Translocation of hepatic cytosol androgen receptor to the nucleus in vivo in male rats

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Summary. Testosterone, which was injected s.c. into adult male rats castrated 15 h prior to the injection, decreased the number of androgen-specific binding sites in the cytosol at 30 min. Coincidentally, a substantial increase was observed in the nucleus. The decreased number of the sites in the cytosol was restored to the initial level at 60 min; on the contrary, a decrease was observed in the nucleus.

In general, a steroid hormone binds to receptor protein in the cytosol of the target cells. This interaction between the steroid and its receptor is believed to result in the conformational alteration of the receptor, and then the steroid-receptor complex translocates into the nucleus. Thus, the steroid-dependent gene-action is expressed through the binding with chromatin and the synthesis of m-RNA in the nucleus. Most investigations as to the mode of binding of the sex steroid-receptor complex with the nucleus have dealt with sex organs such as the uterus, the prostate and the epididymis[1-4], while the androgen binding component has been considered to be absent from the liver[5]. Recently, we have demonstrated the presence of a macromolecular component, which binds specifically with testosterone and androstenedione in male rat liver, which is considered to be a non-target organ[6,7]. Chromatography on a Sephadex column, Scatchard analysis, competition study, the stability to various enzymes and heat, and sucrose density gradient centrifugation analysis have revealed that this component is a protein which has a sedimentation coefficient of approximately 10 S and a moderate affinity ($Kd = 1.13 \times 10^{-6}$ M) for testosterone. If this component in the cytosol is an androgen receptor, it can be translocated into the nucleus. In the present study, distribution of the androgen-binding component in the hepatic cytosol and nucleus of castrated rats following injection of testosterone was investigated.

Materials and methods. Male adult Wistar rats weighing 200–250 g were castrated by the abdominal route 15 h prior to sacrifice. The rats were injected s.c. with testosterone propionate and were decapitated 30 min or 60 min after the injection. The liver was perfused with cold saline solution and the cytosol fraction was prepared as described previously[8] and was salted out with neutralized saturated ammonium sulfate (the final concentration = 30%) and stirred for 40 min, followed by standing for 30 min. The preparation was centrifuged at 25,000 × g for 10 min and, then the precipitate was suspended in 0.01 M Tris-HCl buffer (pH 7.4), containing 0.0015 M EDTA and 0.001 M NaN_3 (TEN buffer). The solution was used as 'partially purified cytosol'. For preparation of the nuclear fraction, the liver was homogenized in 6 vol. of 0.05 M Tris-HCl buffer (pH 7.55), containing 2.2 M sucrose, 0.005 M $MgCl_2$, 0.015 M KCl and 0.2% Triton X-100 (nuclear purification buffer) with a polytron PT 10-35. The resulting homogenate was filtered through 2 layers of cheese cloth. The filtrate obtained was layered on the nuclear purification buffer and centrifuged at 75,000 × g for 2 h in a SW rotor (Hitachi RPS 25-2). The precipitate was washed in 0.05 M Tris-HCl buffer (pH 7.55), containing 0.25 M sucrose, 0.01 M $MgCl_2$ and 0.025 M KCl (0.25 M sucrose buffer). The suspension was centrifuged at 800 × g for 10 min and the washed precipitate was resuspended in 0.25 M sucrose buffer. Such suspensions served as 'nuclear fractions'. Aliquots of 'cytosol fractions' were assayed for protein by the method of Lowry et al.[9]. DNA was fractionated from the aliquots of nuclear suspension by the method of Schmidt-Thannhauser-Schneider[10] and measured by the diphenylamine method[11].

Exchange assay of cytosol and nuclear fractions was performed as follows. 1.0 ml (2 mg protein) of the partially purified liver cytosol was incubated with 20 µl (12.5 pmoles) of [1,2-^{3}H] testosterone (sp. act. 40 Ci/mmole) in the presence of 20 µl (12.5 nmoles) of nonlabeled testosterone or TEN buffer for 2 h in ice, and then for 1 h at 30 °C. 750 µl (equivalent to 0.75 g liver) of nuclear fraction was incubated with 20 µl (25 pmoles) of [1,2-^{3}H] testosterone in the presence of 20 µl (25 nmoles) of nonlabeled testosterone or 0.25 M sucrose buffer for 1 h at 30 °C. For the measurement of testosterone-bound component in

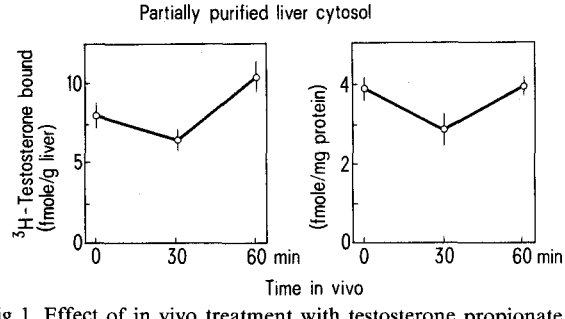

Fig. 1. Effect of in vivo treatment with testosterone propionate on the [^{3}H]-testosterone binding ability of hepatic cytosol in vitro in castrated male rats. Testosterone propionate (1.0 mg as testosterone) or vehicle alone was injected s.c. into adult male rats castrated 15 h prior to the injection. The animals were killed 30 or 60 min later and the androgen-specific binding sites of the partially purified cytosol were determined by exchange assay. The results are given as the mean ± SD of 4–5 animals. In the left panel, the value at 30 min significantly differs from that at 0 min ($p < 0.05$, by Student's t-test) and from that at 60 min ($p < 0.01$). In the right panel, the value at 30 min significantly differs from those at 0 and 60 min. (both, $p < 0.01$).

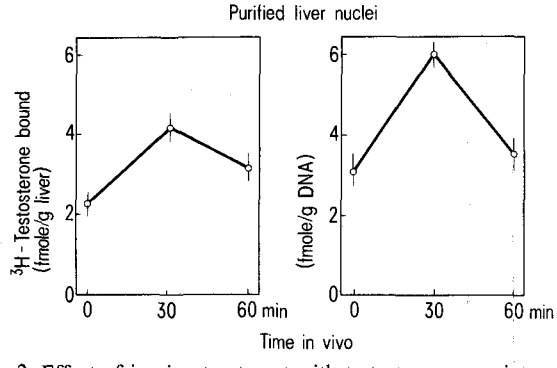

Fig. 2. Effect of in vivo treatment with testosterone propionate on the [^{3}H]-testosterone binding ability of hepatic nuclei in vitro in the castrated male rats. See the legend of figure 1 for the detailed treatment of rats. The androgen-specific binding sites of the purified nuclei were determined by exchange assay. The value at 30 min differs from those at 0 and 60 min ($p < 0.01$).

the cytosol fraction, the incubates were treated with Dextran-coated charcoal suspension according to Beato and Feigelson[12]. The mixture was centrifuged for 10 min at 3,000 rpm at 4 °C. To the supernatant obtained, the phase combining system (PCS) scintillator (Amersham) diluted with toluene (PCS: toluene, 2:1 v/v) was added. For the nuclear fraction, the incubate was centrifuged at 800 × g for 10 min. The pellet obtained was washed with 0.25 M sucrose buffer followed by centrifugation at 800 × g for 10 min. This procedure was repeated twice. The washed pellet was solubilized with 0.4 ml 5 M urea-2 M NaCl with vigorous vortexing, and then the mixture was kept overnight at 0-2 °C. To the extract was added PCS scintillator diluted with toluene. The radioactivity was analyzed as described[8].

Results and discussion. The testosterone treatment decreased the initial high level of cytosol [^{3}H] testosterone-binding sites (figure 1) and contrarily, it increased substantially the initial low level of nuclear [^{3}H] testosterone-binding sites (figure 2). As the binding sites of the component which appeared in the nuclei were occupied by the non-radioactive steroid, the exchange assay was employed for the measurement of occupied receptors. The exchange assay has been used for the measurement of several steroid hormone receptors and provides a method for the evaluation of cytosol and nuclear receptors[13].

The distribution of binding sites between the cytosol and the nucleus suggested that the binding sites which appeared in the nuclei following the injection of testosterone are related to the disappearance of binding sites from the cytosol. Such a pattern of distribution and the appearance of the binding sites occupied by injected testosterone are consistent with the possibility that the androgen-specific binding sites of rat liver are androgen receptors which can be translocated to the nucleus. The decrease in cytosol receptor is transient and the cytosol receptor replenishes within 1 h. This rapid recovery may partially be due to a process that involves receptor recycling from the nucleus. Although the fate of the receptor translocated into the nucleus is not well known, it may possibly be inactivated by nuclear protease[14] and it may re-enter the cytosol after it dissociates from testosterone. It was of interest to note that the finding obtained here is similar to the distribution of estrogen-binding sites in the cytosol and the nucleus of the liver 30 and 60 min after ethinyl estradiol treatment in female rats[15].

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Plasma corticosterone fluctuations during the oestrous cycle of the house mouse

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Summary. The circadian rhythm of corticosterone concentration in the mouse persists throughout the oestrous cycle, but concentrations are significantly elevated at proestrus and oestrus.

It has been shown that activity of the pituitary-adrenal axis varies with the oestrous cycle in the rat. Buckingham[1] and Raps[2] have shown that plasma corticosterone levels are higher both in the morning and evening of the day of pro-oestrus than at other times in the cycle, although there is no interruption in the normal circadian pattern of variation. However, Champlin[3] found no difference in plasma corticosterone at different stages of the cycle in mice. The purpose of this study was to re-examine the mouse for plasma corticosterone variations during the oestrous cycle.

Since Schwartz[4] has shown that 5-day cycling rats differ in their hormonal balance from 4-day cyclers only mice on 4-day cycles were used. These were induced by the presence of males, as 4-day cycles are otherwise uncommon in mice (Bingel and Schwartz[5]).

Materials and methods. 200 virgin TO mice (A. Tuck and Sons) were randomly assigned to 6 groups for blood sampling at 4-h intervals. They were housed individually, each with a male in an inner wire cage, given food and water ad libitum and maintained at 22 ± 1 °C on a 14:10 h light:dark cycle. The animals were left undisturbed for 2 weeks, then each was blood-sampled once, by retro-orbital puncture[6] after ether anaesthesia. Sampling, in a room adjacent to the animal room, was complete within 3 min of entry to the animal room. Only 3 entries per session were made; sessions were spread over 3 days. Heparinized blood was centrifuged within 45 min of collection, and the plasma frozen and stored at − 20 °C until assayed.

Corticosterone was determined by a radioimmunoassay similar to that used by Gross et al.[7] but using an initial