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Tissue steroid sulfatase levels, testosterone and blood pressure

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Abstract

The objective of this study was to examine the response of tissue steroid sulfatase (STS) levels in hypertensive rat strains, when blood pressure (BP) was lowered by different techniques at an early age. A 4×3 factoral design was used, in which males ($n = 6–8$) from four rat strains (WKY, SHR, SHR/a, SHR/y) at 4 weeks of age, were randomly assigned to one of three treatment groups: a hydralazine group, a castration group and a control group. BP was measured by the tail cuff technique and verified by tail catheter at the end of the experiment. BP was significantly reduced by both treatments in the hypertensive strains (SHR, SHR/a, SHR/y) compared to respective control groups. At 15–17 weeks of age, animals were euthanized and heart, kidney, adrenal glands and liver were assayed for STS levels. The major trend in tissue STS was that castration significantly lowered: adrenal, heart and liver STS in specific strains. In conclusion, castration and hydralazine significantly lowered the BP in the hypertensive rat strains, but only castration consistently lowered STS levels across strains implicating testosterone as a regulator of tissue STS.

Keywords: Castration; Hydralazine; Kidney; Liver; Heart; Adrenal gland

1. Introduction

Steroid sulfatase (STS) (Locus Sts; E.C.3.1.6.2) is an important enzyme in steroid metabolism because it activates most steroids and increases the available pool of precursors that produce the biologically active steroids. STS activity is present in most tissues and is especially high in tissues with high dihydroepiandrosterone activity. STS is also important in many developmental pathways, such as, embryogenesis for neurosteroid synthesis, immune response development and differentiation of the skeletal system [1]. Our laboratory has been interested in studying the mechanisms and genes that cause hypertension and we have found that spontaneously hypertensive rats (SHR) have higher STS levels than normotensive WKY rats in the adrenal glands, testes, liver and brain [2]. The consomic strains, SHR/y and SHR/a, which are derived from crossing SHR and WKY have blood pressure (BP) that is borderline hypertensive between SHR and WKY. STS levels in the testes, adrenal glands, liver and brain of these consomic strains also lie between the values of SHR and WKY [2]. Recently, we administered an STS inhibitor for 10 weeks (age 5–15 weeks) to these four strains of rats and found that it slightly lowered blood pressure but markedly lowered plasma testosterone in the hypertensive strains [3]. Our original study was the first to examine tissue STS levels and blood pressure in SHR and WKY and to our knowledge, the current study is the first to lower blood pressure by two different means while measuring tissue STS levels.

In the mouse, the STS gene is on the X and Y chromosome (pseudoautosomal region; PAR). In the rat, it is on the X chromosome (not PAR) and has no identified Y chromosome locus [4]. In the human, there are two STS loci, one active X chromosome and one Y chromosome pseudogene [5]. Mutations at the human STS locus are frequent and often involve sizeable deletions [6].

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Since an increase in STS activity causes an increase in active circulating steroids in animals [7], we postulated there might be a relationship between tissue STS levels and blood pressure because elevated serum steroids, like testosterone and corticosterone, have been implicated in hypertension [8–14]. Therefore, the objectives of this study were to: (1) determine whether tissue STS levels were secondary to blood pressure differences; and (2) determine if there was a Y chromosome effect on STS tissue levels.

2. Methods

The experimental design consisted of four strains of male rats (SHR, SHR/a, WKY, SHR/y) with three treatments: controls, castrated and hydralazine ($n = 6$–8 males per group). SHR/a rats are derived from an SHR mother and originally had a WKY father and sons were backcrossed for 17 generations to SHR females. SHR/y rats originally had an SHR father and sons were backcrossed for 17 generations to a WKY female [2]. A typical cage ($40 \times 50 \times 20$ cm) used processed bedding (P.J. Murphy Sani-Chips, Montville, NJ) and housed two males. Each strain was housed in a separate animal room with temperature (23–24°C) and humidity (50–70%) maintained during a 12:12 h light:dark cycle (06:00–18:00 h, light; 18:00–06:00 h, dark). The cages were cleaned once a week and food (rat chow-Prolab Rat/Mouse/Hamster 3000 Formula by PMI Feeds, Inc., St. Louis, MO) and water were supplied continuously.

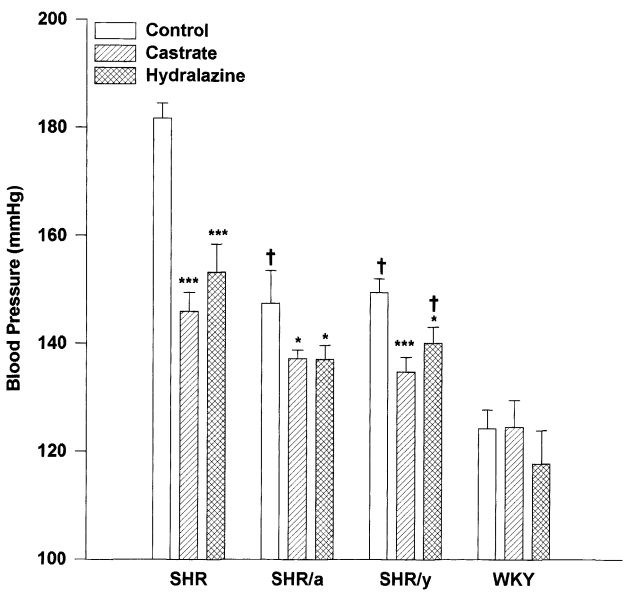

Fig. 1. Blood pressure averaged for the last 4 weeks by strain and treatment (means, ± S.E.M., *$P < 0.05$, ***$P < 0.001$ compared to respective controls, †$P < 0.05$ for between strains same treatment, SHR/a versus SHR and SHR/y versus WKY. Two-way ANOVA for strain: $F = 58$, df = 3, $P < 0.001$, treatment: $F = 21.5$, df = 2, $P < 0.001$), interaction not significant.

Hydralazine, an antihypertensive drug, was added to the drinking water (80 mg/l) beginning when the rats were 4-weeks-old [15]. Castrations were performed at 4 weeks of age [16]. In all groups, BP was measured bi-weekly starting at 8 weeks through 15 weeks by the tail cuff method. whereby the rats were warmed to 37–40°C in a chamber for 20 min to dilate the tail artery [17]. At the end of the study (15–17 weeks) BP was verified in half the animals using the tail catheterization technique [18]. Briefly, the animals were anesthetized with Brevital (50 mg/kg, i.p.) and the ventral tail artery cannulated (PE 10) and filled with heparinized saline. After the animal recovered and was mobile (≈ 2–3 h) a pressure recording was taken using a Micro-med system [18].

Retro-orbital blood [19] was collected during the last 2 weeks under Brevital anesthetic (50 mg/kg, I.P., E. Lilly, Indianapolis, IN) and used to analyze serum testosterone by RIA (BioRad, Laboratories, Hercules, CA). At 15 weeks of age, the adrenal glands, heart, liver and kidney were removed, frozen at −70°C and analyzed for STS activity levels, as we previously reported [2].

Non-radiolabeled estrone sulfate (ES; Sigma, St. Louis, MO) and tritium labeled ES (New England Nuclear, Dupont Chemical Co.) were used as substrates for all STS assays. The reaction cocktail contained 50 000 cpm of labeled ES per reaction, which was mixed with 100 μM unlabeled ES in 0.1 M Tris–HCl, pH 7.2. We had previously found no differences in the levels of activity between freshly collected tissue and tissue that had been frozen and thawed several times [2]. All activity measurements were normalized per microgram of protein. Protein concentrations in the tissue homogenate supernatants were measured using BioRad Protein Assay Kit (Sigma) as the standard. All STS measurements were performed in duplicate and averaged.

Data was analyzed using two-way ANOVA, follow up Bonferonni or Student's t-tests and Pearson's correlation coefficient (SigmaStat, Jandel Scientific Software, San Rafael, CA). Significance was assumed if $P < 0.05$. All animal protocols were approved by the University of Akron, Institutional Animal Care and Use Committee and meet current NIH animal welfare guidelines.

3. Results

Fig. 1 shows that both castration and hydralazine treatment significantly reduced BP in the hypertensive strains (SHR, SHR/a, SHR/y) but not the normotensive strain (WKY) (strain: $F = 58$, df = 3, $P < 0.001$, treatment: $F = 21.5$, df = 2, $P < 0.001$, interaction: $F =$

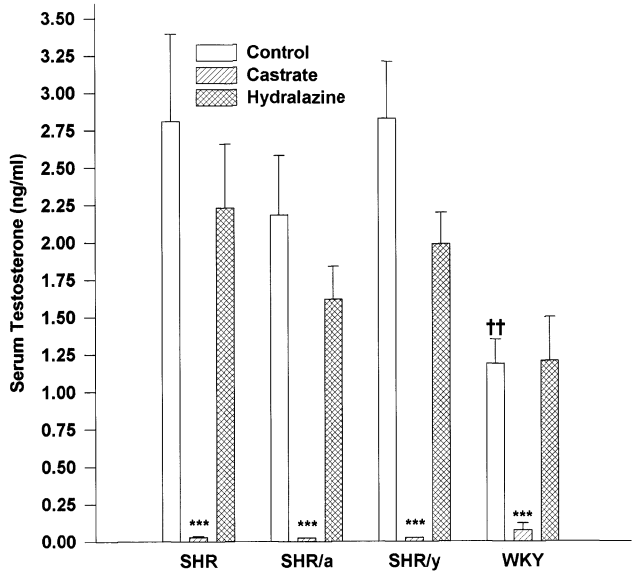

Fig. 2. Serum testosterone by strain and treatment (means, ± S.E.M., ***$P < 0.001$ compared to respective control, two-way ANOVA: strain: $F = 5.6$, df = 3, $P < 0.001$, treatment: $F = 79.4$, df = 2, $P < 0.001$, interaction: $F = 2.3$, df = 6, $P < 0.05$), ††$P < 0.01$, WKY control versus SHR/y control.

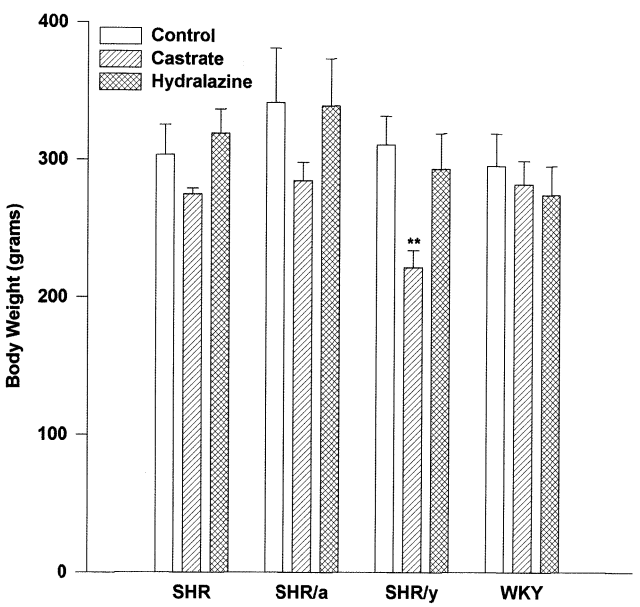

Fig. 3. Final body weight by strain and treatment (means, ± S.E.M., two-way ANOVA: strain: $F = 3.0$, df = 3, $P < 0.05$, treatment: $F = 6.5$, df = 2, $P < 0.01$, interaction: not significant, **$P < 0.01$ compared to control.

5, df = 6, $P < 0.001$). Also SHR/a animals had significantly lower BP than their counterpart SHR; and SHR/y had significantly higher BP than their counterpart WKY. The correlation between final tail cuff BP and tail artery BP was significant ($r = 0.91$, $P < 0.01$).

Fig. 2 shows the testosterone (T) levels by strain and treatment and the two way ANOVA was significant for both strain and treatment (strain: $F = 5.6$, df = 3, $P < 0.01$, treatment: $F = 79.4$, df = 2, $P < 0.001$, interaction: not significant). Hydralazine did not significantly lower T in any strain, but control T was significantly less in WKY as compared to SHR/y. The correlation of final BP and T level on all strains and treatments showed a positive correlation ($r = 0.41$, $P < 0.001$).

Fig. 3 shows significant strain ($F = 3.0$, df = 3, $P < 0.05$) and treatment ($F = 6.5$, df = 2, $P < 0.01$, interaction not significant) effects for body weight. The treatment effect was fairly consistent across all strains with an average 15% decrease caused by castration while hydralazine did not affect body weight. The strain effect was due to the SHR/y group having an overall reduced body weight in the castrate group.

Fig. 4 shows the tissue STS levels. The adrenal STS showed a significant treatment effect ($F = 3.1$, df = 2, $P < 0.05$) and an interaction ($F = 4.0$, df = 6, $P < 0.01$) but no strain effect (Fig. 4A). Castration lowered STS by an average of 40%. The exception was the SHR/a strain, in which castration did not change STS levels. Hydralazine significantly lowered adrenal STS in all strains except WKY in which it was significantly elevated. The only significant strain difference was the SHR/y hydralazine group whixh had lower STS levels than the elevated WKY group ($P < 0.05$). There were no significant strain or treatment effects with regard to kidney STS levels (Fig. 4B). In the heart, there were both significant strain ($F = 5.8$, df = 3, $P < 0.01$) and treatment ($F = 4.1$, df = 2, $P < 0.05$) effects without significant interactions (Fig. 4C). Castration generally caused a reduction in heart STS levels (SHR/a exception) compared to controls and the data for each strain is as follows: (SHR = 18% decrease, SHR/a = 11% increase, SHR/y = 69%, $P < 0.05$ decrease, WKY = 60%, $P < 0.05$ decrease), while hydralazine did not significantly change heart STS levels. Liver STS levels showed both strain ($F = 5.4$, df = 3, $P < 0.01$) and treatment ($F = 9.9$, df = 2, $P < 0.001$) effects with no significant interaction (Fig. 4D). The treatment effect was mainly due to castration lowering liver STS by an average of 46%, whereas hydralazine did not effect STS levels.

4. Discussion

Both castration and hydralazine treatment decreased BP (no significant difference between HYZ and castrate groups) in all strains with elevated BP (SHR, SHR/a, SHR/y). Castration lowered adrenal and liver STS levels in most strains and heart STS in two strains, but not in the kidney, however, hydralazine did not consistently change tissue STS levels in spite of lowering BP. Only in the adrenal did hydralazine lower STS levels in three-quarters of strains. Therefore, BP does not appear to primarily influence tissue STS level. Although BP did

not decrease as much with hydralazine as with castration, the data support the idea that T is an important factor influencing tissue STS level. Hydralazine lowers BP by acting peripherally as a vasodilator and has not been reported to affect serum T levels. Hydralazine did not change T levels even though BP was lowered. STS levels were not consistently changed by hydralazine treatment. The exact mechanism for castration influencing STS levels are not known, however, STS has been shown to be regulated by both steroids and electrolytes. For example, phosphate ions inhibit placental and ovarian STS [20] and steroids can inhibit STS [21]. The most potent inhibitor among the C_{19} steroids was 5α-androstane-3α, 17β-diol. Therefore, the loss of T and its precursors because of castration may have contributed to the sharp decline in STS activity in several tissues. For instance, in a clinical study the kinetics of inhibition of testicular steroid sulfatase by free steroids was consistent with partial inhibition and suggested that modulation of STS by free steroids may regulate release of free steroid precursors of T [22]. The interactions for the control of steroid sulfate metabolism are very complex [21] and involve not only an on–off locus controlled by pregnenolone and progesterone, but also dihydroepiandrosterone sulfate [23]. Steroid sulfatase activity is an integral part of steroid hormone action because changes in enzyme activity occur when steroid hormones and regulators bind to steroid specific regulatory sites on this enzyme [23]. For instance, in the adrenal gland, ACTH can regulate STS activity and stressed animals have higher adrenal STS [24] and plasma corticosterone than controls [25,26].

The only other study involving BP and steroid sulfates showed that SHR rats had increased hepatic cortisol sulfotransferase activity [27]. The increase in hepatic sulfotransferase activity may be the result of the elevated BP. Recently, Valigora et al. in our laboratory showed that a steroid sulfatase inhibitor, estrone-3-*O*-sulfamate, when administered chronically to these same strains of rats, resulted in reduced BP in the hypertensive strains along with a reduction in plasma T and corticosterone [3]. However, the inhibitor had estrogenic activity that elevated plasma estrogen levels that may have caused a BP lowering effect [28].

The decreased STS levels in specific tissues cannot be explained by interaction with body weight, since in three of the four strains there was no significant change of body weight with castration. The STS activity found in control tissues in this study were comparable to those found in the same strains in our previous study [2]. Also, the relationship between the SHR and WKY strains were consistent with our previous results showing that SHR had slightly higher adrenal STS levels than WKY [2] but not in liver. The age of the rats in

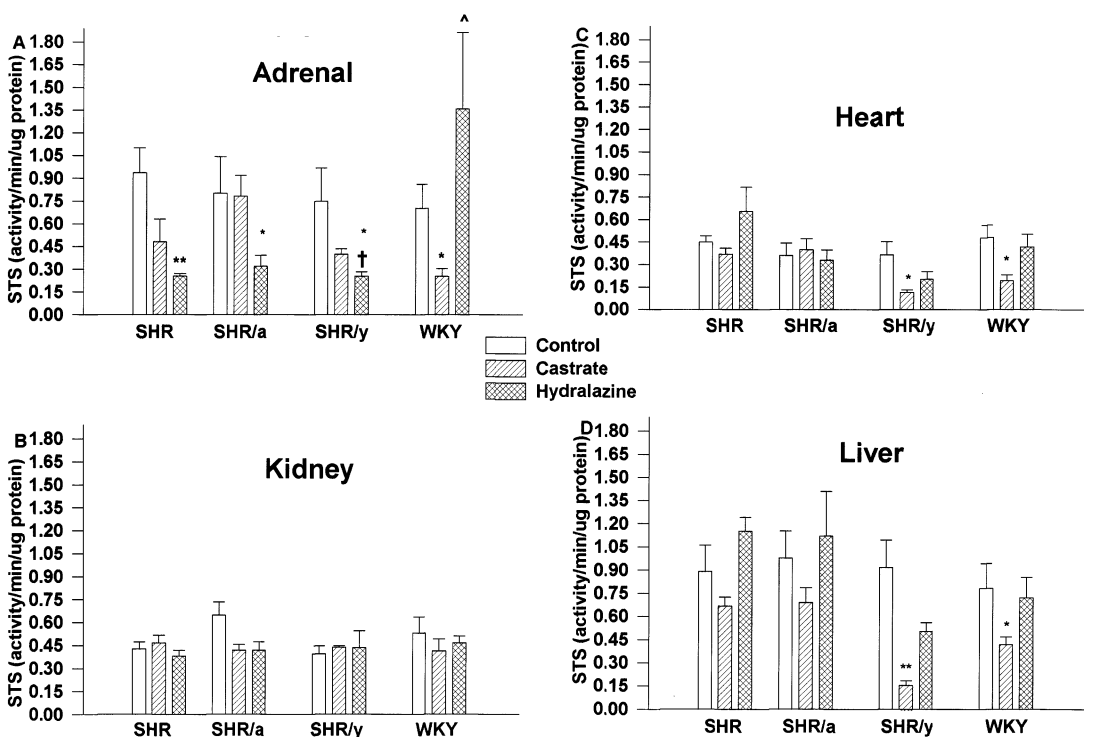

Fig. 4. Steroid sulfatase levels in specific tissues by strain and treatment (means, ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to respective control, †$P < 0.05$ between strain comparison for same treatment: $\hat{}P < 0.05$ for WKY HYZ versus castrate, SHR/a versus SHR and SHR/y versus WKY. Two-way ANOVA by tissue, adrenal-treatment: $F = 3.1$, $P = 0.05$, strain not significant, interaction: $F = 4.0$, df = 6, $P < 0.01$. Kidney, not significant. Heart-treatment: $F = 4.1$, df = 2, $P = < 0.05$, strain: $F = 5.8$, df = 3, $P < 0.01$, interaction not significant. Liver-treatment: $F = 9.9$, df = 2, $P < 0.01$, strain: $F = 5.4$, df = 3, $P < 0.01$, interaction not significant.

the previous study was 12 weeks and in this study the age was 15–17 weeks which could have altered the STS levels because as T decreases with age, STS values could decrease.

With regards to the possibility that the Y chromosome may influence tissue STS levels, there does not appear to be a relationship. We showed previously that the Y chromosome from SHR (present in SHR and SHR/y strains) increases BP [17,29] which has also been verified in other labs [30,31]. It also causes an early T rise [32] and increases indices of sympathetic nervous system activity [18]. Therefore, we hypothesized that tissue STS levels could be influenced by genes on the Y chromosome. However, the SHR/a and WKY strains without the hypertensive Y chromosome had similar tissue STS levels as SHR/y and SHR, suggesting that the Y chromosome does not influence STS levels.

In conclusion, STS is not a secondary response to blood pressure changes, but T does influence STS levels in a tissue specific manner and the SHR Y chromosome does not influence STS levels.

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