Effect of Androgen on Ornithine Decarboxylase Activity in Androgen-Dependent Mouse Mammary Tumor (Shionogi Carcinoma 115) and Its Androgen-Independent Subline (CS 2)

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Abstract

The activity of ornithine decarboxylase in androgen-dependent mouse mammary tumor (Shionogi Carcinoma 115) was reduced to 25% by castration of tumor-bearing mice and restored to the normal level 12 h after administration of testosterone or 5α-dihydrotestosterone. Administration of estradiol-17β to the tumor-bearing castrated mice also stimulated the enzyme activity while progesterone and cortisol had little effect. On the other hand, the enzyme activity was affected by neither castration nor androgen injection to CS 2, which is a subline of SC 115 and completely independent of androgen for growth. The inhibition of ornithine decarboxylase activity in SC 115 by injecting α-difluoromethylornithine did not affect the enhancement of RNA polymerase I activity by androgen, showing independent elevation of the levels of the two enzymes by androgen.

Androgen-dependent mouse mammary tumor (Shionogi Carcinoma 115: SC 115) was established by Minesita and Yamaguchi (1964, 1965). Recently, during serial transplantation of the tumor, two sublines were obtained termed Chiba subline 1 (CS 1) and Chiba subline 2 (CS 2), which showed a partial and a complete loss, respectively, of androgen dependency for the growth of the tumor (Wakisaka et al., 1980; Fuse et al., 1983; Suzuki et al., 1983a). From the experiments with these tumors, we found that the degree of dependency was correlated with the increase in RNA polymerase I activity (Suzuki et al., 1983a, b).

Ornithine decarboxylase (EC 4. 1. 1. 17) is known as an enzyme which plays a role in the biosynthesis of polyamines that are especially rich in proliferating cells and regulated by circulating androgen in androgen-dependent tissues (for reviews; Jänne et al., 1978; Tabor and Tabor, 1984). In mouse kidney, for example, the activity of ornithine decarboxylase was increased a few hundred-fold by androgen through a rapid accumulation of mRNA (Isomaa et al., 1983; Berger et al., 1984). Several workers also suggested that ornithine decarboxylase may play a role in the regulation of RNA polymerase I activity by enhancing the syn-
thesis of polyamines (Danzin et al., 1979; Moruzzi et al., 1975) or by acting as the initiating factor in RNA polymerase I (Russell and Manen, 1982) in mammalian tissues. It was also reported in the case of Physarum polycephalum that ornithine decarboxylase was, after phosphorylation, capable of stimulating the transcription of ribosomal genes by RNA polymerase I (Kuehn et al., 1979; Atmar and Kuehn, 1981).

In view of these facts, it seemed worthwhile to examine how the activity of ornithine decarboxylase is affected by circulating androgen in the androgen-dependent and -independent tumors and whether there is any relationship between the enzyme and RNA polymerase I in these tissues.

Materials and Methods

**Chemicals**

DL-[1-14C] ornithine (57.3 mCi/mmol) and [3H] UTP were obtained from New England Nuclear, Boston, MA, U.S.A. DFMO was supplied by Merrell Dow Research Institute, Cincinnati, OH, U.S.A.

**Animals and Tumors**

Adult male mice of DD/S strain weighing approximately 30 g at transplantation of tumors were used throughout the experiments. Castration was performed on the 16th day after tumor transplantation. All mice were sacrificed on the 20th day after tumor implantation. Testosterone and other steroids were administered subcutaneously each in 0.4 ml of 30% ethanol-saline at a specified time before sacrifice. DFMO dissolved in saline was administered subcutaneously (12 mg in 0.1 ml in each injection). SC 115 and CS 2 used for the present study were 318th-344th and 56th-66th generations, respectively.

**Tissue preparation and assay of ornithine decarboxylase activity**

The tissue preparation was carried out at 0-4°C. After necrotic tissues were removed, the tumor tissues were homogenized with 0.25 M sucrose buffer containing 25 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, and 1 mM dithiothreitol. Preparation of cytosol and assay for ornithine decarboxylase activity were performed by the method of Noguchi et al. (1979). One unit of enzyme activity was defined as the amount of forming 1 pmol of CO₂/h under the above conditions.

**Assay for RNA polymerase I**

Preparation of nuclei and assay for RNA polymerase I were performed as described previously (Suzuki et al., 1983a, b).

**Protein determination**

The protein concentration was determined by the Coomassie-blue binding method of Bradford (1976) using bovine serum albumin as standard.

**Results**

To observe the effect of androgen on the ornithine decarboxylase activity of SC 115 and CS 2, tumor-bearing mice were castrated and testosterone (0.2 mg/mouse) was injected into the animals (Fig. 1). In the case of SC 115, the enzyme activity declined to a value of 25% of that of intact animals 3 days after castration. A single dose of testosterone restored the activity to almost the normal level at 12 h after hormone treatment, followed by a decrease toward the castrated level. On the other hand, the activity of ornithine decarboxylase of CS 2 tissue, which was about 50% of that of SC 115, was not influenced by castration or testosterone treatment.

Table 1 shows changes in the ornithine decarboxylase activity of SC 115 12 h after injection of various steroid hormones to castrated tumor-bearing animals. The maximum increase (3.5-3.6 fold) was accomplished by the administration of testosterone (0.2 mg) and DHT (0.02 or 0.2 mg). The increase after injection with estradiol-17β was statistically significant, but progesterone and cortisol did not seem to affect the enzyme activity.

The possibility that the increase in
ornithine decarboxylase activity after testosterone treatment may be involved in the regulation of RNA polymerase I activity was tested by the inhibition of ornithine decarboxylase activity using a specific inhibitor, DFMO. DFMO was injected every 3 h after testosterone administration to castrated mice bearing SC 115. Twelve and 18 h after testosterone administration, the activities of ornithine decarboxylase in cytosol and RNA polymerase I in nuclei were determined. As shown in Fig. 2, DFMO almost completely inhibited the ornithine decarboxylase activity, but did not affect the testosterone-induced enhancement of RNA polymerase I activity.

### Discussion

The results shown in Fig. 1 indicate that the regulation of ornithine decarboxylase

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Table 1. Hormonal specificity on stimulation of ornithine decarboxylase activity of SC 115 12 h after a single dose of steroids into tumor-bearing castrated mice

<table>
<thead>
<tr>
<th>Steroid</th>
<th>mg/mouse</th>
<th>Activity of Ornithine Decarboxylase (units/mg protein)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castrated for 3 days</td>
<td>0.0</td>
<td>211±53</td>
<td>100</td>
</tr>
<tr>
<td>+ Testosterone</td>
<td>0.2</td>
<td>765±85*</td>
<td>363</td>
</tr>
<tr>
<td>+ DHT</td>
<td>0.02</td>
<td>730±62*</td>
<td>346</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>737±85*</td>
<td>349</td>
</tr>
<tr>
<td>+ Estradiol-17β</td>
<td>0.002</td>
<td>617±47*</td>
<td>292</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>496±134**</td>
<td>235</td>
</tr>
<tr>
<td>+ Progesterone</td>
<td>0.02</td>
<td>419±133</td>
<td>199</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>237±67</td>
<td>112</td>
</tr>
<tr>
<td>+ Cortisol</td>
<td>0.02</td>
<td>433±101</td>
<td>205</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>308±93</td>
<td>146</td>
</tr>
</tbody>
</table>

DHT: 5α-dihydrotestosterone

Three to five experiments were performed and the values are expressed as the mean±S. E. *, p<0.01; **, p<0.05 compared to castrated mice without hormone.
by androgen occurs in androgen-dependent SC 115, but not in androgen-independent CS 2. A similar situation in the case of RNA polymerase I has already been reported (Suzuki et al., 1983a, b). Since both enzymes play an important role in the rapid proliferation of cells (Jänne et al., 1978; Tabor and Tabor, 1984), it is conceivable that such regulation of the activities of the two enzymes participates in the androgen-dependency of SC 115 on growth. Fig. 1 also indicates that CS 2 maintained a low but constant level of activity of ornithine decarboxylase. The activity of ornithine decarboxylase in CS 2 seems to represent the basal level, to which the androgen-dependent activity of the enzyme is added in SC 115.

In view of the finding obtained in kidney (Kontula et al., 1984), it is reasonable to consider that the transcription of the ornithine decarboxylase gene in SC 115 is controlled by the androgen receptor (Fuse et al., 1983). However, the precise mechanism, together with the reason why the androgen receptor in CS 2 (Fuse et al., 1983) does not work, remain to be clarified.

It is noteworthy that a slight but significant enhancement of ornithine decarboxylase activity in SC 115 was observed following the administration of estradiol-17β, as in the case of RNA polymerase I (Suzuki et al., 1983a, b). These findings are in agreement with recent information (Noguchi et al., 1984) that the present strain of SC 115 has the potency to respond to estrogen, changing from the original strain (1964) whose growth was inhibited by estrogen.

Remarkable is the finding that testosterone-induced activity of RNA polymerase I in SC 115 was not inhibited by blocking ornithine decarboxylase activity with DFMO (Fig. 2). In comparison with the time course of RNA polymerase I after injection of androgen reported previously (Fig. 3 in the paper of Suzuki et al., 1983a), the enhancement of ornithine decarboxylase activity does not seem to precede the
stimulation of RNA polymerase I activity after the hormone injection. These results, together with the previous findings in liver tissue that there was no antigenic cross-reactivity between the two enzymes (Seely et al., 1984) and that ornithine decarboxylase activity was not inactivated by protein kinase II (Seely et al., 1984) or transglutaminase (submitted for publication), do not favor the view (Kuehn et al., 1979; Atmar and Kuehn, 1981) that the enhancement of ornithine decarboxylase activity or modification of the enzyme protein is a prerequisite to RNA polymerase I stimulation. It is likely that both increases are separate responses to the primary stimulus by androgen.

Acknowledgements

The authors wish to thank Drs. S. Hayashi and T. Kameji of Jikei University School of Medicine for their valuable advice and Drs. P. P. McCann and W. J. Hudak of Merrell Dow Research Institute for the donation of DFMO. This work was supported in part by Grants-in-Aid for Cancer Research from the Ministry of Health and Welfare and from the Ministry of Education, Science and Culture, and for Scientific Research from the Ministry of Education, Science and Culture, Japan.

References


proliferation in rat liver. *J. Biochem.* (Tokyo) 85, 953–959.