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The Effect of Different Types of Hepatic Injury on the Estrogen and Androgen Receptor Activity of Liver

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Abstract *Mammalian liver contains receptors for both estrogens and androgens. Hepatic regeneration after partial hepatectomy in male rats is associated with a loss of certain male-specific hepatic characteristics. In this study we investigated the effects of lesser forms of hepatic injury on the levels of estrogen and androgen receptor activity in the liver. Adult male rats were subjected to portacaval shunt, partial portal vein ligation, hepatic artery ligation, or two-thirds partial hepatectomy. Another group of animals was treated with cyclosporine. At the time of sacrifice the livers were removed and used to determine the estrogen and androgen receptor activity in the hepatic cytosol. A significant reduction ($p < 0.05$) in the hepatic cytosolic androgen receptor activity and a slight increase in the estrogen receptor activity occurred following total portosystemic shunting. Partial ligation of the portal vein, which produces a lesser degree of portosystemic shunting, had no effect on the levels of the estrogen and androgen receptor activity present within hepatic cytosol. Cyclosporine-treated animals had significantly greater ($p < 0.01$) levels of estrogen receptor activity in the hepatic cytosol compared to vehicle-treated control animals. Levels of estrogen and androgen receptor activity within the hepatic cytosol remained unchanged after ligation of the hepatic artery. The reduction in the cytosolic estrogen and androgen receptor activity in the liver after partial hepatectomy was confirmed. In summary, certain types of hepatic injury are associated with profound changes in the estrogen and androgen receptor content within the liver.*

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Certain functions of the mammalian liver are known to display a sexual dimorphism.[1-3] Receptors for both estrogens and androgens have been identified in both rat and human liver, emphasizing that the liver is an important sex-hormone-responsive organ.[4-7] Furthermore, hepatic regeneration after partial hepatectomy in male rats is associated with a loss of certain male-specific hepatic characteristics and the acquisition of a more female-like pattern.[4,8] The changes seen include reduced testosterone and hepatic androgen receptor levels and increased plasma estradiol levels and hepatic estrogen receptor activity.[8] Moreover, a relocation of cytosolic estrogen receptors to the hepatic nucleus occurs and is thought to play a fundamental role in the initiation of the regenerative response.[9] The present experiments were designed to assess the effects of lesser forms of hepatic injury on the levels of estrogen and androgen receptor activity in the liver.

Materials and Methods

Animals and Chemicals

Adult male Wistar and Lewis rats weighing 200–300 g were purchased from Harlan Sprague Dawley (Indianapolis, IN). New England Nuclear (Boston, MA) provided the [^{3}H]estradiol (99 Ci/mmol), ^{3}H-1881, and unlabeled R1881. Diethylstilbestrol, sodium molybdated, bovine serum albumin, and Tris base were purchased from Sigma Chemical Company (St. Louis, MO). Absolute ethanol and ACS scintillation fluid were purchased from U.S. Industrial Chemicals Company (Tuscola, IL) and Amersham (Arlington Heights, IL), respectively. All other chemicals were obtained from Fisher Chemical Company (Pittsburgh, PA).

Surgical Procedures

The rats were assigned randomly to the following treatment groups:

Group 1. Six Wistar male rats were treated with cyclosporine 25 mg/kg orally each day for 7 days. On the eighth day the rats were anesthetized with ether and weighed, and the livers were removed for measurement of hormone receptor activity.

Group 2. Six Wistar male rats were subjected to partial portal vein ligation as described previously.[10] The portal vein was exposed via a midline abdominal incision. A 20-gauge needle was held along side the length of the portal vein and two 3-O silk ligatures were tied around the needle and the portal vein. The needle was then carefully slipped out of the ligatures, allowing the portal vein to expand to a diameter equal to a 20-gauge needle. At 8 weeks postoperatively the animals were anesthetized with ether and weighed, and the livers were removed and used for measurement of the hormone receptor levels.

Group 3. Six Lewis male rats were subjected to a standard end-to-side portacaval shunt using conventional microsurgical techniques. At 8 weeks postoperatively

the animals were anesthetized with ether and weighed, and the livers were removed and used for measurement of hormone receptor levels.

Group 4. Six Lewis male rats were subjected to hepatic artery ligation. On the tenth postoperative day the rats were anesthetized with ether and the livers removed for measurement of the hormone receptor levels.

Group 5. Wistar male rats were subjected to standard two-thirds partial hepatectomy.[11] Groups of 4 rats were anesthetized with ether at 6, 24, 48, and 72 h postoperatively and weighed, and the liver remnants were removed for subsequent measurement of hormone receptor levels.

Preparation of Cytosol

The livers were removed as described above, weighed, and homogenized in 4 vol of ice-cold buffer consisting of 0.25 M sucrose, 1.5 mM EDTA, 10 mM mercaptoethanol, and 10 mM Tris–HCl (pH 7.4) using a Brinkman polytron homogenizer. The homogenate was centrifuged at 103,000*g* for 1 h at 4°C and the supernatant used for all cytosolic assays.

Estrogen and Androgen Receptor Assays

The activity of the cytosolic estrogen receptors within the liver was determined by measuring the specific binding of a saturating concentration of [^{3}H]estradiol.[4] The hepatic cytosol was diluted 1:1 with a buffer consisting of 40 mM sodium molybdate, 1.5 mM EDTA, and 10 mM Tris–HCl (pH 7.4) to stabilize the estrogen receptor. Total [^{3}H]estradiol binding was measured by mixing 200 μL of the diluted cytosol with 25 μL 30 mM [^{3}H]estradiol and 25 μL ethanol. To determine the nonspecific binding, parallel assays were performed in which the ethanol was replaced with 25 μL 3 μM unlabeled diethylstilbestrol dissolved in ethanol. The mixture was allowed to incubate for 2 h at 4°C. Unbound steroid was removed from the mixture by adding 0.4 mL 1% dextran-coated charcoal and used to terminate the reaction. The mixture was centrifuged for 5 min at 1500*g* at 4°C, and the supernatant carefully transferred to a scintillation vial containing 8 mL ACS scintillation fluid. The radioactivity was measured in a Packard Tri-Carb 460 CD Liquid Scintillation System (Downers Grove, IL).

The assay for cytosolic androgen receptor activity was similar to that described above for the estrogen receptor with only minor variation.[8] Tritiated R1881, a synthetic androgen, was used as the labeled steroid to determine the total binding and unlabeled R1881 was used to determine nonspecific binding. Binding of the R1881 to glucocortrioid receptors was blocked by adding 5 μM triamcinolone acetonide to each tube utilized for measurement of the androgen receptor. After an overnight incubation at 4°C, the reaction was terminated by adding 1% dextran-coated charcoal. The mixture was centrifuged at 1500*g* for 5 min at 4°C and the supernatant carefully transferred to a scintillation vial containing 8 mL ACS scintillation fluid. The radioactivity was measured in a Packard Tri-Carb 460 CD Liquid Scintillation System (Downers Grove, IL).

Statistical Methods

Cytosolic protein concentration was determined using the method of Lowry with bovine serum albumin being used as the standard.[12]

All data are presented as mean values ± SEM. Statistical analysis of the data was performed using a Student *t* test. A *p* value of 0.05 or less was considered to represent a significant difference.

Results

The effects of cyclosporine and olive oil vehicle treatment on the hepatic cytosolic estrogen and androgen receptor activity are shown in Figure 1. The level of estrogen receptor activity in the hepatic cytosol of rats treated with the olive oil vehicle was similar to that of control animals. The cyclosporine-treated rats had significantly greater levels of estrogen receptor activity than did both the olive oil-treated and the control animals ($p < 0.01$). The cyclosporine-treated rats and the olive oil-treated rats had similar levels of androgen receptor activity, but both groups had significantly lower levels of androgen receptor activity than did the control animals ($p < 0.05$).

The changes in the hepatic sex hormone receptor activity after partial portal vein ligation and sham operation are shown in Figure 2. The levels of estrogen and androgen receptor activity in the hepatic cytosol at 8 weeks after partial portal vein ligation and after sham operation were similar.

At 8 weeks after surgery, rats subjected to portacaval shunt (Fig. 3) had greater levels of hepatic estrogen receptor activity as compared to sham operated animals, but the difference was not statistically significant. However, there were significantly greater levels of cytosolic estrogen receptor activity in the liver after portacaval shunt compared to the pretreatment levels ($p < 0.05$). In contrast, significantly lower levels of androgen receptor activity were present after portosystemic shunting compared to that present in sham operated animals ($p < 0.05$).

The effects of hepatic artery ligation on the hepatic sex hormone receptor

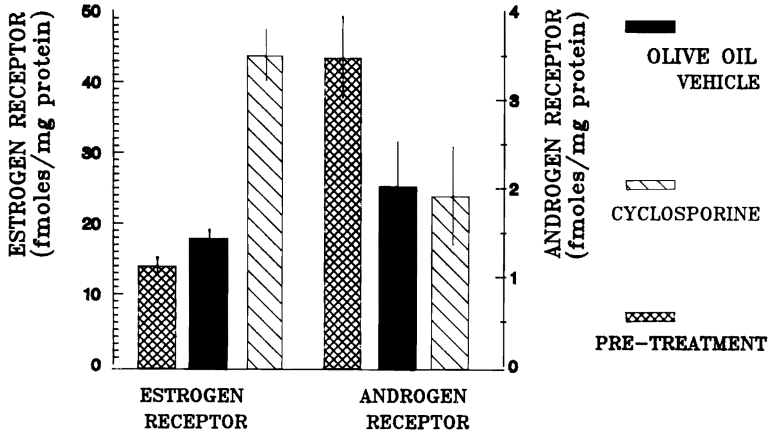

Figure 1. Hepatic estrogen and androgen receptor activity (fmol/mg protein) in male cyclosporine- and olive oil vehicle-treated control rats ($n = 6$ animals). $^{**}p < 0.01$.

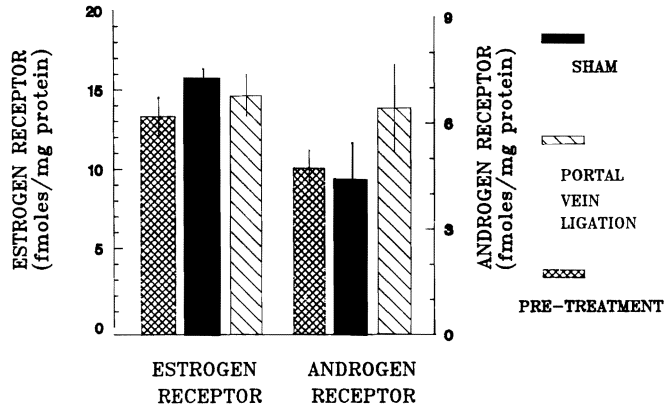

Figure 2. Estrogen and androgen receptor activity (fmol/mg protein) in the liver of male rats after either partial portal vein ligation or sham operation ($n = 6$ animals).

activity are shown in Figure 4. The levels of the estrogen and androgen receptor activity in the hepatic cytosol at 10 days after ligation of the hepatic artery were similar to those of controls after sham operation and in unoperated controls.

Both estrogen and androgen receptor activity in hepatic cytosol were lower at 6 h after partial hepatectomy compared to baseline preoperative levels (Fig. 5) ($p < 0.05$). Thereafter the activity levels of both receptors increased. The androgen receptor activity was back to preoperative levels at 48 h following operation. The estrogen receptor activity was still reduced significantly compared to baseline control values at 48 h after partial hepatectomy ($p < 0.05$).

Discussion

That the liver is a sex hormone-responsive organ has been established only recently.[4–7] However, the precise function of the estrogen and androgen receptors within the liver has not been determined. An increase in estrogen receptor activity and a decrease in androgen receptor activity occurs in the liver after partial

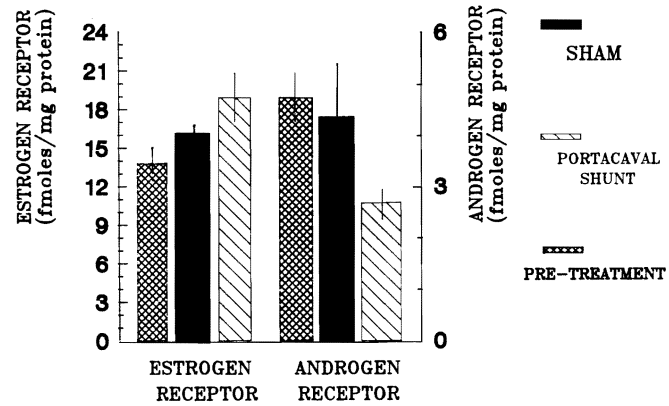

Figure 3. Estrogen and androgen receptor activity (fmol/mg protein) in the liver after either surgical portacaval shunt or sham operation ($n = 6$ animals). $^*p < 0.05$.

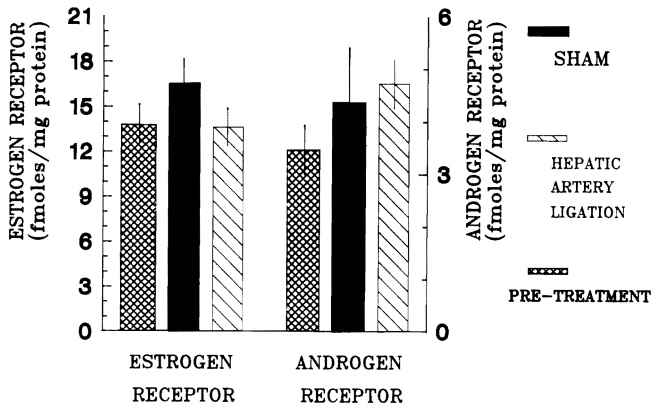

Figure 4. Estrogen and androgen receptor activity (fmol/mg protein) in the liver after either ligation of the hepatic artery or sham operation ($n = 6$ animals).

hepatectomy and is associated with the initiation of hepatic regeneration.[8] Specifically, the transfer of the estrogen receptor from the hepatic cytosol to the nucleus coincides with the observed increase in deoxyribonucleic acid synthesis in regenerating liver and is thought to play a critical role in the control of the regenerative response.[9]

In the present study, the effects of lesser forms of hepatic injury, including a total absence of portal blood supply, a decreased portal blood supply, ischemia due to hepatic artery ligation, and drug toxicity, on the activity of the estrogen and androgen receptors in hepatic cytosol were determined. The results were compared with those obtained in response to a two-thirds partial hepatectomy.

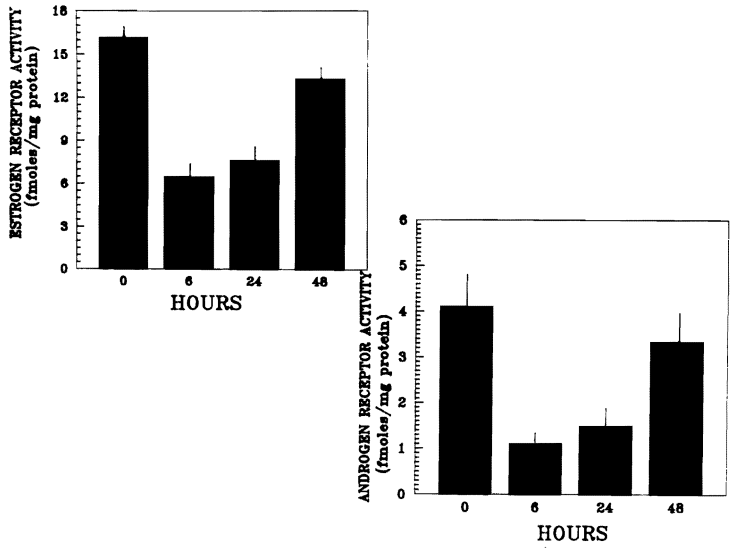

Figure 5. Estrogen and androgen receptor activity (fmol/mg protein) in male rat hepatic cytosol after partial hepatectomy ($n = 4$–6 animals per time per group). $^{*}p < 0.05$; $^{**}p < 0.01$.

The most dramatic changes in the levels of sex hormone receptor activity in the hepatic cytosol occurred in rats subjected to a two-thirds partial hepatectomy. The decrease in estrogen and androgen receptor activity observed in the hepatic cytosol during regeneration has been described previously.[8,9] This reduction in cytosolic estrogen receptor is associated with an increase in total hepatocyte estrogen receptor with a marked increase in binding of the receptor.

Less dramatic changes in the cytosolic content of sex hormone receptor levels were detected after portacaval shunting. A significant reduction in the hepatic cytosolic androgen receptor activity and slight increase in the estrogen receptor activity occurred following this procedure. Total diversion of the portal blood with a surgical shunt is associated with liver atrophy.[13] Thus, it was not surprising that the changes in hepatic receptor activity described in regenerating liver were not seen following this procedure. Whether similar receptor changes are seen at times earlier as well as later than 8 weeks following portal vein diversion is not known as yet.

Partial ligation of the portal vein which produces a lesser degree of portal vein shunting had no effect on the levels of the estrogen and androgen receptor activity present within hepatic cytosol. Interestingly, the feminization, which includes an increase in serum estradiol levels and a reduction in serum testosterone levels, that is associated with chronic liver disease is thought to be due to least in part to portosystemic shunting.[14] The origin of the increased estrogen levels in such cases is an increased plasma production occurring as a result of peripheral aromatisation of androstenedione to estrone and to a lesser degree estradiol.[15] The present findings demonstrate that the feminization that occurs after portosystemic shunting is also evident within the liver and is evidenced by an increased estrogen receptor activity occurring in conjunction with a reduction in the androgen receptor activity.

Levels of estrogen and androgen receptor activity within hepatic cytosol remained unchanged after ligation of the hepatic artery. This implies that the arterial blood supply of the liver is not essential to maintain the sex hormone receptor activity of the liver.

Cyclosporine, an immunosuppressive agent used widely after solid organ transplantation, is known to be mildly hepatotoxic. Other untoward effects attributed to cyclosporine use include hirsutism, increased uterine bleeding, breast fibroadenomas, and diabetes mellitus. Each of these appears to be mediated at least in part hormonally. Thus, it was not surprising that cyclosporine altered the hormone receptor activity present within the liver. Previous studies have shown that prolactin plays an important role in the regulation of the cytosolic content of estrogen receptors in the liver and prolactin levels increase after cyclosporine administration.[16,17] Specifically, the reduction in hepatic cytosolic estrogen receptor content seen after hypophysectomy can be restored at least partially by the administration of exogenous prolactin.[16] Thus, the increase in serum prolactin levels that occurs with cyclosporine administration may be responsible, at least in part, for the enhanced activity of the estrogen receptor observed in this study following cyclosporine administration. Interestingly, the cyclosporine-treated rats and the olive oil vehicle-treated rats had similar levels of androgen receptor activity present in their hepatic cytosol. Both groups, however, had less androgen receptor than was present prior to treatment. In this regard it is important to

recall that vegetable oils, including olive oil, have been shown to contain estrogen-like materials and to produce estrogen-like responses in gonadectomized animals.[18] Thus, the minor estrogenizing effect of the olive oil vehicle may account for the androgen receptor activity reduction seen in these two groups of animals as compared to their pretreatment status.

In summary, the hepatic injury associated with partial ligation of the portal vein and ligation of the hepatic artery does not significantly affect the level of the cytosolic estrogen and androgen receptor activity present in the liver. In contrast, significant changes in both the estrogen and androgen receptor activity present in hepatic cytosol occur after partial hepatectomy and are compatible with a partial feminization of the liver and a regenerative response. Feminization of the liver, including a decrease in the level of the androgen receptor activity and an increase in the level of estrogen receptor activity, was also noted after portacaval shunt. Finally, cyclosporine, which was suspected of having some hormonal effect and is known to be hepatotoxic, caused a significant increase in the level of the estrogen receptor activity but did not alter the level of the androgen receptor activity in the liver.

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