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The Presence of High-Affinity, Low-Capacity Estradiol-17β Binding in Rainbow Trout Scale Indicates a Possible Endocrine Route for the Regulation of Scale Resorption

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High-affinity, low-capacity estradiol-17β (E_2) binding is present in rainbow trout scale. The K_d and B_{max} of the scale E_2 binding are similar to those of the liver E_2 receptor (K_d is 1.6 ± 0.1 and 1.4 ± 0.1 nM, and B_{max} is 9.1 ± 1.2 and 23.1 ± 2.2 fmol × mg protein^{-1}, for scale and liver, respectively), but different from those of the high-affinity, low-capacity E_2 binding in plasma (K_d is 4.0 ± 0.4 nM and B_{max} is 625.4 ± 63.1 fmol × mg protein^{-1}). The E_2 binding in scale was displaced by testosterone, but not by diethylstilbestrol. Hence, the ligand binding specificity is different from that of the previously characterized liver E_2 receptor, where E_2 is displaced by diethylstilbestrol, but not by testosterone. The putative scale E_2 receptor thus appears to bind both E_2 and testosterone, and it is proposed that the increased scale resorption observed during sexual maturation in both sexes of several salmonid species may be mediated by this receptor. No high-affinity, low-capacity E_2 binding could be detected in rainbow trout gill or skin.

INTRODUCTION

Sexual maturation in female teleosts is stimulated by a gonadotropin-induced increase in plasma estradiol-17β (E_2) levels (Nagahama, 1987). E_2 stimulates the liver to produce vitellogenin, a yolk protein precursor which binds significant amounts of calcium. Vitellogenin is subsequently sequestered by the oocytes, processed, and stored to provide nutrition for the embryo. The accumulation of calcium in the maturing oocytes during this period elevates the calcium demand of the female and is met by calcium mobilization from internal and/or external sources. Although the precise role of the calcium bound to vitellogenin and accumulated in the oocytes remains to be elucidated, it is likely that the calcium is important for the normal development of the embryo.

Scales can contain approximately 20% of the total body calcium of teleost fish (Berg, 1968; Fleming, 1974). There is ample evidence that scale resorption increases during sexual maturation of several salmonid species (Crichton, 1935; Järvi and Menzies, 1936; van Someren, 1937; Takagi, 1990; Persson *et al.*, 1998) and that E_2 treatment induces calcium mobilization from scales in goldfish (*Carassius auratus*), killifish

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(*Fundulus heteroclitus*), and rainbow trout (*Oncorhynchus mykiss*) (Mugiya and Watabe, 1977; Carragher and Sumpter, 1991; Persson *et al.*, 1994). The E_2-induced calcium mobilization from rainbow trout scales is due to an increased scale osteoclast activity (Persson *et al.*, 1995, 1997), and an increased scale osteoclast activity has been observed during sexual maturation of Atlantic salmon, *Salmo salar* (Persson *et al.*, 1998).

E_2 treatment has also been demonstrated to increase whole-body calcium uptake from the environment (Persson *et al.*, 1994). This may occur across the gills, skin, fins, and intestine. The gills are considered to account for a large part of the calcium uptake by freshwater fish (Mashiko and Jozuka, 1962; Perry and Wood, 1985; Flik *et al.*, 1985a), and active calcium uptake mechanisms have been demonstrated in the gills of several teleost species (Flik *et al.*, 1984, 1985b; Flik and Perry, 1989). Further, some environmental calcium uptake can occur across skin and fins (Mashiko and Joshuka, 1962; Perry and Wood, 1985), and it has been shown that teleost skin is capable of active calcium uptake, with the calcium transporting capacity increasing with the density of mitochondria-rich cells (Marshall *et al.*, 1992; McCormick *et al.*, 1992).

The action of E_2 is likely to be mediated by an intracellular and/or a membrane-bound receptor(s). The presence of E_2 receptors and E_2 receptor mRNA have been demonstrated in the liver of several teleost species (Le Menn *et al.*, 1980; Slope *et al.*, 1984; Lazier *et al.*, 1985; Pottinger, 1986; McPherson *et al.*, 1988; Pakdel *et al.*, 1989; Pottinger and Pickering, 1990; Smith and Thomas, 1991; Hernández *et al.*, 1992; Campbell *et al.*, 1994; MacKay *et al.*, 1996) and in rainbow trout brain and pituitary (Salbert *et al.*, 1991; Pakdel *et al.*, 1994). Further, a high-affinity E_2 binding component has been identified in teleost plasma (Fostier and Breton, 1975; Le Menn *et al.*, 1980; Lazier *et al.*, 1985; Pasmanik and Callard, 1986; Pottinger, 1986; Pottinger and Pickering, 1990; Laidley and Thomas, 1994). In salmonids, this high-affinity E_2 binding component in plasma generally has an order of magnitude lower affinity for E_2 than the liver E_2 receptor. In addition, a low-affinity E_2 binding component has been found in plasma of rainbow trout and Atlantic salmon (Fostier and Breton, 1975; Lazier *et al.*, 1985).

To elucidate whether the E_2-induced increase in scale osteoclast activity and total-body calcium uptake is mediated by a direct action of E_2 on scale, gill, and skin, this study investigated the presence of high-affinity, low-capacity E_2 binding in these tissues of rainbow trout.

MATERIALS AND METHODS

Fish. Juvenile rainbow trout (87 ± 6 g) were obtained from a local hatchery, Antens Laxodling AB, Anten, Sweden. The fish were acclimated to aerated, recirculating fresh water ($[Ca^{2+}] \approx 0.5$ mM) at 10° for at least 12 days prior to killing and fed (diets by EWOS) 1.5% body weight × day^{-1} (EWOS).

Sampling. The fish were killed by a blow to the head and bled by draining blood from the caudal vessels into heparinized syringes. Thereafter, all procedures were performed on ice. The blood was centrifuged immediately to obtain plasma. The heart was exposed, the caudal fin was severed at the caudal peduncle, and the fish was perfused with 5 ml homogenization buffer (20 mM Tris, 3 mM $MgCl_2$, 330 mM sucrose, and 10 mM monothioglycerol, pH 7.9) through the heart. The gill arches were dissected out, rinsed in homogenization buffer, and blotted dry, and the soft tissue was scraped off using a glass slide. The body surface was blotted dry, the mucus was scraped off, and the scales were sampled using a scalpel. Each scale lies in a separate scale pocket and is covered by the epidermis, and, on removal, a piece of epidermis remains attached to the scale surface. The liver was dissected out, and a piece of skin from the head, containing no visible scales, was removed. All tissues sampled were weighed and immediately frozen in 2 ml homogenization buffer on dry ice, as were the plasma samples.

Cytosol preparation. The tissues were thawed, and liver (about 1.2 g) and gill (about 0.8 g) were homogenized in 5 ml homogenization buffer using an ultraturrax (Intermed Disp 25) for 3 × 10 s. Scales (about 0.8 g) and skin (about 0.1 g) were homogenized in 5 and 3 ml homogenization buffer, respectively, using a glass–glass motorized homogenizer. The scales were homogenized until well fragmented to rupture all associated cells. The teleost scale, which consists of a calcified layer and a fibrillary plate, is

formed by osteoblasts and resorbed by osteoclasts attached to the scale surface, and is generally considered to be acellular, although in some species cells have been observed within the fibrillary plate, suggesting a certain acellularization (see Persson, 1997). Protease inhibitor cocktail (0.5 ml containing 0.5 mg bacitracin, 1 μg aprotinin, 40 μg benzamidine, 20 μg *o*-phenanthroline) was added, and the homogenates were centrifuged at 1000*g* for 30 min at 4°. The resulting supernatants were spun at 48,000*g* for 2 h at 4° to obtain the cytosol fraction. To remove endogenous steroids, dextran-coated charcoal (DCC; 5% charcoal and 0.5% dextran in homogenization buffer) was added to the cytosols in the ratio 1:3 (DCC:cytosol). After a 10-min incubation at 4°, the DCC was spun down at 35,000*g* for 20 min at 4°. The centrifugation was repeated to ensure the removal of all DCC, and the resulting supernatants were used in the E_2 radioreceptor assay.

Quantification of E_2 binding. Binding of E_2 to liver (which was included as a positive control), scale, gill, and skin cytosols ($n = 10$) was assessed as described below. Duplicate 100-μl aliquots of each cytosol were incubated with 0.5, 1, 3, 6, and 12 nM [2,4,6,7-^{3}H(N)]E_2 ([^{3}H]E_2, 84.1 Ci $\times$ mmol^{-1}, NEN), with and without a 500-fold excess of E_2 to a final volume of 200 μl, giving nonspecific and total binding, respectively. After an overnight incubation at 4°, unbound steroid was removed by adding 500 μl DCC (0.5% charcoal and 0.05% dextran). The samples were incubated for 10 min and spun at 2000*g* for 15 min at 4°. Five hundred microliters of each supernatant were removed, added to a scintillation vial containing 2.5 ml Optiphase Hisafe 3 scintillation fluid (Wallac), and counted in a liquid scintillation counter (Wallac 1409). Specific binding was calculated as the difference between total and nonspecific binding. The equilibrium dissociation constant (K_d), the binding capacity (B_{max}), the Hill coefficient, and the number of E_2 binding sites were assessed using EBDA/LIGAND software (McPherson, 1985).

Ligand binding specificity. The ligand binding specificity was determined in liver and scale cytosols by incubating each cytosol (100 μl; $n = 4$) in duplicate with 5 nM [^{3}H]E_2 and 0-, 1-, 10-, 100-, 500-, and 1000-fold excess E_2, testosterone, 4-pregnene-17α20β-diol-3-one (17α,20β-P), cortisol, or diethylstilbestrol (DES) to a final volume of 200 μl. The cytosols were assayed and bound ligand was assessed as described above.

E_2 binding in plasma. To demonstrate that the high-affinity, low-capacity E_2 binding component in the scale cytosol is distinct from the high-affinity, low-capacity E_2 binding in plasma, and thus not due to plasma contamination, plasma was diluted 20 times and treated with DCC (5% charcoal and 0.5% dextran) to remove endogenous steroids as described for the cytosols. The quantification of the E_2 binding was performed as described for the cytosols. However, to obtain saturation of the high-affinity plasma E_2 binding site, the [^{3}H]E_2 concentrations were increased to 2, 4, 8, 19, and 30 nM.

Ammonium sulfate precipitation. To further characterize the high-affinity, low-capacity E_2 binding components in liver, scale, and plasma, saturated ammonium sulfate (SAS) precipitation was performed. Juvenile rainbow trout (103 $\pm$ 6 g) were obtained, kept, and sampled, and cytosol was prepared as described above. Plasma, liver, and scale cytosol were pooled into three batches per tissue with 10 fish in each pool. SAS precipitation was performed on each pool at 4° with constant shaking by adding SAS (in 0.01 M Tris, pH 7.0) dropwise (50 μl) to 2.5 ml sample to give a final concentration of 0, 20, 25, 30, 35, 40, 50, or 60% SAS. The samples were incubated at 4° with constant shaking for 30 min and centrifuged at 12,000*g* for 20 min at 4°. The pellets were dissolved in 2.5 ml homogenization buffer, and the E_2 binding was quantified as described above.

Protein determination. Protein concentration was determined in all cytosols, plasma, and SAS precipitates by the method of Lowry *et al.* (1951).

Statistical analyses. Differences in K_d and B_{max} were assessed by a one-way ANOVA followed by a Newman–Keuls test. The data from the ammonium sulfate precipitation experiments were ranked, and an ANOVA and a Newman–Keuls test were performed on the ranked data. Deviation of the Hill coefficients from one was assessed by a one-sample *t* test. Differences in the inflexion point of ligands observed to displace ^{3}[H]E_2 were tested by a one-way ANOVA. The validity of a one- versus two-binding-site model was assessed by EBDA/LIGAND software (McPherson, 1985). Statistical significance was set to $P < 0.05$. The results are presented as means $\pm$ SE.

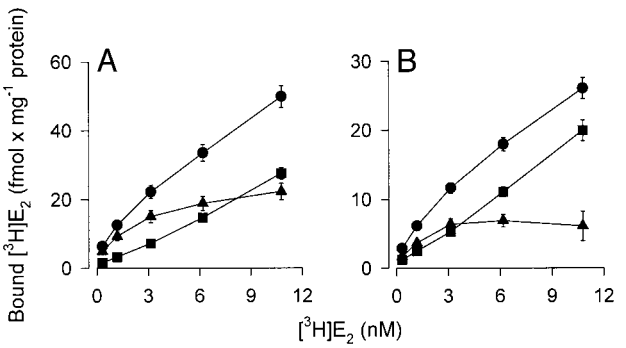

FIG. 1. Total (●), specific (▲), and nonspecific (■) binding of [^{3}H]estradiol-17β ([^{3}H]E_2) by rainbow trout liver (A) and scale (B) cytosol (n = 10). Data are presented as means ± SE.

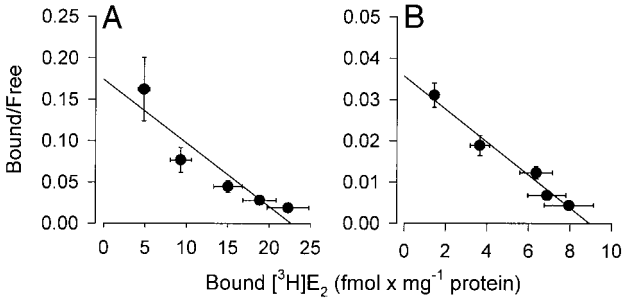

FIG. 2. Scatchard plot of [^{3}H]estradiol-17β ([^{3}H]E_2) bound to rainbow trout liver (A) and scale (B) cytosol (n = 10). Data are presented as means ± SE.

RESULTS

Quantification of E_2 binding. Rainbow trout liver and scale cytosols bound [^{3}H]E_2 in a specific and saturable manner (Figs. 1A and 1B). Models for one versus two binding sites were assessed by EBDA/LIGAND software (McPherson, 1985), and only one E_2 binding site appeared to be present in both liver and scale cytosol. There was no difference between liver and scale K_d or B_{max} ($P > 0.05$, Table 1). The Hill coefficients for liver and scale did not differ significantly from one ($P > 0.05$, Table 1). The Scatchard plots for liver and scale cytosols are presented in Figs. 2A and 2B, respectively. No specific binding was detected in gill and skin cytosols.

Ligand binding specificity. The [^{3}H]E_2 binding in the liver cytosol was displaced by E_2 and DES, but not by testosterone, cortisol, or 17α,20β-P (Fig. 3A), whereas the [^{3}H]E_2 binding in the scale cytosol was displaced by E_2 and testosterone, but not by DES, cortisol, or 17α,20β-P (Fig. 3B). There was no difference between the inflexion points of E_2 and DES in liver ($P > 0.05$), or between the inflexion points of E_2 and testosterone in scale ($P > 0.05$).

E_2 binding in plasma. Rainbow trout plasma was found to bind [^{3}H]E_2 in a specific and saturable manner (Fig. 4A). The Scatchard plot for plasma is presented in Fig 4B. The plasma K_d and B_{max} were different from those of liver and scale ($P < 0.05$, Table 1), and the plasma Hill coefficient was different from one ($P < 0.05$, Table 1).

Ammonium sulfate precipitation. The high-affinity, low-capacity E_2 binding component in liver started to precipitate at 20% SAS, whereas that of scale and

TABLE 1

Characteristics of High-Affinity, Low-Capacity Estradiol-17β Binding in Liver and Scale Cytosol and Plasma of Rainbow Trout

	K_d (nM)	B_{max} (fmol × mg^{-1} protein)	Hill coefficient
Liver	1.4 ± 0.1[a]	23.1 ± 2.2[a]	1.11 ± 0.05
Scale	1.6 ± 0.1[a]	9.1 ± 1.2[a]	0.92 ± 0.06
Plasma	4.0 ± 0.4[b]	625.4 ± 63.1[b]	0.91 ± 0.03*

Note. Different letters indicate significant differences between tissues $P < 0.05$). Data are presented as means ± SE (n = 10).

* Indicates that the Hill coefficient was significantly different from 1 in plasma ($P < 0.05$), but not in either scale or liver cytosol ($P > 0.05$).

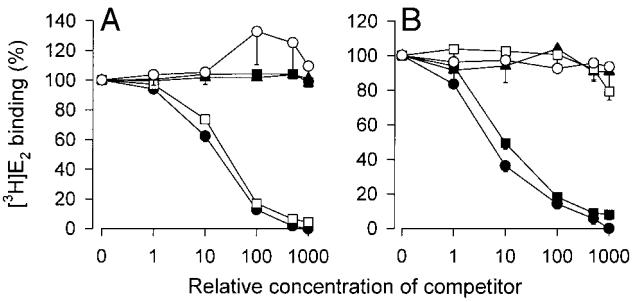

FIG. 3. Ligand specificity of the [^{3}H]estradiol-17β ([^{3}H]E_2) binding component in rainbow trout liver (A) and scale (B) cytosol. Data are expressed as the percentage [^{3}H]E_2 bound at a given relative concentration of competitor. Estradiol-17β (●), testosterone (■), cortisol (▲), diethylstilbestrol (□), 4-pregnene-17α20β-diol-3-one (○) (n = 4). Data are presented as means ± SE.

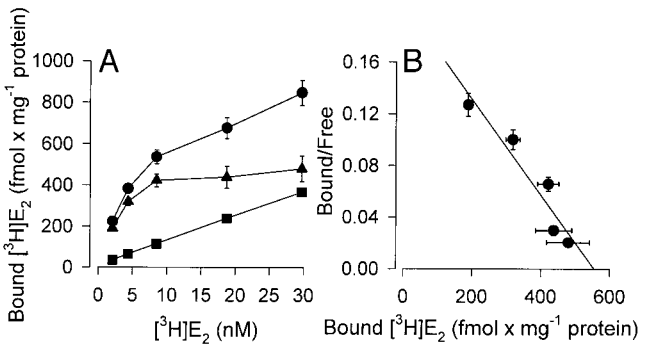

FIG. 4. Total (●), specific (▲), and nonspecific (■) binding of [^{3}H]estradiol-17β ([^{3}H]E_2) by rainbow trout plasma (A) and Scatchard plot of [^{3}H]E_2 bound to rainbow trout plasma (B) (n = 10). Data are presented as means ± SE.

plasma precipitated at 30 and 40% SAS, respectively (Table 2). The liver, scale, and plasma K_d precipitated by 0, 40, 50, or 60% SAS differed, as did B_{max} ($P < 0.05$; Table 2) with the exception of B_{max} at 40 and 50% SAS, where there was no difference between liver and scale ($P > 0.05$; Table 2).

DISCUSSION

This study demonstrates that high-affinity, low-capacity E_2 binding is present in juvenile rainbow trout scale and liver cytosols. The K_d of the E_2 binding in liver agrees with previously reported data on hepatic E_2 receptors in teleosts (Lazier *et al.*, 1985; Pottinger, 1986; McPherson *et al.*, 1988; Pottinger and Pickering, 1990; Smith and Thomas, 1991; Campbell *et al.*, 1994; MacKay *et al.*, 1996), and the B_{max} obtained is similar to previously reported values for adult male rainbow trout, as well as immature rainbow trout of both sexes, but lower than those reported for maturing female rainbow trout (Pottinger and Pickering, 1990; Campbell *et al.*, 1994; MacKay *et al.*, 1996). The ligand specificity of the high-affinity, low-capacity E_2 binding in liver cytosol is also in agreement with previous studies, as [^{3}H]E_2 was displaced by E_2 and DES, but not by testosterone, cortisol, or 17α,20β-P (Lazier *et al.*, 1985; Pottinger, 1986; McPherson *et al.*, 1988; Pottinger and Pickering, 1990; MacKay *et al.*, 1996). Further, only one high-affinity E_2 binding site appeared to be present in liver cytosol, which is in agreement with previous studies on the well-characterized hepatic E_2 receptor and suggests that the E_2 binding characterized for juvenile rainbow trout liver in this study is equivalent to the salmonid liver E_2 receptor described previously (Lazier *et al.*, 1985; McPherson *et al.*, 1988).

The high-affinity, low-capacity E_2 binding in scale cytosol has a K_d similar to the liver E_2 receptor K_d reported in this study and by others (see references above). The E_2 binding capacity of the scales is similar to that of the liver, and only one high-affinity E_2 binding site appeared to be present in scale cytosol. However, the ligand specificity of the E_2 binding in scale cytosol is different from that of the liver, as E_2 is displaced by testosterone, but not by DES. Despite the difference in specificity, the scale E_2 binding displays high affinity and low capacity, suggesting that the E_2 binding observed in scale cytosol is a putative E_2 receptor. Additional evidence for the existence of E_2

TABLE 2

Equilibrium Dissociation Constants and Binding Capacity of Ammonium Sulfate-Precipitated High-Affinity, Low-Capacity Estradiol-17β Binding in Liver and Scale Cytosol and Plasma of Rainbow Trout

Organ	Parameter	0% SAS	20%	25%	30%	35%	40%	50%	60%
Liver	K_d	0.8 ± 0.1^a	2.5 ± 0.4	7.6 ± 5.6	1.9 ± 0.2	1.3 ± 0.2	1.5 ± 0.1^a	1.6 ± 0.1^a	1.4 ± 0.1^a
Scale	K_d	1.4 ± 0.1^b	n.p.	n.p.	18.0 ± 10.4	7.0 ± 4.4	9.1 ± 3.2^b	5.2 ± 0.8^b	4.8 ± 0.6^b
Plasma	K_d	8.2 ± 1.0^c	n.p.	n.p.	n.p.	n.p.	25.1 ± 6.2^c	40.0 ± 5.1^c	51.0 ± 15.9^c
Liver	B_{max}	30.3 ± 10.6^a	75.2 ± 23.2	160.0 ± 97.2	71.4 ± 16.1	61.4 ± 17.2	67.0 ± 15.6^a	42.8 ± 10.3^a	29.7 ± 7.3^a
Scale	B_{max}	106.3 ± 22.2^b	n.p.	n.p.	107.6 ± 43.0	51.8 ± 15.8	44.1 ± 1.7^a	58.1 ± 13.8^a	115.3 ± 21.3^b
Plasma	B_{max}	450.9 ± 63.0^c	n.p.	n.p.	n.p.	n.p.	222.5 ± 35.3^b	451.7 ± 12.2^b	668.1 ± 99.0^c

Note. Different letters indicate significant differences between tissues ($P < 0.05$). Data are presented as means ± SE (n = 3 and each n comprises 10 pooled fish); n.p., not precipitated.

receptors in rainbow trout scale is the detection of E_2 receptor mRNA in this tissue (Armour *et al.,* 1997). However, it is yet not known whether the putative E_2 receptor described in this study corresponds to the E_2 receptor mRNA found by Armour *et al.* (1997). This, as well as the number of isoforms present in scales, needs further investigation. Further, it has been reported that E_2, but not DES, can interact with the androgen receptor and the androgen receptor coactivator and activate androgen target genes in yeast and human prostrate cells (Yeh *et al.,* 1998). Therefore, it is also important to characterize the E_2 and androgen receptor coactivators and the complexity of hormone receptor activation of target genes.

As in scales, E_2 receptor mRNA has recently been detected in rainbow trout bone (Armour *et al.,* 1997). The demonstration of E_2 binding and E_2 receptor mRNA in teleost calcified tissues is consistent with the existence of E_2 receptors in mammalian and avian osteoclasts, osteoblasts, and osteoclast- and osteoblast-like cells (Eriksen *et al.,* 1988; Komm *et al.,* 1988; Pensler *et al.,* 1990; Oursler *et al.,* 1991, 1994; Brubaker and Gay, 1994; Fiorelli *et al.,* 1996; Shamay *et al.,* 1996; Hoyland *et al.,* 1997).

Plasma of several salmonids, including rainbow trout, contains an E_2 binding component (Fostier and Breton, 1975; Lazier *et al.,* 1985; Pottinger, 1986; Pottinger and Pickering, 1990). As the plasma E_2 binding is displaced by testosterone, but not by DES, it might be that the high-affinity, low-capacity E_2 binding by the scale cytosol fraction could be due to plasma contamination. However, the K_d of the high-affinity, low-capacity E_2 binding component in plasma is significantly higher than that of both liver and scale, which is in agreement with previously reported studies on E_2 binding in salmonid liver and plasma (Lazier *et al.,* 1985; Pottinger, 1986; Pottinger and Pickering, 1990). To obtain saturation of the E_2 binding in plasma in this study, the [^{3}H]E_2 levels had to be increased to 2–30 nM (0.5–12 nM was used for liver and scale). Further, the Hill coefficient for plasma binding is less than one, implying negative cooperativity between multiple binding sites and thus the presence of more than one E_2 binding site (a Hill coefficient equal to one indicates no cooperativity, a Hill coefficient higher than one indicates positive cooperativity, and a Hill coefficient lower than one indicates negative cooperativity (Fersht, 1985)). This is in agreement with previous studies showing salmonid plasma to contain both high- and low-affinity E_2 binding sites (Fostier and Breton, 1975; Lazier *et al.,* 1985; Pottinger, 1986; Pottinger and Pickering, 1990). No high-affinity, low-capacity E_2 binding could be detected in either skin or gill tissue, which is highly vascularized. This further indicates that the perfusion carried out prior to sampling successfully eliminated plasma from the tissues studied and that the steroid binding in scales is not due to plasma contamination. The SAS precipitation also confirms that the E_2 binding in scale is different from that in plasma, as the scale binding component had a higher affinity for E_2 and precipitated at a different SAS concentration than that of plasma. The E_2 binding affinity and capacity for the scale E_2 binding component were in the same range as that of the liver E_2 receptor at all SAS concentrations, implying that the scale E_2 binding component is a true receptor. However, as the liver E_2 receptor precipitated at 20% SAS and the scale E_2 binding component at 30% SAS and the ligand binding specificity is different, it may be that the two receptors are not identical. There is evidence that several E_2 receptor subtypes and isoforms are present in teleosts, as in mammals (Tchoudakova *et al.,* 1999; Chang *et al.,* 1999). However, it remains to be elucidated whether the E_2 receptor characterized in this study is a novel subtype/isoform or whether it corresponds to one of the previously reported subtypes or isoforms.

The ligand binding specificity of the scale binding site, with similar affinity for E_2 and testosterone, is notable. However, a testosterone receptor which binds both E_2 and testosterone has previously been identified in the skin of male brown trout, *S. trutta,* where the binding of testosterone was displaced by E_2 (Pottinger, 1987). In the present study, there was no high-affinity, low-capacity E_2 binding by rainbow trout skin. However, it is unclear whether the skin samples used by Pottinger (1987) contained scale tissue.

Scale resorption increases during sexual maturation in several salmonid species (Crichton, 1935; Järvi and Menzies, 1936; van Someren, 1937; Takagi, 1990; Persson *et al.,* 1998). In females, this is believed to be caused by an E_2-induced increase in scale osteoclast activity (Persson *et al.,* 1995, 1997, 1998), and E_2 has recently been shown to increase scale osteoclast activ-

ity *in vitro* (Suzuki *et al.*, 2000). Further, E_2 has been demonstrated to down-regulate the expression of osteonectin (a scale matrix protein) mRNA in a goldfish scale cell line, suggesting an E_2-induced decrease in scale formation (Lehane *et al.*, 1999). The present study demonstrates the existence of high-affinity, low-capacity E_2 binding in scales, and it is suggested that the E_2-induced increase in scale osteoclast activity observed both *in vivo* and *in vitro* is mediated by a direct action of E_2 on the scale cells. However, no attempt has been made to separate osteoclasts from other cells (osteoblasts and fibroblasts) associated with the scales. Therefore, it is not possible at present to locate the high-affinity E_2 binding to a particular cell type. However, it seems likely that the putative E_2 receptor is located in the osteoclasts and/or osteoblasts, as no high-affinity, low-capacity E_2 binding was observed in fibroblast-containing skin.

The cause of the increased scale resorption observed in male salmonids during their sexual maturation has received little attention. It has been speculated that the scales are resorbed to provide calcium for the remodeling of the bones in the jaw and skull, as the secondary sexual characteristics develop (Tchernavin, 1938; Persson *et al.*, 1998). In male teleosts, several androgens are involved in the maturation of the gonads and the development of the secondary sexual characteristics (Borg, 1994), but the effects of androgens on scale metabolism have not been investigated. The present study suggests that testosterone binds to scale with an affinity similar to that of E_2. Thus, it may be speculated that the increased scale resorption observed in male and female salmonids during sexual maturation is mediated through the same receptor. In addition, plasma testosterone levels increase during sexual maturation of both male and female salmonids with peak values about 1 month before spawning (Scott and Sumpter, 1983; Olsson *et al.*, 1987; Norberg *et al.*, 1989), implying that the scale resorption observed in females during this phase can be affected by both E_2 and testosterone.

No high-affinity, low-capacity E_2 binding could be detected in rainbow trout gill or skin. Thus, it may be suggested that the E_2-induced increase in calcium uptake reported previously (Persson *et al.*, 1994) is not mediated by a direct action of E_2 on these tissues. Hence, it may be hypothesized that the E_2-induced increase in calcium uptake from the water is an indirect effect, mediated by other endocrine factors acting on the calcium uptake mechanisms.

In conclusion, high-affinity, low-capacity E_2 binding exists in rainbow trout scale. This putative receptor appears to bind both E_2 and testosterone and is thus proposed to be stimulated by both hormones. It is suggested that the increased scale resorption observed during sexual maturation in both sexes of several salmonid species may be mediated by this receptor.

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