Expressions of Sulfated Glycoprotein 2 and pSvr-1 Genes and Involution of Steroid Hormone-Dependent Rat Tissues

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Abstract. To further survey the molecular mechanisms underlying the involution of steroid hormone-dependent rat tissues, we undertook experiments to test whether or not any significant correlation between the tissue involution and expressions of rat sulfated glycoprotein 2 (SGP-2) and pSvr-1 genes, which had been initially cloned from the Sertoli cells and the seminal vesicles, respectively, and then identified as androgen repressed messages both in the ventral prostate and in the seminal vesicles, could be observed in steroid hormone-dependent rat tissues. Expressions of these genes were stimulated within 48 h after castration of animals both in the ventral prostate and in the seminal vesicles as reported previously, but not significantly altered by ovariectomy in the uterus. Expressions of these genes in the thymus were significantly repressed by the administration of dexamethasone and/or cycloheximide. Although the roles of expressions of SGP-2 and pSvr-1 genes in steroid hormone-dependent tissues remain unclear, their presence might become useful molecular markers of tissue involution not only in androgen-dependent rat tissues but also in glucocorticoid-dependent ones, and also provide excellent model systems for the study of negative regulation mechanism of gene expression by steroid hormones.

IT HAS been suggested that the involution of steroid hormone-dependent tissues is an actively initiated process involving a genetically programmed series of events [1–4]. Although the mechanisms underlying the process are not yet fully understood, changes in a variety of metabolic processes including both stimulation and inhibition of synthesis of RNAs and proteins, genomic DNA fragmentation, growth inhibition and cell death, seem to accompany them.

Recently, Buttyan et al. [5] suggested that the expression of TRPM-2 gene, which had been initially cloned from the ventral prostate of castrated rats as a testosterone repressed message-2 [6] and shown to have highly homologous sequences to rat sulfated glycoprotein 2 (SGP-2) gene [7], might serve as a sensitive molecular marker of the onset of programmed cell death in many types of mammalian cells. More recently, Bettuzzi et al. [8] identified an androgen-repressed message in the ventral prostate of castrated rats as SGP-2 mRNA and another androgen-repressed cDNA clone pSvr-1 was isolated from the seminal vesicles of castrated rats [9].

From these, in the present study, we undertook experiments to test whether or not expression of the SGP-2 and the pSvr-1 genes might become sensitive molecular markers for the involution of steroid hormone-dependent rat tissues. We show that the expression of these genes is stimulated by castration of the animals both in the seminal vesicles and in the ventral prostate as reported [8, 9] but not significantly altered by ovariectomy in the uterus. Furthermore, administration of dexamethasone to adrenalectomized rats resulted in a significant decrease in expressions of these genes in the thymus.
Materials and Methods

Animals

Wistar rats were used throughout the experiments. Castration (330–340 g b.w.), adrenalectomy (180–200 g b.w.) or ovariectomy (170–190 g b.w.) was performed as described previously [9, 10]. In experiments on the effect of dexamethasone and/or cycloheximide on gene expression in the thymus, animals, which had been adrenalectomized for 10 days, received a subcutaneous injection of dexamethasone (100 µg/100 g b.w.) and/or cycloheximide (1 mg/100 g b.w., [11]).

Isolation of RNA and Northern blot analysis

Tissue RNA was prepared by the method of Chirgwin et al., [10]. For Northern blot analysis, 5 µg of tissue RNA was subjected to electrophoresis in 1.2% agarose gel containing formaldehyde [11], transferred onto a nitrocellulose filter and hybridized to cDNA probes of the SGP-2 (1.9 kb Hind III/EcoRI fragments from pSP64-60HE, [7], pSvr-1 (0.8 kb PstI/PstI fragments [9] or human β-actin (1.8 kb fragments from pHFβA-1, [14], which had been labeled by the random primer method using α-32-P-dCTP. Hybridization and washing procedures were performed as described by Thomas [15] with the exception that polyethylene glycol (10%) was included in the hybridization mixtures [16]. At the end of the washing period, the filters were removed and exposed to XAR-5 films at -80°C with intensifying screens. Quantitation of the relative expression of the transcripts was determined by densitometry (UltraScan 2202, LKB).

Special reagents

α-32-P-dCTP was purchased from ICN Biomedicals, (Irvine, USA). Restriction enzymes and random primer labeling kits were obtained from TAKARA SHUZO (Kyoto, Japan). Other chemicals were purchased from Nacarai Tesque (Kyoto, Japan).

Results

Expressions of SGP-2 and pSvr-1 genes in the seminal vesicles, the ventral prostate, the uterus and the thymus

The involution of steroid hormone-dependent tissues is well known. As shown in Table 1, castration or ovariectomy of rats brings about a significant decrease in tissue wet weight of the male accessory sex organs and the uterus: within 48 h after operation, the tissue weight of the ventral prostate, the seminal vesicles and the uterus decreased to 48, 74 and 27% of that of the intact controls, respectively. On the other hand, within 3 h after the administration of dexamethasone to adrenalectomized rats at a dose of 100 µg per 100 g body weight, a rapid and significant decrease in the thymus wet weight was also observed. Under these experimental conditions, we undertook experiments to examine expressions of SGP-2 and pSvr-1 genes by Northern blot analysis. As shown in the Fig. 1, pSvr-1 hybridized to a mRNA of approximately 1,700 nucleotides in length, while SGP-2 probe hybridized to at least two mRNA species of approximately 1,700 nucleotides in length: a major RNA with a higher molecular weight and a minor one with a lower molecular weight. Messages from SGP-2 and pSvr-1 genes were markedly induced within 48 h after castration of animals both in the ventral prostate and in the seminal vesicles as reported previously [8, 9], while in the uterus of ovariectomized rats, no significant increase in the expressions of these genes was observed: the expressions remained unchanged 48 h after operation and even fell to approximately 50% of those of the intact control thereafter. Contrary to our expectations, the administration of glucocorticoid to adrenalectomized rats resulted in repression of these genes to approximately 30% of those in the thymus of adrenalectomized animals. Under the experimental conditions described above, significant

<table>
<thead>
<tr>
<th>Tissue Wet Weight (g)</th>
<th>Seminal Vesicle</th>
<th>Ventral Prostate</th>
<th>Uterus</th>
<th>Thymus</th>
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<tbody>
<tr>
<td></td>
<td>C48</td>
<td>O48</td>
<td>O96</td>
<td>A D3</td>
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<td>311</td>
<td>220</td>
<td>264</td>
<td>126</td>
<td>196</td>
</tr>
<tr>
<td>±16</td>
<td>±16</td>
<td>±20</td>
<td>±18</td>
<td>±27</td>
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<td>(4)</td>
<td>(4)</td>
<td>(5)</td>
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Table 1. Involution of steroid hormone-dependent rat tissues

a) Values are expressed as means ± SEM. Figures in parentheses indicate numbers of animals.
b) Intact male rats (I) or male rats castrated for 48 h (C48).
c) Intact female rats (I) or female rats ovariectomized for 48 h (O48) or 96 h (O96).
d) Male rats adrenalectomized for 10 days (A) and then treated with dexamethasone (100 µg/100 g b.w.) for 3 h (D3).
Fig. 1. Effect of castration, ovariectomy or dexamethasone administration on expressions of SGP-2 and pSvr-1 genes in steroid hormone-dependent rat tissues. Five micrograms each of tissue RNAs from the seminal vesicles, the ventral prostate, the thymus and the uterus of intact, castrated (48 h), ovariectomized (48 and 96 h), adrenalectomized (10 days) or adrenalectomized (10 days) and then dexamethasone-treated (3 h) animals, were electrophoresed on 1.2% denaturing agarose gels containing 0.66 M formaldehyde. RNAs were transferred onto nitrocellulose filters. Filters were hybridized to 32-P-labeled cDNA sequences from SGP-2 [7], pSvr-1 [9] or human \( \beta \)-actin [14]. i: intact animals, C48: animals castrated for 48 h, O48 and O96: animals ovariectomized for 48 and 96 h, A: animals adrenalectomized for 10 days, D3: animals adrenalectomized for 10 days and then treated with dexamethasone (100 \( \mu g/100 \) g b.w.) for 3 h. Arrowheads (\( \uparrow \)) in the right of upper panel indicate two RNA species approximately 1,700 nucleotides in length. 28 S and 18 S in the left indicate the positions of ribosomal RNAs.

Changes in the expression of the \( \beta \)-actin gene were also observed: approximately 30 and 10% decreases in the relative concentrations of the mRNA were observed in the seminal vesicles of castrated rats and in the thymus of dexamethasone-treated rats, respectively, while in the ventral prostate and in the uterus, significant increases were also observed (approximately 120% increase in the ventral prostate and approximately 20% increase in the uterus of rats ovariectomized for 48 h).

Effect of cycloheximide and/or dexamethasone on expressions of SGP-2 and pSvr-1 genes in the thymus

Among tissues in which expressions of SGP-2...
pSvr-1 genes were significantly altered (Fig. 1), the response in the thymus was the most rapid. Within 3 h after castration or ovariectomy, no significant change in the expressions of these genes could not be observed (data not shown). We therefore undertook experiments to examine the effect of cycloheximide on expressions of these genes in the thymus as a way to elucidate the molecular mechanisms, since a toxic effect of the protein synthesis inhibitor seems to become serious in prolonged treatment of the animals. Expressions of SGP-2 and pSvr-1 genes reached approximately 30% of those of the adrenalectomized control within 3 h after administration of dexamethasone
at a dose of 100 µg per 100 g body weight, while the administration of cycloheximide alone, at a
dose of 1 mg per 100 g body weight, which had been shown to inhibit more than 90% of the
incorporation of 3H-leucine into tissues [11], brought about comparable inhibitions to those due
to dexamethasone alone (Fig. 2). The concomitant administration of dexamethasone and cyclohex-
imide did not enhance their inhibitory effects significantly. Under the experimental conditions, a
significant change in the expressions of the β-actin
gene was also observed (approximately 10% de-
crease).

Discussion

Although additional factors might be involved, numerous studies have been conducted on the
roles of circulating steroids in maintaining the homeostasis of steroid hormone-dependent tissues
by controlling cell proliferation as well as cell death.

To further survey the molecular mechanisms underlying this physiologically important event, in
the present study we concentrated on experiments to test whether or not any significant correlation
between the tissue involution and expressions of SGP-2 and pSvr-1 genes could be observed in
steroid hormone-dependent rat tissues. Expressions of these genes in the male accessory sex
organs are markedly induced after castration [8, 9] and seem to become sensitive molecular markers
of the involution of these tissues as reported concerning the TRPM-2 gene [5], while in the
uterus, no change in expressions of these genes could be observed even under experimental condi-
tions where involution of the tissue became signifi-
cant (Table 1 and Fig. 1). Administration of
dexamethasone to adrenalectomized rats evoked a
rapid and significant decrease in the thymus wet
weight accompanying a repression of these genes
(Table 1 and Fig. 1). These results suggest that the
expressions of SGP-2 and pSvr-1 genes in androgen-
and glucocorticoid-dependent tissues could be efficiently suppressed by androgen or
glucocorticoid, but in the estrogen-dependent
tissues, estrogen could not evoke any significant
effect on expressions of these genes. Although the
function of the products encoded by these genes
remain nuclear at present, their presence might
become useful molecular markers of tissue involu-
tion not only in androgen-dependent tissues but
also in glucocorticoid-dependent ones.

As a way to elucidate the molecular mechanisms
of expressions of these genes, the effect of cyc-
loheximide and/or dexamethasone on expressions
of SGP-2 and pSvr-1 genes in the thymus was
examined, since the repression of these genes in
the thymus was most rapid among the tissues
examined and a toxic effect of the inhibitor of
protein synthesis seemed to become serious the
longer the rats were treated (Fig. 2). From the
findings that the administration of cycloheximide
alone caused almost comparable repression of
these genes to that by dexamethasone alone, and
concomitant administration of cycloheximide and
dexamethasone did not enhance their inhibitory
effects, it is suggested that the effects of androgen
and glucocorticoid on expressions of these genes
might be mediated by other gene products reg-
ulated by androgen or glucocorticoid. This situa-
tion is very analogous to a model recently prop-
osed by Rories and Spelsberg [17] to explain the
differences in response times observed with va-
rious steroid responsive genes. They classify ster-
oid-responsive genes into two groups: the early
responders represent genes that directly bind
steroid receptors at their cognate cis-acting ele-
ments, steroid response elements, and whose
products may be regulatory proteins. Such regula-

In summary, although the role of the expres-
sions of SGP-2 and pSvr-1 genes in steroid
hormone-dependent rat tissues remains unclear at
present, they might become useful molecular
markers of tissue involution not only in androgen-
dependent tissues but also in glucocorticoid-
dependent ones, and also provide excellent model
systems for the study of molecular mechanisms of
gene expression by steroid hormones.
References


