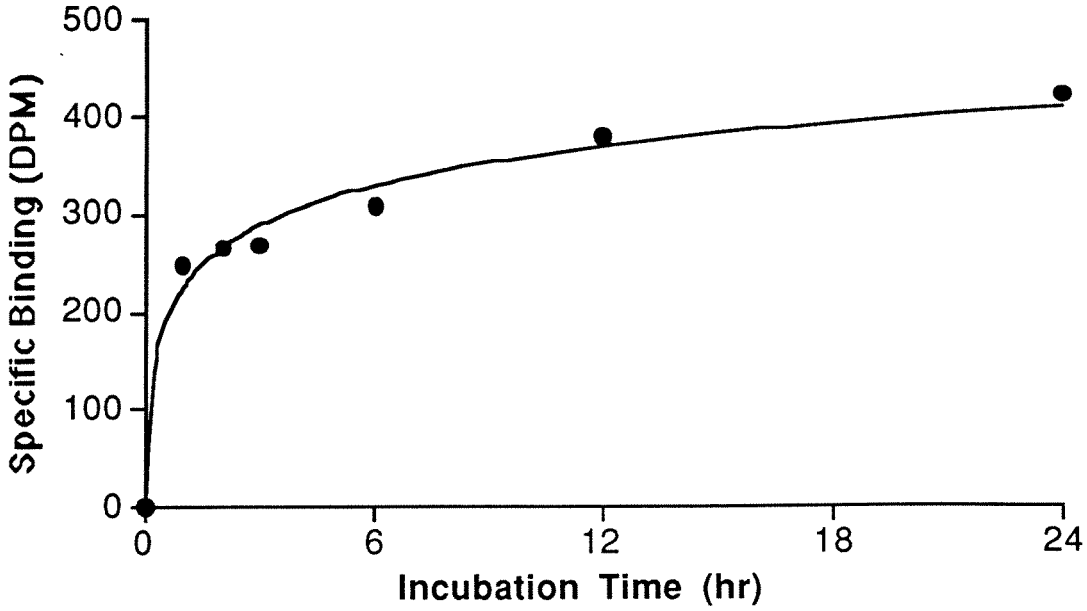

Fig. 2. Specific binding of [^{3}H]testosterone in ovarian cytosol of Pacific lamprey. Cytosol was incubated for the indicated times at 4 C in the absence (Total Binding) or presence (Non-specific Binding) of 500-fold excess radioinert testosterone. Specific binding was calculated as described for Fig. 1. Each point represents the mean of triplicate determinations.

Steroid Binding. Specific Binding for ^{3}H-mibolerone and ^{3}H-testosterone were readily detected in both ovarian and testicular cytosols from adult Pacific lamprey (Fig. 3). However, very low or no Specific Binding for ^{3}H-11-ketotestosterone and ^{3}H-estradiol was detected in gonadal cytosols from adults (data not shown). No Specific Binding of any of the steroids was detected in nuclear extracts or juvenile cytosol (data not shown).

Saturation Binding. Saturation binding assays for ^{3}H-mibolerone binding in adult ovarian cytosol indicated a dissociation constant, K_d, of 4.8 ± 1.0 nM and the maximum number of binding sites, B_{max}, of 19.6 ± 3.5 fmol/mg protein (mean ± SE; n=2; Fig. 4). Saturation binding experiments with ^{3}H-mibolerone were not conducted on adult testicular cytosol.

Saturation binding assays for ^{3}H-testosterone binding indicated a Kd of 2.3 ± 0.8 nM and $Bmax$ of 16.8 ± 2.5 fmol/mg protein (n=4) in adult ovarian cytosol (Fig. 5a); and a K_d of 2.5 nM and B_{max} of 478.6 fmol/mg protein in adult testicular cytosol (n=1; Fig. 5b).

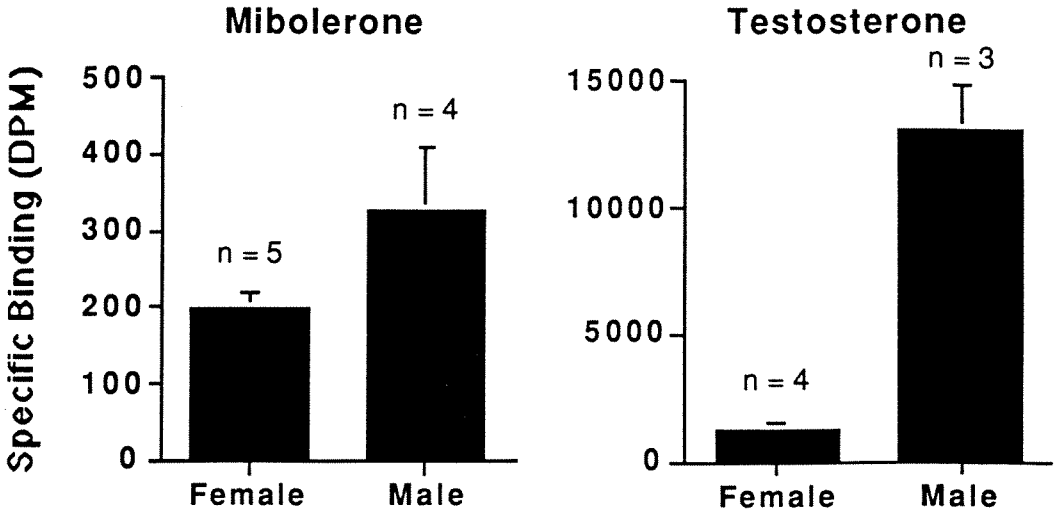

Fig. 3. Specific binding of [^{3}H]mibolerone and [^{3}H]testosterone in testicular and ovarian cytosol of Pacific lamprey. Cytosol was incubated overnight at 4 C in the absence (Total Binding) or presence (Non-specific Binding) of 500-fold excess radioinert mibolerone or testosterone. Specific binding was calculated as described in Fig. 1. Each bar represents the mean from the indicated number of experiments.

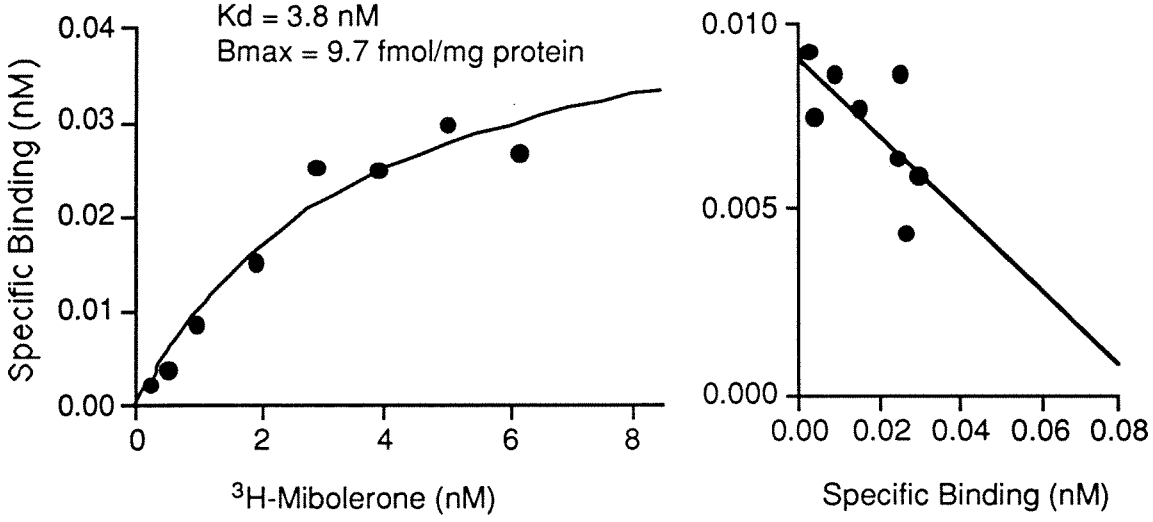

Fig. 4. Saturation analyses of [^{3}H]mibolerone binding in ovarian cytosol of Pacific lamprey. Cytosol extracts were incubated with increasing concentrations of [^{3}H]mibolerone overnight at 4 C in the absence (Total Binding) or presence (Non-specific Binding) of 500-fold excess radioinert mibolerone. Specific binding was calculated as described in Fig. 1. A scatchard plot of the specific binding data after transformation is shown. Each point represents the mean of triplicate determinations of a representative experiment from two experiments.

Steroid Specificity of Binding. Testicular cytosolic binding of testosterone demonstrated steroid specificity. Of the naturally occurring steroids,

dihydrotestosterone (DHT) was the most potent competitor for ^{3}H-testosterone (Fig. 6a); however, displacement of specific binding to less than 50% occurred only at between 10- and 100-fold excess of DHT, and did not occur even at 500-fold excess for 11-ketotestosterone (KT), estradiol (E2), progesterone (P4), and 15α-hydroxytestosterone (15α-T). Of the synthetic androgens tested, 17α-methyltestosterone (MT) was the most potent competitor for ^{3}H-testosterone (Fig. 6b), whereas mibolerone (Mb) and 17α-methyldihydrotestosterone (MDHT) were considerably less potent competitors. Displacement of specific binding to less than 50% occurred at between 2- and 100-fold excess MT, and at between 100- and 500-fold excess Mb or MDHT.

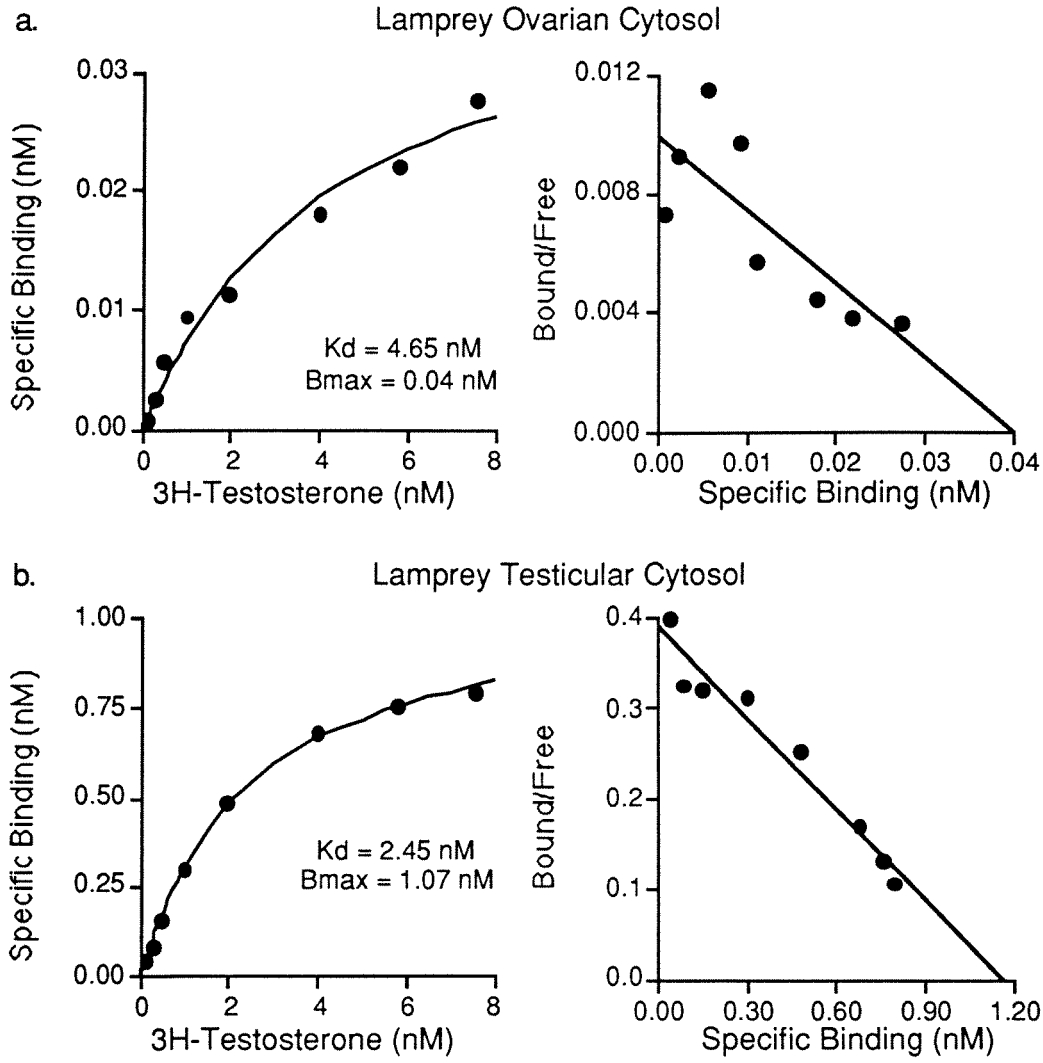

Fig. 5. Saturation analyses of [^{3}H]testosterone binding in ovarian (a) and testicular (b) cytosol of Pacific lamprey. Cytosolic extracts were incubated overnight at 4 C in the absence (Total Binding) or presence (Non-specific Binding) of 500-fold excess radioinert testosterone. Specific binding (the difference between Total and Non-specific Binding) is shown. Each point represents the mean of triplicate determinations. Scatchard plots of the transformed data are shown.

Discussion

We have demonstrated for the first time that androgen binding sites exist in the gonads of a member of the Petromyzontidae--the adult Pacific lamprey. We have established methods for measuring gonadal binding of both a synthetic androgen, mibolerone, and a naturally occurring androgen, testosterone, in cytosolic extracts of ovaries and testes from adults, and the binding characteristics (namely, high affinity, low capacity, and steroid specificity) of these sites are similar to those of steroid receptors. The binding site for mibolerone in the ovaries had high affinity and low capacity, but Scatchard plots of the data were not linear which indicated that more than one binding site may have been present. The binding site for testosterone in both ovarian and testicular cytosol had high affinity and low capacity binding site for testosterone and many more sites present in testicular cytosol than in ovarian cytosol. Furthermore, the binding of testosterone in testicular cytosol is highly steroid-specific; only 17α-methyltestosterone displaced ^{3}H-testosterone at excess concentrations similar to those of radioinert testosterone.

Androgen receptors have been characterized in the gonads of coho salmon (Fitzpatrick *et al.* 1994). The characteristics of the binding sites from lamprey gonads differs somewhat from those of coho salmon in that mibolerone was bound with about 10-fold less affinity in lamprey ovaries than in coho ovaries. Such results could be due to species differences; however, they could also be due to developmental differences. The coho ovarian cytosols were prepared from juvenile animals (Fitzpatrick *et al.* 1994); whereas the lamprey ovarian cytosols were prepared from adults. The abundance of yolk proteins in adult ovaries may interfere with mibolerone binding.

Testosterone binding in lamprey ovarian cytosol was over six times higher (1244 ± 301 vs 197 ± 19 dpm), and in testicular cytosol was almost 40 times higher (13046 ± 1764 vs 327 ± 83 dpm) than that for mibolerone in comparable tissues. Such high binding of testosterone, especially in testicular cytosol, led us to concentrate our efforts on characterizing testosterone binding. Both ovarian and testicular cytosol demonstrated the presence of saturable, high affinity binding sites. The affinity for testosterone was similar for binding sites in either ovaries or testes; however, there were many more binding sites in testicular cytosol. The number of testosterone binding sites in testicular cytosol exceeded the number of binding sites in female cytosol by over 100-fold.

Androgen receptors have been characterized from the brains of goldfish, *Carassius auratus* (Pasmanik and Callard 1988) and the skin of brown trout, *Salmo trutta* (Pottinger 1987, 1988). Pasmanik and Callard (1988) found that goldfish brains had androgen binding sites with equal affinity for ^{3}H-testosterone or ^{3}H-

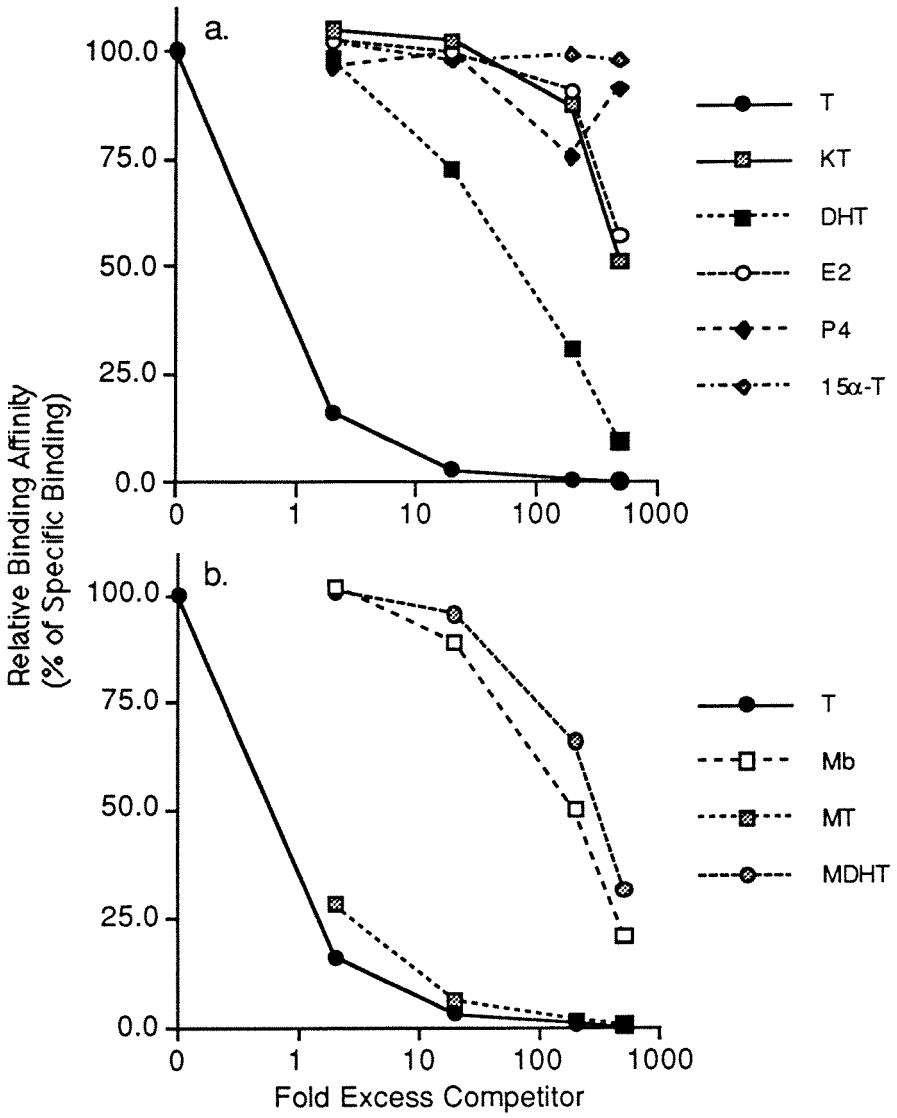

Fig. 6. Specificity of steroid binding in testicular cytolsol of Pacific lamprey. Cytosolic extracts were incubated with [^{3}H]testosterone overnight at 4 C in the absence (Total Binding) or presence of excess radioinert competitor steroid (displayed on the x-axis). Specific Binding was calculated as the difference between Total Binding and binding in the presence of 500-fold excess testosterone. Displacement of binding (in percentage) is expressed as the difference between Total Binding and binding in the presence of competitor, divided by the Specific Binding. Each point represents the mean of triplicate determinations. a. Naturally occurring steroids: testosterone (T), 11-ketotestosterone (KT), dihydrotestosterone (DHT), estradiol (E2), progesterone (P4), and 15α-hydroxytestosterone (15α-T). b. Synthetic steroids: mibolerone (Mb), 17α-methyltestosterone (MT), and 17α-methyldihydrotestosterone (MDHT).

dihydrotestosterone; however, DHT had limited capacity to compete for ^{3}H-testosterone binding in lamprey testes. Therefore, the lamprey testosterone binding site may differ considerably from testosterone binding sites in other fish species. The unusual steroid 15α-hydroxytestosterone, which has been shown to

be produced *in vitro* by the gonads of various lamprey species (Kime and Rafter 1981; Kime and Callard 1982), and was suggested to be the major androgen produced by lampreys, did not displace testosterone binding in testicular cytosol. This finding suggests that the question of the nature and role of androgens in lampreys remains unresolved.

Katz *et al.* (1982) found that only two of 77 blood samples from sexually mature sea lampreys *Petromyzon marinus* contained testosterone. Total androgens (testosterone, 11-ketotestosterone, and DHT) in plasma from adult sea lampreys were measurable but low and unfluctuating during final maturation (Sower *et al.* 1985), which suggested to some researchers that 'classical' androgens have a limited role in lamprey physiology (Kime 1993). However, Kime and Larsen (1987) found concentrations of testosterone in excess of 1 ng/ml in plasma from gonadectomized River lampreys, *L. fluviatilis*, which suggested to these authors that there was a nongonadal source for testosterone (and estradiol). The presence of a highly specific binding site in the testes of Pacific lampreys found in the current study indicates that at least one part of the signal transduction system for androgens is present in the gonads of lampreys. It is possible that this binding site plays no role in final maturation if androgens are not present, as has been suggested by other workers. However, the binding site could be a remnant of androgen action at some other life stage, e.g. gonadal differentiation.

If the testosterone binding site characterized in Pacific lamprey testes plays a role in gonadal differentiation, then the competition studies suggest that the selection of synthetic steroids to invert the sex or hormonally sterilize lampreys must be made carefully. Displacement of ^{3}H-testosterone binding at low concentrations occurred only with the use of 17α-methyltestosterone; mibolerone and 17α-methyldihydrotestosterone were much less effective. Mibolerone and 17α-methyldihydrotestosterone have been shown to be more potent masculinizing agents in tilapia (Torrans *et al.* 1988) and salmon (Piferrer *et al.* 1993) than 17α-methyltestosterone; although, the latter is widely used as a masculinizing agent. In coho salmon ovaries, all three of these synthetic androgens were nearly equipotent competitors for the ^{3}H-mibolerone receptor (Fitzpatrick *et al.* 1994) which was consistent with the potency of these compound for masculinizing salmon. The effectiveness of 17α-methyltestosterone for displacing ^{3}H-testosterone binding suggests that this synthetic androgen is the logical first choice for testing as a masculinizing agent in lampreys.

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References

Bergstedt, R.A. and J.G. Seelye (1992). Current and proposed alternative methods for the control of sea lampreys. U.S. Fish and Wildlife Service Report, Hammond Bay Biological Station, April 1992.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Donaldson, E.M. and T.J. Benfey (1987). Current status of induced sex manipulation. In: Proceedings of the Third International Symposium on the Reproductive Physiology of Fish, (D.R. Idler, L.W. Crim, and J.M. Walsh, eds.), pp. 108-119, Memorial University, Newfoundland.

Fitzpatrick, M.S. and C.B. Schreck (1991). Sex-specific gonadal proteins in salmonids. In: Proceedings of the Fourth International Symposium on Reproductive Physiology of Fish (A.P. Scott, J.P. Sumpter, D.E. Kime, and M.S. Rolfe, eds.), p. 230, FishSymp 91, Sheffield.

Fitzpatrick, M.S., W.L. Gale, and C.B. Schreck (1994). Binding characteristics of an androgen receptor in the ovaries of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. 95: 399-408.

Hanson, L.H. and P.J. Manion (1980). Sterility method of pest control and its potential role in an integrated sea lamprey (Petromyzon marinus) control program. Can. J. Fish. Aquat. Sci. 37: 2108-2117.

Hardisty, M.W. and B.J.R. Taylor (1965). The effects of sex hormones on the ammocoetes larva. Life Sci. 4: 743-747.

Hunter, G.A. and E.M. Donaldson (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.

Katz, Y., L. Dashow, and A. Epple (1982). Circulating steroid hormones of anadromous sea lampreys under various experimental conditions. Gen. Comp. Endocrinol. 48: 261-268.

Kime, D.E. (1993). 'Classical' and 'non-classical' reproductive steroids in fish. Rev. Fish Biol. Fish. 3: 160-180.

Kime, D.E. and G.V. Callard (1982). Effect of gonadectomy and hypophysectomy on plasma steroid levels in male and female lampreys (*Lampetra fluviatilis*, L.). Gen. Comp. Endocrinol. 68: 189-196.

Kime, D.E. and L.O. Larsen (1987). Formation of 15-hydroxylated by the testes and other tissues of the sea lamprey, *Petromyzon marinus, in vitro.* Gen. Comp. Endocrinol. 46: 267-270.

Kime, D.E. and J.J. Rafter (1981). Biosynthesis of 15-hydroxylated steroids by gonads of the River lamprey, *Lampetra fluviatilis, in vitro.* Gen. Comp. Endocrinol. 44: 69-76.

O'Malley, B.W. and M.-J. Tsai (1992). Molecular pathways of steroid receptor action. Biol. Reprod. 46, 163-167.

Pasmanik, M. and G.V. Callard (1988). A high abundance androgen receptor in goldfish brain: characteristics and seasonal changes. Endocrinology 123: 1162-1171.

Piavis, G.W. (1961). Embryological stages in the sea lamprey and effects of temperature on development. U.S. Fish Wildl. Bull. 61: 111-143.

Piferrer, F., I.J. Solar, and E.M. Donaldson (1993). Effects of natural, synthetic, aromatizable, and nonaromatizable androgens in inducing male sex differentiation in genotypic female chinook salmon (*Oncorhynchus tshawytscha*). Gen. Comp. Endocrinol. 91: 59-65.

Pottinger, T.G. (1987). Androgen binding in the skin of mature brown trout, *Salmo trutta.* Gen. Comp. Endocrinol. 66: 224-232.

Pottinger, T.G. (1988). Seasonal variation in specific plasma- and target-tissue binding of androgens, relative to plasma steroid levels, in brown trout, *Salmo trutta* L. Gen. Comp. Endocrinol. 70: 334-344.

Sower, S.A., E. Plisetskaya, and A. Gorbman (1985). Changes in plasma steroid and thyroid hormones and insulin during final maturation and spawning of the sea lamprey, *Petromyzon marinus.* Gen. Comp. Endocrinol. 58: 259-269.

Thorgaard G.H. and S.K. Allen (1987). Chromosome manipulation and markers in fishery management. In "Population Genetics and Fishery Management" (N. Ryman and F. Utter, eds.), pp. 319-331, Washington Sea Grant Program, University of Washington Press, Seattle.

Torrans, L., F. Merriwether, F. Lowell, B. Wyatt, and P.D. Gwinup (1988). Sex-reversal of Oreochromis aureus by immersion in mibolerone, a synthetic steroid. J. World Aquaculture Soc. 19: 97-102.