Effects of 17-β-Estradiol and ICI 182 780 on Hair Growth in Various Strains of Mice

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17-β-Estradiol (10 nmol per 200 μl acetone) applied topically twice weekly to the clipped dorsal surface of C57BL/6 or C3H female mouse skin prevented hair growth, as previously described in the CD-1 mouse strain. Twice weekly topical application of the estrogen receptor antagonist, ICI 182 780 (10 nmol per 200 μl acetone), induced the telogen-anagen transition and produced early pigmentation appearance in skin and hair growth in C57BL/6 and C3H female mice. Whereas twice weekly topical application of 10 nmol 17-β-estradiol blocked hair growth, the intraperitoneal administration of this dose twice weekly did not block hair growth, suggesting a direct cutaneous effect of 17-β-estradiol. We also evaluated the effect of 17-α-estradiol, 17-β-estradiol, and ICI 182 780 on hair growth in male mice. As observed in female mice, 17-β-estradiol was a potent inhibitor of hair growth and ICI 182 780 stimulated hair growth; however, unlike the results previously observed in female mice, 17-α-estradiol was a potent inhibitor of hair growth in male mice. These results demonstrate that (i) the route of administration of 17-β-estradiol is critical for its ability to block hair growth; (ii) C57BL/6 and C3H mice, two commonly employed mouse strains for hair growth studies, responded to 17-β-estradiol and ICI 182 780 in a manner similar to that described in CD-1 mice; and (iii) the hair follicles of male and female mice respond similarly to 17-β-estradiol and ICI 182 780, but display striking sex differences in the response to 17-α-estradiol on hair growth. ***Key words: alopecia/dermal papilla/estrogen receptor/hair follicle. Journal of Investigative Dermatology Symposium Proceedings 4:285–289, 1999***

The hair follicle is a complex structure that is influenced by systemic factors as well as by signals derived from within the skin itself (Ebling *et al*, 1991; Hardy, 1992). A variety of hormones, including androgens, glucocorticoids, and estrogens, have been shown to alter hair follicle biology. Androgen receptors are expressed in the hair follicle (Blauer *et al*, 1991) and recently dihydrotestosterone (DHT) has received much attention due to its role in androgenic alopecia (Diani *et al*, 1992; Kaufman, 1996). Whereas many hormones may alter hair growth through a classic endocrine mechanism, the hair follicle itself has the metabolic capacity to synthesize a number of hormones, including testosterone, DHT, estrone, and 17-β-estradiol, from precursor steroids initially derived from the serum. For example, aromatase, 17β-hydroxysteroid dehydrogenase, 3α-hydroxysteroid dehydrogenase, 3β-hydroxysteroid dehydrogenase, and 5α-reductase are expressed within the hair follicle (for review see Sawaya, 1991). Thus, steroid hormones can be produced locally within the skin and can influence hair follicle biology through a paracrine mechanism.

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Although less studied than androgens, prolonged administration of various estrogens has been shown to retard hair growth in rats and mice (Hooker and Pfeiffer, 1943; Houssay, 1953; Johnson, 1958), dogs (Williams *et al*, 1946), and guinea pigs (Jackson and Ebling, 1972). Estrogen binding proteins or putative estrogen receptors, as detected by Scatchard analysis, have been detected in human (Hasselquist *et al*, 1980; Punnonen *et al*, 1980) and mouse skin (Uzuka *et al*, 1978), and based on [^{3}H]-estrogen binding are present in mouse and rat skin (Stumpf *et al*, 1974; Bidmon *et al*, 1990). Using an immunohistochemical approach and antibodies directed toward the estrogen receptor-α, our laboratory found that estrogen receptor-α is expressed in the nuclei of the dermal papilla cells of CD-1 mouse skin, and topical treatment of CD-1 mouse skin with 17-β-estradiol arrests follicles in telogen, whereas topical treatment with the estrogen receptor antagonist, ICI 182 780, caused the telogen follicle to enter anagen and initiate hair growth (Oh and Smart, 1996). These results indicate that an estrogen receptor pathway within the dermal papilla regulates the telogen-anagen transition of the hair follicle in CD-1 mice.

Because CD-1 mice are not frequently used in hair growth studies, we wanted to determine if 17-β-estradiol and ICI 182 780 could also modulate hair growth in strains of mice frequently employed in hair growth studies. Therefore, we have examined the effect of topically applied 17-β-estradiol and ICI 182 780 on hair growth in female C3H and C57BL/6 mice and compared these results with those obtained with female CD-1 mice. We also evaluated the effects of 17-β-estradiol, 17-α-estradiol, and ICI 182 780 on hair growth in male mice. In addition, we administered 17-β-estradiol intraperitoneally in an attempt to determine if the

effects of topically applied 17-β-estradiol were due to a direct cutaneous effect or an indirect systemic effect.

MATERIALS AND METHODS

Topical treatment with 17-β-estradiol, 17-α-estradiol, or ICI 182 780 Five-week-old female CD-1, C57BL/6, or C3H mice with the exact date of birth and 5-wk-old male CD-1 mice with the exact date of birth were purchased from Charles River Laboratories (Raleigh, NC). Mice were fed rodent chow (Purina Mills, Richmond, IN) and water *ad libitum* and were kept on a 12 h light/dark cycle. The hair on the dorsal region (approximately 4 × 2.5 cm area) was clipped with electric clippers. For hair growth studies, beginning on week 6 the mice were treated on the clipped dorsal surface with 10 nmol 17-β-estradiol (Sigma, St Louis, MO), 10 nmol 17-α-estradiol (Sigma), or 10 nmol ICI 182 780, a pure estrogen receptor antagonist (Zeneca Pharmaceuticals, Cheshire, U.K.), in 200 μl acetone or acetone alone twice weekly for up to 20 wk of age. Hair regrowth was categorized as that covering 50% or more of the clipped dorsal area or as full hair growth, which is defined as the complete regrowth of hair over the entire clipped dorsal surface.

Localization of estrogen receptor in mouse skin The dorsal skin area from 6-wk-old untreated mice was excised and fixed for 24 h in cold 10% neutral buffered formalin, then changed to cold 70% ethanol and processed and embedded in paraffin. Mouse uterus was collected and used as a positive control for estrogen receptor staining. Tissue sections (5 μm) were deparaffinized and placed in 3% H_2O_2 for 10 min to quench the endogenous peroxidase activity and then washed with automation buffer (Biomeda, Foster City, CA). The sections were treated with trypsin (0.15 mg per ml in automation buffer) for 4 min at room temperature followed by two washes with automation buffer and then incubated with DNAase (0.25 mg per ml in automation buffer) for 3 min at room temperature, followed by two washes with automation buffer. The sections were blocked with 10% normal goat serum and incubated with the prediluted ER-ICA rat monoclonal antibody (Abbott Laboratories, North Chicago, IL) overnight at 4°C. Slides were washed twice with automation buffer and incubated with biotinylated goat antirat IgG (Boehringer Mannheim, Indianapolis, IN) at a dilution of 1:50 for 60 min at room temperature. After washing with automation buffer twice, the sections were incubated with peroxidase (HRP)-conjugated streptavidin (1:20 dilution, BioGenex, San Ramon, FL) for 30 min at room temperature. The samples were washed with automation buffer followed by a 0.05 M Tris-HCl (pH 7.5) wash and incubated with 3,3′-diaminobenzidine tetrahydrochloride for 10 min in the dark. Slides were rinsed with 0.05 M Tris-HCl buffer and some samples were counterstained in hematoxylin. Samples were dehydrated in a graded series of ethanol and xylene and permanently mounted with Permount. Uterine cells demonstrated characteristic nuclear staining; however, no estrogen receptor staining was observed when control antibody was used or when the primary antibody was omitted in both skin and uterine samples. In addition, we utilized another monoclonal antibody to the estrogen receptor, ER1D5 (Immunotech, Westbrook, ME) and obtained the same results as with ER-ICA.

RESULTS

As previously reported, immunohistochemical staining for the estrogen receptor in CD-1 female mouse skin revealed intense and specific staining of the nuclei of cells within the dermal papilla of a telogen follicle, as shown in the counterstained section (**Fig 1*a***) (Oh and Smart, 1996). A noncounterstained section is shown in **Fig 1(*b*)** to better demonstrate the areas and levels of estrogen receptor expression. Very light estrogen receptor staining was observed in the cells of the outer root sheath in the isthmus of the telogen follicle, as well as in some nuclei of dermal fibroblasts.

The hair follicles of CD-1 female mice are highly synchronous from birth to approximately 12 wk of age. CD-1 female mice enter their second telogen at 6 wk of age and remain in telogen until approximately 9 wk, when they enter their third anagen. To evaluate the effects of 17-β-estradiol and ICI 182 780 on the telogen-anagen transition we utilized 6-wk-old mice that had entered their second telogen. The dorsal hair of 6-wk-old CD-1 female mice was clipped with electric clippers and the dorsal surface treated twice weekly with topical applications of either 10 nmol 17-β-estradiol, or 10 nmol ICI 182 780 in 200 μl of acetone or acetone vehicle alone from the sixth week of age to the fifteenth

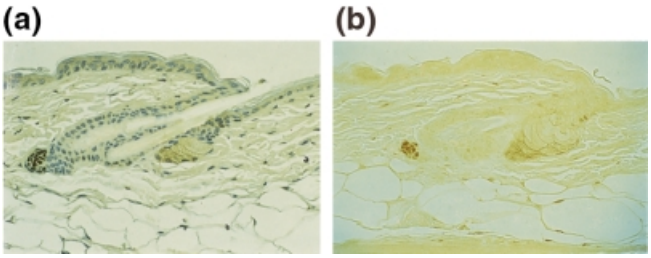

Figure 1. Immunohistochemical localization of estrogen receptor in mouse skin. Estrogen receptor immunohistochemical staining was conducted with or without hematoxylin counterstaining. (*a*) Telogen hair follicle with counterstaining; (*b*) telogen hair follicle without counterstaining.

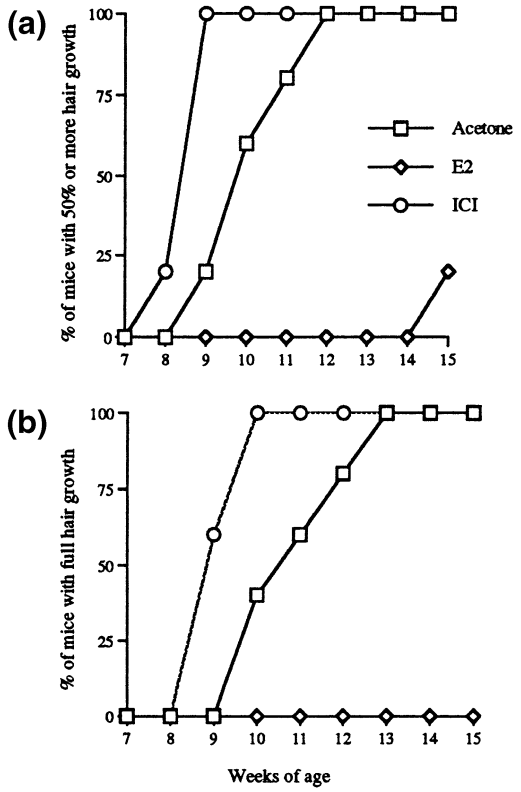

Figure 2. Effect of 17-β-estradiol and ICI 182 780 on hair regrowth in CD-1 female mice. Six-week-old female CD-1 mice (5–6 mice per group) were treated on the clipped dorsal surface twice weekly with topical applications of 10 nmol 17-β-estradiol (E2), 10 nmol ICI 182 780 (ICI), or acetone vehicle for the indicated number of weeks. (*a*) Percentage of mice with 50% or more hair growth; (*b*) percentage of mice with full hair growth.

week of age. As shown in **Fig 2(*a*)**, 20% of CD-1 female mice treated with the acetone vehicle beginning on week 6 displayed partial hair regrowth on the clipped dorsal surface at 9 wk of age, and by 12 wk of age all of the mice displayed hair regrowth on at least 50% of the previously clipped dorsal surface. As shown in **Fig 2(*b*)**, full hair regrowth in acetone-treated mice followed a similar time course as partial hair regrowth but was delayed by 1–2 wk. In comparison and as previously described (Oh and Smart, 1996), only 20% of mice treated with 17-β-estradiol demonstrated partial hair growth and none displayed full hair growth. Treatment with ICI 182 780 had the opposite effect of 17-β-estradiol, as it stimulated hair regrowth and all mice displayed a full coat of hair by 10 wk of age. We have previously reported that the histologic examination of 17-β-estradiol-treated mouse skin revealed that the hair follicles

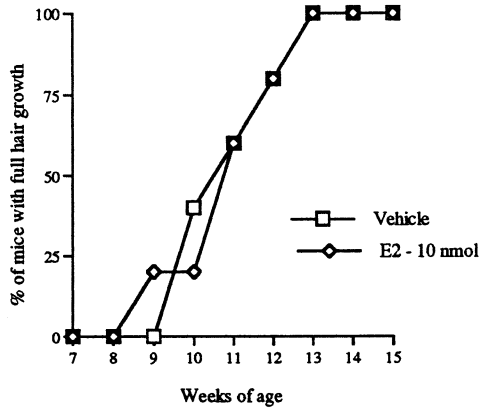

Figure 3. Intraperitoneal administration of 17-β-estradiol does not block hair growth. 17-β-estradiol (10 nmol) (E2) or corn oil vehicle alone was injected intraperitoneally into 6-wk-old female CD-1 mice (five mice per group) twice weekly for 9 wk.

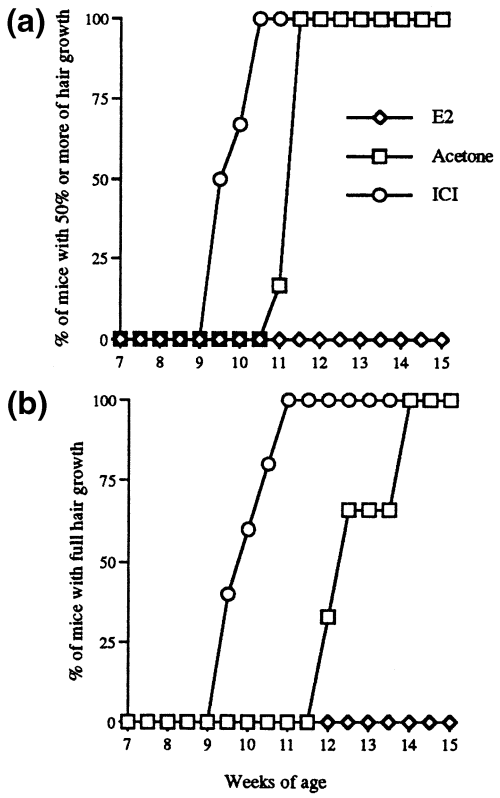

Figure 4. Effect of 17-β-estradiol and ICI 182 780 on hair regrowth in C57BL/6 female mice. Six-week-old female C57BL/6 mice (5–6 mice per group) were treated on the clipped dorsal surface twice weekly with topical applications of 10 nmol 17-β-estradiol (E2), 10 nmol ICI 182 780 (ICI), or acetone vehicle for indicated number of weeks. (*a*) Percentage of mice with 50% or more hair growth; (*b*) percentage of mice with full hair growth.

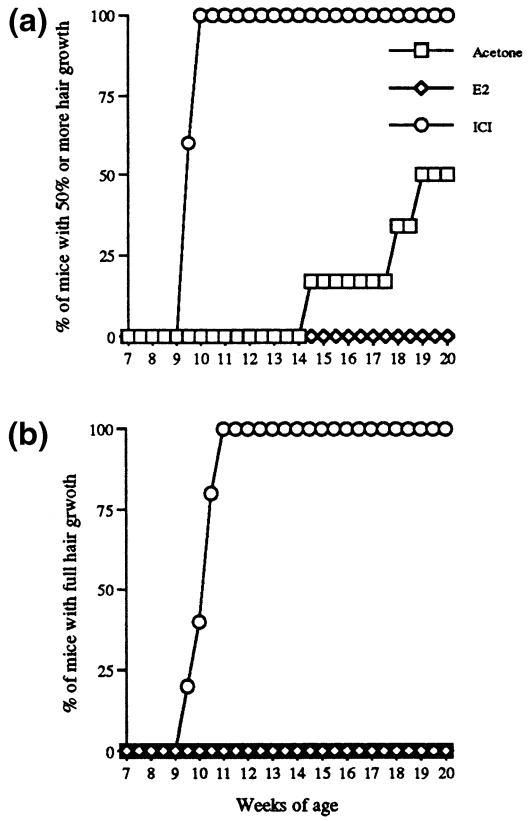

Figure 5. Effect of 17-β-estradiol and ICI 182 780 on hair regrowth in C3H female mice. Six-week-old female C3H mice (5–6 mice per group) were treated on the clipped dorsal surface twice weekly with topical applications of 10 nmol 17-β-estradiol (E2), 10 nmol ICI 182 780 (ICI), or acetone vehicle for indicated number of weeks. (*a*) Percentage of mice with 50% or more hair growth; (*b*) percentage of mice with full hair growth.

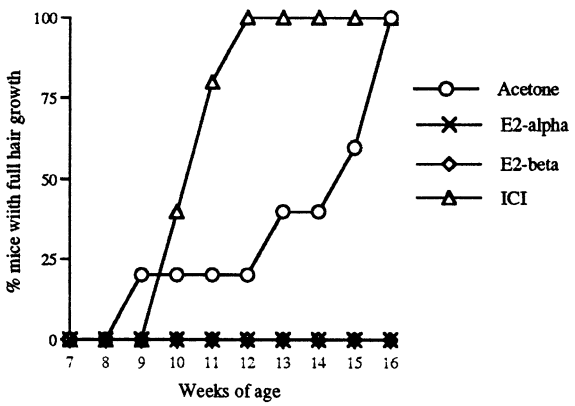

Figure 6. Effect of 17-β-estradiol, 17-α-estradiol, and ICI 182 780 on hair regrowth in CD-1 male mice. Six-week-old male CD-1 mice (5–6 mice per group) were treated on the clipped dorsal surface twice weekly with topical applications of 10 nmol 17-β-estradiol (E2-beta), 10 nmol 17-α-estradiol (E2-alpha), 10 nmol ICI 182 780 (ICI), or acetone vehicle for indicated number of weeks.

in the treated area remained in telogen, whereas histologic examination of ICI 182 780-treated skin revealed that the hair follicles in the treated area had entered early anagen within 1 wk of treatment (Oh and Smart, 1996). To begin to determine if the effects of topically applied 17-β-estradiol were due to a direct cutaneous effect or due to an indirect systemic effect, we evaluated the effect of intraperitoneal administration of 17-β-estradiol on hair growth. Intraperitoneal injection of 17-β-estradiol (10 nmol per 200 μl corn oil, twice weekly) at a dose and frequency equal to that used in the dermal application was without effect on hair growth, indicating that the inhibitory effect of dermally applied 17-β-estradiol on hair growth was due to its action within the skin and was not of an indirect systemic nature (**Fig 3**).

Because CD-1 mice are not frequently used in hair growth studies, we wanted to determine the effects of ICI 182 780 and estrogen on hair regrowth in C3H and C57BL/6 mice, two strains of mice that are frequently used in hair growth studies. In addition, C3H and C57BL/6 mice are pigmented and pigmentation changes

in skin provide an early indication of the telogen-anagen transition. As shown in **Fig 4**, female C57BL/6 responded to ICI 182 780 and 17-β-estradiol as observed in CD-1 female mice; however, the initiation of hair regrowth in the vehicle-treated control mice was slightly delayed when compared with CD-1 mice. 17-β-Estradiol treatment completely blocked hair regrowth, whereas treatment with ICI 182 780 produced full hair regrowth 3 wk earlier than control mice. Pigmentation changes in the skin of ICI-treated mice were observed within 1 wk of ICI 182 780 treatment, whereas pigmentation changes did not occur until 4.5 wk of acetone treatment in the control mice. Estrogen-treated mice did not display any pigmentation changes throughout the experimental period. Thus, ICI 182 780 and estrogen produce effects in C57BL/6 mice that are similar to CD-mice; however, the effects of ICI on full hair regrowth occur earlier in C57BL/6 mice relative to their acetone controls.

C3H mice, an agouti mouse strain, are also frequently used in hair growth studies. As shown in **Fig 5**(***a***), by 20 wk of age only 50% of CH3 female mice treated with the acetone vehicle displayed hair regrowth on at least 50% of the previously clipped dorsal area, and the remaining 50% of the acetone-treated mice displayed patchy hair growth that covered less than 50% of the clipped dorsal area (data not shown). In contrast, 100% of the ICI-treated mice displayed hair growth on 50% or greater of the clipped area at 10 wk of age, and by 11 wk of age all mice regrew a full coat of hair (**Fig 5**). Pigmentation changes in the skin were observed in 40% and 100% of the ICI 182 780 treated mice at 1 wk and 4 wk of treatment, respectively. In comparison, the earliest pigmentation change observed in the vehicle-treated control group occurred in one mouse at 6 wk of treatment (12 wk of age). None of the 17-β-estradiol treated group mice displayed pigmentation changes or hair regrowth.

Because both ICI 182 780 and 17-β-estradiol were efficacious in female CD-1, C3H, and C57BL/6 mice and as previous studies (Oh and Smart, 1996) had shown that 17-α-estradiol was without effect in CD-1 female mice, we wanted to assess the influence of 17-α-estradiol, 17-β-estradiol, and ICI 182 780 on hair growth in male mice. As shown in **Fig 6**, male CD-1 mice responded similarly to female CD-1 mice in that ICI 182 780 stimulated hair regrowth and 17-β-estradiol blocked hair growth; however, in contrast to female mice, 17-α-estradiol blocked hair growth in male mice. Immunohistochemical staining of 7-wk-old male skin with an antibody to estrogen receptor-α demonstrated the presence of estrogen receptor-α in the dermal papilla of the telogen follicle (data not shown), similar to that observed in female mice.

DISCUSSION

The results presented in this manuscript demonstrate that the effects of topical application of 17-β-estradiol and ICI 182 780 on hair growth are not restricted to the CD-1 mouse. It was previously suggested that the effects of 17-β-estradiol and ICI 182 780 may be unique to the CD-1 mouse and that C3H and C57BL/6 mice do not respond (Stenn *et al*, 1998). Indeed, C3H and C57BL/6 female mice responded to ICI 182 780 treatment by regrowing hair, whereas those mice treated with 17-β-estradiol responded by not growing hair. The observed early pigmentation changes in both ICI 182 780-treated C3H and C57BL/6 mice provide additional evidence that the effects of ICI 182 780 on the telogen-anagen transition are rapid. Hair regrowth in CD-1 and C57BL/6 vehicle-treated control mice was synchronous and followed a similar time course, whereas regrowth in C3H vehicle-treated mice was delayed and these mice never demonstrated full hair regrowth. This lack of full hair regrowth in C3H mice may represent an innate difference in the initiation of the third anagen or could represent a strain-specific effect of topically applied acetone. Regardless, it is clear that both C3H and C57BL/6 mice respond to topically applied 17-β-estradiol and ICI 182 780 in a manner similar to that reported for CD-1 female mice (Oh and Smart, 1996).

Although it is important to demonstrate that 17-β-estradiol blocks hair growth and ICI 182 780 stimulates hair regrowth in male mice, it is most remarkable that there is a striking sexual dimorphism with respect to the effect of 17-α-estradiol. In many biologic systems, 17-α-estradiol is an inactive stereoisomer of 17-β-estradiol, with little or no estrogenic activity, and yet in other systems it possesses potent estrogenic activity. In previous studies using female mouse skin (Oh and Smart, 1996), 17-α-estradiol had little to no inhibitory effect on hair regrowth, whereas in this studies using male mice, 17-α-estradiol blocked hair growth. The reason for this sex difference is unknown; however, based on preliminary data from our laboratory this difference is not due to the ability of male mice to isomerize 17-α-estradiol to 17-β-estradiol (unpublished data). Perhaps the gender specific response is due to the differential expression of a steroid hormone coactivator and/or corepressor, which can form complexes with the estrogen and/or androgen receptor and alter transcription of the hair growth signal within the dermal papilla.

Previously we reported that the intraperitoneal administration of ICI 182 780 at a dose and frequency equal to that employed in the topical studies had no effect on hair growth (Oh and Smart, 1996). Here we report that the intraperitoneal administration of 17-β-estradiol at a dose and frequency used in the topical studies had no effect on hair growth, suggesting a direct cutaneous effect of topically applied estrogen. Others have reported an inhibitory effect on hair growth when estrogens are injected subcutaneously on a daily basis (Hooker and Pfeiffer, 1943; Houssay, 1953) or when estrogen administration is continuous and prolonged through the use of subcutaneous implants (Johnson, 1958). Whether the inhibitory effects on hair growth induced by daily subcutaneous injection or chronic estrogen treatment through the use of implants are of a direct cutaneous nature remains to be determined.

Based on Scatchard analysis, estrogen receptors are expressed in human skin (Hasselquist *et al*, 1980; Punnonen *et al*, 1980). Recently, estrogen receptor immunohistochemical staining of human scalp biopsies collected from patients with alopecia areata and androgenic alopecia revealed estrogen receptor staining in the dermal papilla of some specimens (Wallace and Smoller, 1998). Recently, Lachgar *et al* (1998) provided immunohistofluorescence evidence for estrogen receptor expression in dermal papilla within human scalp specimens. It remains to be determined to what extent estrogen or ICI 182 780 can influence hair growth in humans. The elucidation of the downstream effectors of estrogen receptor action may also represent potential common convergence points between species.

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