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Steroid sulfatase inhibitor alters blood pressure and steroid profiles in hypertensive rats

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Abstract

Our hypothesis is that the steroid sulfatase gene (*Sts*) may indirectly contribute to the modulation of blood pressure (BP) in rats with genetic hypertension. The steroid sulfatase enzyme (STS) catalyzes the conversion of estrone sulfate, dehydroepiandrosterone sulfate, cholesterol sulfate and glucocorticoid sulfates to their active nonconjugated forms. This causes the elevation of biologically active steroids, such as glucocorticoids, mineralcorticoids as well as testosterone, which may lead to increased BP. The main objective was to examine the effects of a steroid sulfatase inhibitor on blood pressure and steroid levels in rats with hypertensive genetic backgrounds. Three treatment groups, 5–15 weeks of age were used: controls, estrone and STS inhibitor (estrone-3-*O*-sulfamate), ($n = 8$ per group). BP was taken weekly by tail cuff, and serum testosterone (T), estrogens (E), and plasma corticosterone (C) levels were measured by radioimmunoassay. BP was significantly reduced by the STS inhibitor in the strains with genetically elevated BP. Also the inhibitor alone significantly reduced plasma corticosterone in all strains compared to estrone treatment with a concomitant as well as significant rise in estrogens and reduction in testosterone and body weight.

Keywords: Testosterone; Estrogen; Corticosterone; Body weight; Y chromosome

1. Introduction

The Spontaneously Hypertensive Rat (SHR) has a Y chromosome locus [1,2] and multiple autosomal loci that contribute to elevated blood pressure (BP) [3,4]. The Y chromosome hypertensive locus requires both an androgen receptor and testosterone for maximum expression [5]. In some mammals the steroid sulfatase locus (*Sts*) is on the Y chromosome, although the rat *Sts* is on the X chromosome [6] with no identified Y chromosome homolog. The steroid sulfatase (STS) enzyme catalyzes the conversion of estrone sulfate, dehydroepiandrosterone sulfate, cholesterol sulfate, and glucocorticoid sulfates to their nonconjugated forms [7,8]. Drugs have been developed to block STS activity since steroid levels, specifically estrogen, are involved in the progression of certain breast cancers [5]. Several research groups have developed steroid sulfatase inhibitors, and estrone-3-*O*-sulfamate has been shown to be the most potent sulfatase inhibitor to date [9]. Because of the broad specificity of STS with all steroid sulfates and the interaction of many steroids with BP, we propose that the *Sts* locus in the hypertensive SHR strain could be an important background modifier accelerating hypertension resulting from the SHR Y chromosome locus [4].

Our laboratory has shown that STS activity levels are higher in the testes, adrenal glands, liver, and hypothalamus of adult SHR males compared to normotensive Wistar Kyoto (WKY) males [7]. Hypothetically, this would increase the nonconjugated, or active form, of many steroids and lead to increased

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BP. If any of the glucocorticoids, mineralcorticoids, or androgens are abnormally elevated, mechanisms have been reported, which would link them with elevated BP [5,10,11]. STS activity was also measured in the backcross consomic strains, SHR/y and SHR/a to test for a Y chromosome influence on STS. STS tissue activity levels and BP in these strains were intermediate between those of SHR and WKY [7]. Thus, an STS inhibitor, estrone-3-*O*-sulfamate, may be a valuable tool to evaluate the effect of STS upon BP. However, since a recent report by Elger et al., demonstrated that estrone-3-*O*-sulfamate is estrogenic [12], estrone will be used in the following study as a control.

2. Materials and methods

The hypothesis tested was that systolic BP of hypertensive and borderline hypertensive strains would be lowered by STS inhibitor treatment if STS activity was directly related to BP. If STS activity was a secondary response to BP, the inhibitor would have no effect on BP. The design of the experiment consisted of males of four strains with three treatments: control, estrone injected, and STS inhibitor injected ($n = 6$–8 per group). The four strains used were SHR; WKY; and two Y chromosome consomic strains, SHR/a and SHR/y. SHR/a rats have originally an SHR mother and a WKY father and were derived from 17 generations of backcrossing males to an SHR female. SHR/y rats have originally an SHR father and were backcrossed for 17 generations to a WKY mother. Therefore, all offsprings inherited the Y chromosome from their father's strain and autosomes and X chromosomes from their mother's strain.

A typical cage ($40 \times 50 \times 20$ cm) housed two males with a processed bedding (P.J. Murphy Sani-Chips, Montville, NJ). Each strain was housed in a separate room and temperature ($23 - 24^{\circ}C$) and humidity (50–70%) were maintained during a 12-hour light/dark cycle (6 AM to 6 PM, light; 6 PM to 6 AM, dark). The cages were cleaned once per week, and food (rat chow-Prolab Rat/Mouse/Hamster 3000 Formula by PMI Feeds, St. Louis, MO) and water were supplied continuously.

The estrone treatment group was included because the inhibitor has an estrone backbone with potential physiological effects. The powdered estrone and STS inhibitor were mixed with corn oil for injection. For the estrone group, 150 mg of estrone was mixed with 30 ml^3 of corn oil for an injection (i.p.) of 0.1 ml^3 for every 100 g of body weight. The inhibitor contained 450 mg estrone-3-*O*-sulfamate for every 30 ml^3 of corn oil and 0.1 ml^3 was injected per 100 g body weight. A 1 ml^3 tuberculin syringe with a 21 G precision glide needle was used to deliver the drugs. A needle of this size was required because of the viscosity of the corn oil. The injections were given twice a week on Monday and Friday between 10 AM and 12 PM, starting at 5 weeks of age. In order to observe the effects of the withdrawal of estrone and STS inhibitor on the physiological parameters, the injections were stopped in the WKY and SHR/y strains for 3 weeks after week 15.

BP was measured biweekly by tail sphygmomanometry. The rats were placed in a warming chamber (37–40°C) for 5 min. One at a time, each rat was placed into a plastic restraint and five pressures were recorded. This technique was performed on conscious animals that were calm and vasodilated and did not struggle during the procedure [1]. Also, retroorbital blood samples were taken every 3 weeks when the third week of injections (8 weeks of age) started [13]. Hormones were measured by RIA: plasma corticosterone (ICN Biomedicals, Costa Mesa, CA), serum testosterone (BioRad Laboratories, Hercules, CA) and plasma estrogen (ICN Pharmaceuticals, Costa Mesa, CA). The estrogen kit measured total estrogens with the following percentage cross reactivity: estradiol − 17 β = 100, estrone = 100, estriol = 9, estradiol − 17 α = 7, the remainder of the steroids is < 0.01. The percent coefficient of variation for the range we measured was 5.5 for intra-assay and 10.9 for inter-assay variation. The corticosterone assay had a 100% cross-reactivity with corticosterone and less than 0.5% with the majority of all other steroids. The intra-assay variation in the range we measured was 4.4% and, inter-assay variation was 7.1%. The testosterone assay had a 100% cross reactivity with testosterone, 28.7% with 19-hydroxytestosterone, 15.1% with methyltestosterone, 6.65% with 5α-dihydrosterone and 2% or less with other testosterone derivatives and estrogen, progesterone and corticosterone. The intra-assay variation was 7.4–11.67 and inter-assay variation was 12–17% in the range of our values. BP values were averaged for the last 4 weeks for graphical purposes and the hormone levels were averaged across all time periods. The livers from all strains were sent to Duquesne University and analyzed for steroid sulfatase activity [9]. All animal protocols were approved by the University of Akron, IACUC and met the current NIH animal welfare guidelines.

A two-way ANOVA was used for multiple comparisons of systolic blood pressure, body weight and hormone levels between the four strains and a one-way ANOVA to test within strain effects. Follow-up appropriate *t*-tests, (Student Newman-Keuls and Dunns) were used for group comparisons. Multiple linear regression was performed on BP as the dependent variable and independent variables were: body weight, corticosterone, testosterone and estrogen levels (Sigma Stat, Jandel Scientific Software; San Rafael, CA), and significance was assumed if $p < 0.05$.

3. Results

Fig. 1 shows the BP relationship by strain and treatment (two-way ANOVA: strain, $F = 24.8$, $df = 3$, $p < 0.0001$; treatment, $F = 9.0$, $df = 2$, $p < 0.001$). BP was significantly lowered by the STS inhibitor compared to controls in the SHR, SHR/a and SHR/y strains; however, there was a slight rise ($p < 0.05$) in BP in the WKY inhibitor group. Estrone treatment significantly lowered BP in SHR/y compared to controls ($p < 0.001$) and slightly raised BP in the WKY group ($p < 0.05$). Strain comparisons showed that control SHR/a BP was lower than SHR ($p < 0.01$) and WKY BP was lower than SHR/y ($p < 0.05$). Also, the SHR/a estrone group BP was lower than that of SHR ($p < 0.01$).

Fig. 2 shows the corticosterone (C) relationship by strain and treatment (two-way ANOVA: strain, $F = 7.4$, $df = 3$, $p < 0.0001$; treatment, $F = 7.1$, $df = 2$, $p < 0.001$). One-way ANOVA for treatment within strain showed significant differences in all strains. The STS inhibitor groups had generally lower corticosterone than the estrone groups (in all strains). Estrone lowered corticosterone in the SHR/y and WKY strains (not significant) but raised it in SHR/a and SHR, $p < 0.05$, $p < 0.01$. A correlation coefficient run on corticosterone vs. BP, by treatment, for estrone and STS inhibitor groups separately and pooled together, showed no significance.

Fig. 3 shows the testosterone (T) relationship by strain and treatment (two-way ANOVA: strain, $F = 70$, $df = 3$, $p < 0.0001$; treatment, $F = 95$, $df = 2$, $p < 0.0001$). Both STS inhibitor groups and estrone groups had generally lower testosterone (T) than the control groups ($p < 0.001$).

Fig. 4 shows the estrogen (E) relationship by strain

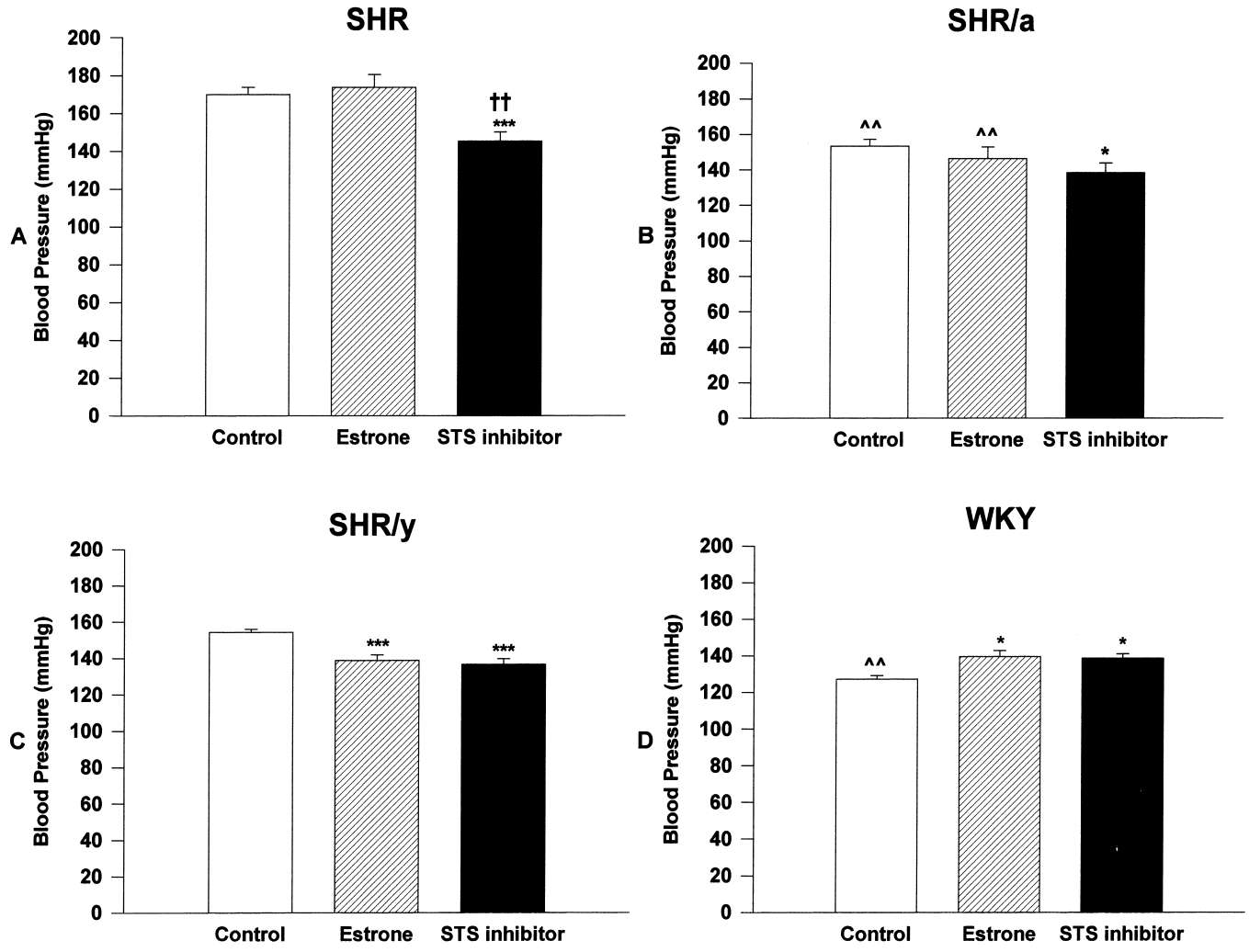

Fig. 1. Average blood pressure of last 4 weeks by strain and treatment (means, ±SEM, * $p < 0.05$, ** $p < 0.01$, *** is $p < 0.001$ compared to respective control, †† is $p < 0.01$ compared to estrone group, ^^ $p < 0.01$ SHR vs. SHR/a for same treatment and SHR/y vs. WKY for same treatment).

and treatment (two-way ANOVA: strain, $F = 4.3$, $df = 3$, $p < 0.01$; treatment, $F = 68$, $df = 2$, $p < 0.0001$). The STS inhibitor significantly increased estrogen in all strains compared to both controls and estrone treatment. Estrone significantly increased estrogen in all groups compared to controls.

The STS inhibitor decreased body weight in all strains as did the estrone treatment (Fig. 5) (two-way ANOVA by strain, $F = 9.4$, $df = 3$, $p < 0.001$; treatment, $F = 119$, $df = 2$, $p < 0.0001$). There were no significant strain differences between SHR vs. SHR/a and WKY vs. SHR/y. The STS inhibitor group had slightly lower weight in SHR/y compared to estrone ($p < 0.05$). Liver steroid sulfatase activity in the rats was decreased by 99% when compared with control rats.

At the end of the experimental period, to verify any hormonal effects, estrone and STS inhibitor injections were withdrawn for 3 weeks in the WKY and SHR/y strains to observe if the hormone levels would return to control values. Testosterone levels that were suppressed by the STS inhibitor rose from 0.1 to 1.5 ng/ml in the SHR/y group and from 0.5 to 0.7 ng/ml in WKY. In a reciprocal manner, the estrogen values decreased from 1700 to 90 pg/ml in SHR/y and from 1300 to 400 pg/ml in WKY. Removal of estrone did not have such a large effect but testosterone increased from 0.2 to 0.6 ng/ml in SHR/y and from 0.2 to 0.38 ng/ml in WKY and estrogen decreased from 500 to 90 pg/ml in SHR/y and from 600 to 250 pg/ml in WKY. Corticosterone values were not significantly changed by drug withdrawal.

Table 1 shows the multiple linear regression analysis using BP as the dependent variable and the independent variables were the final values for: body weight, corticosterone, testosterone and estrogen. Also, calculations for all strains were included in a regression and the R^2 was low due to the normal BP of WKY. The

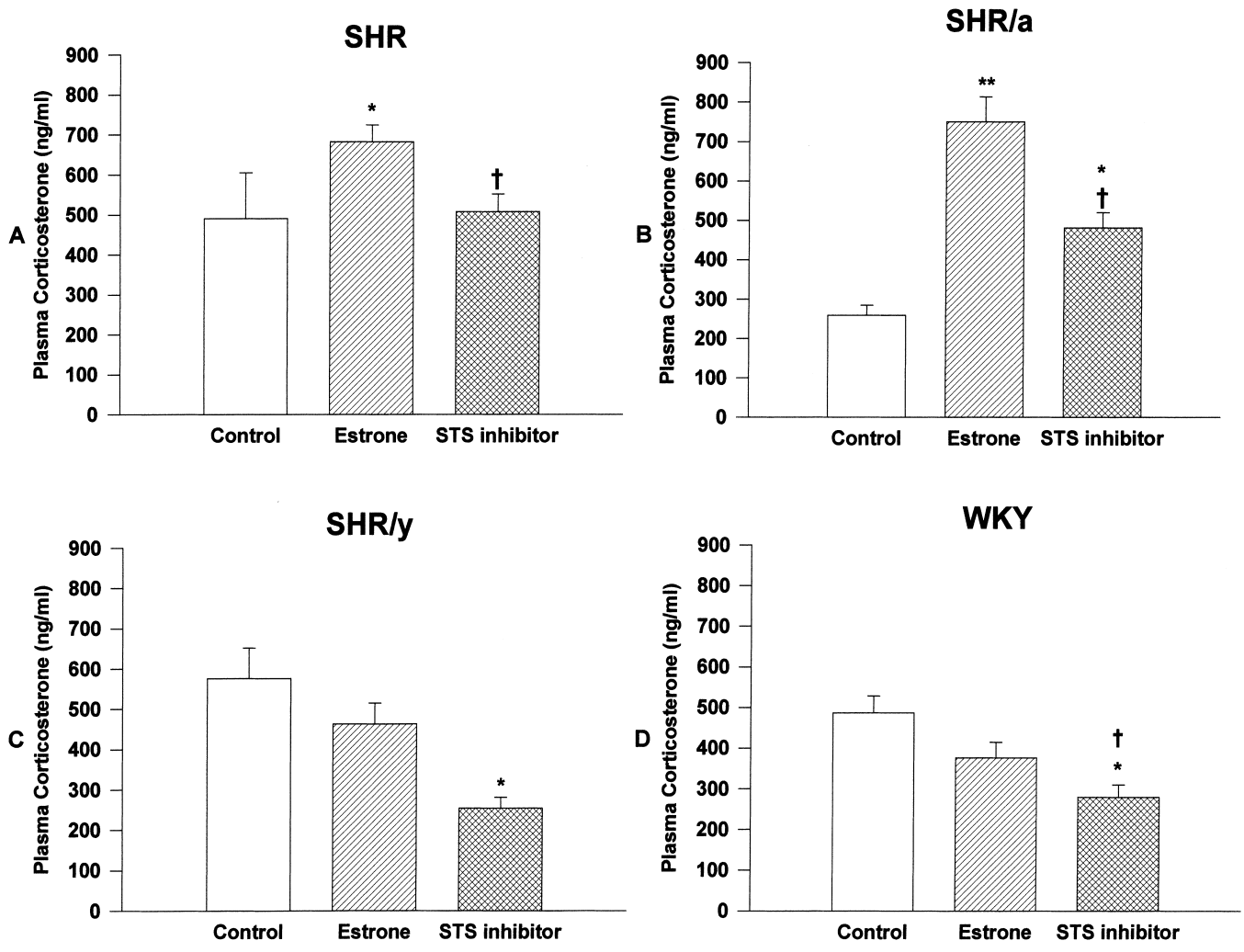

Fig. 2. Average plasma corticosterone for entire period by strain and treatment (means, ±SEM, * $p < 0.05$, ** $p < 0.01$ compared to respective control, † $p < 0.05$ compared to estrone group).

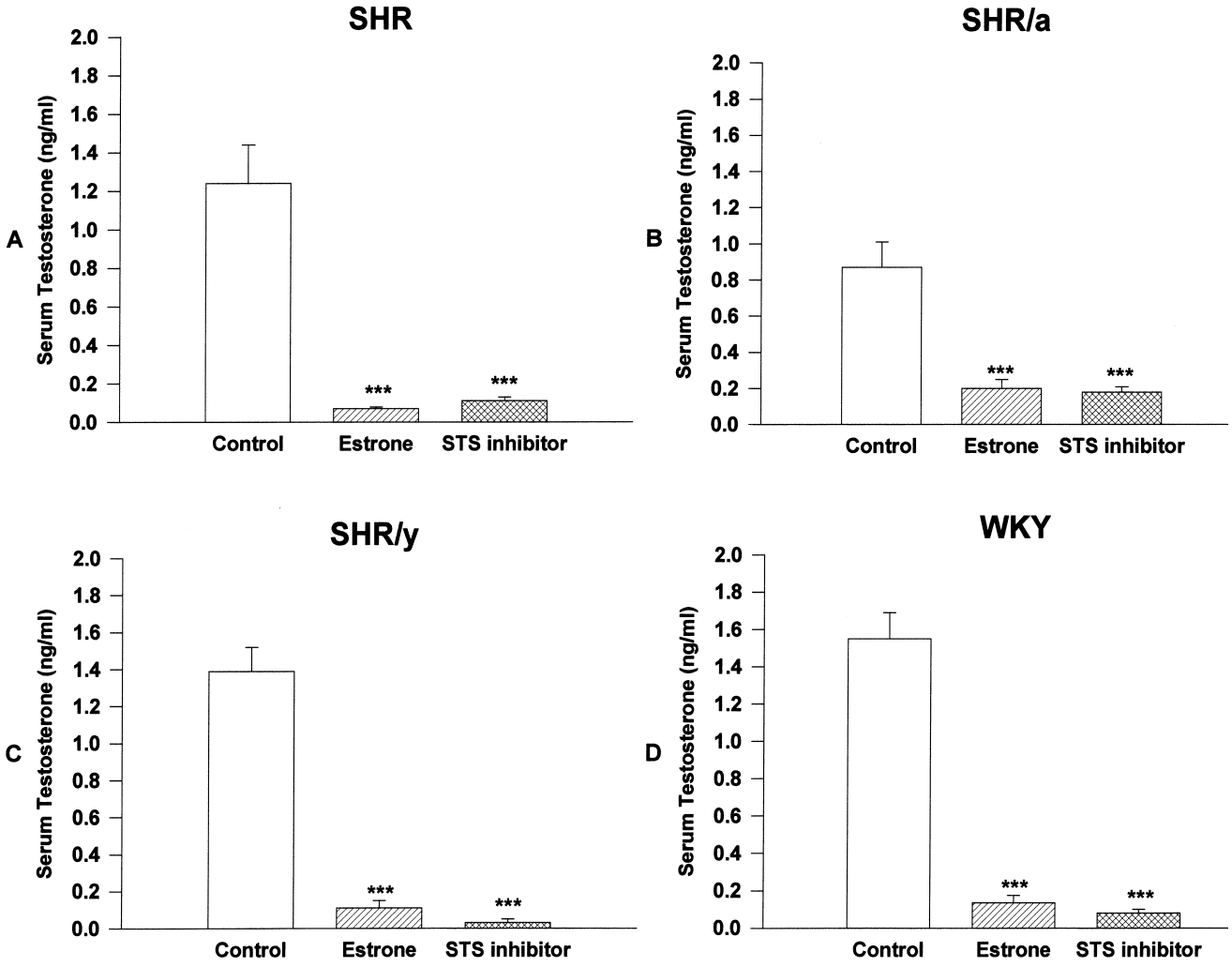

Fig. 3. Average serum testosterone for entire period by strain and treatment (means, ±SEM, *** $p < 0.001$ compared to respective controls).

R^2 value was more than doubled when a similar analysis was performed excluding the normotensive WKY strain.

Table 1
Regression analysis by strains with BP as the dependent variable and independent variables: body weight, serum corticosterone, testosterone and estrogen

Strain	R^2	Adj. R^2	F	P
SHR	0.45	0.31	3.3	0.038
SHR/a	0.68	0.60	8.6	0.0006
WKY	0.29	0.12	1.7	0.189
SHR/y	0.71	0.64	11.2	< 0.0001
All strains	0.19	0.15	4.8	0.0015
All strains without WKY	0.43	0.39	11.6	< 0.0001

4. Discussion

The sulfotransferase enzymes like steroid sulfatase play a role in xenobiotic detoxication, carcinogen activation, prodrug processing, cell signaling pathways, and regulation of intratissue active androgen and estrogen levels [14]. Because the sulfated hormones do not bind to their receptors, the sulfotransferase activity provides an alternative control pathway for regulating intratissue active hormone levels [14]. The inhibitor worked effectively since the liver STS values were decreased by 99% which is comparable to the results reported by Purohit et al. [15]. The STS inhibitor lowered BP in the hypertensive and borderline hypertensive strains as compared to controls. The BP of the control groups of each strain was comparable to our findings using telemetered BP [16]. The STS inhibitor, estrone-3-*O*-sulfamate, has a backbone of estrone which could have an anti-hypertensive effect on the blood vessels. Estrone promotes the deposition of elas-

tin [17,18], increasing distensibility and the up-regulation of nitric oxide, a potent vasodilator [18], both would tend to decrease BP [19,20]. However, a significant BP lowering effect of estrone was only observed in the SHR/y group, so it appears that the STS inhibitor lowered BP by more than just an estrone effect. The effects of the inhibitor and estrone on corticosterone and testosterone were not consistent with the BP lowering effect, but the effects of serum estrogen levels did follow the BP effect. The estrogen levels in the STS inhibitor group were much higher than the estrone group and this could be the mechanism for the further reduction of BP. Since the estrogen assay detects estrone sulfate, this probably accounts for the high estrogen levels in the STS inhibitor groups, but nevertheless, this can be converted to estradiol (E_2), which then could have further vascular physiological effects. Indeed, in men with STS deficiency there was an elevated progesterone level and an elevation in E_2 in response to human chorionic gonadotropin stimulation [21]. Both estrone and E_2 are detected with 100% cross reactivity so we cannot distinguish between them at this time.

When looking at the corticosterone levels between the estrone and STS inhibitor groups of all strains, a general pattern was observed showing reduced levels in the STS inhibitor groups compared to the estrone groups (significant in all but WKY). Because glucocorticoids can alter gene transcription there are complex mechanisms that could influence BP. Angiotensin II a potent vasoconstrictor is increased with elevated glucocorticoids. Also, vascular reactivity to vasoconstrictor is increased by corticosterone and extraneuronal uptake of norepinephrine is inhibited by corticosterone. All these effects tend to promote vasoconstriction and would elevate BP [22]. Since there was a significant decrease of corticosterone in the SHR/y strain, this could account for the decrease in BP. However,

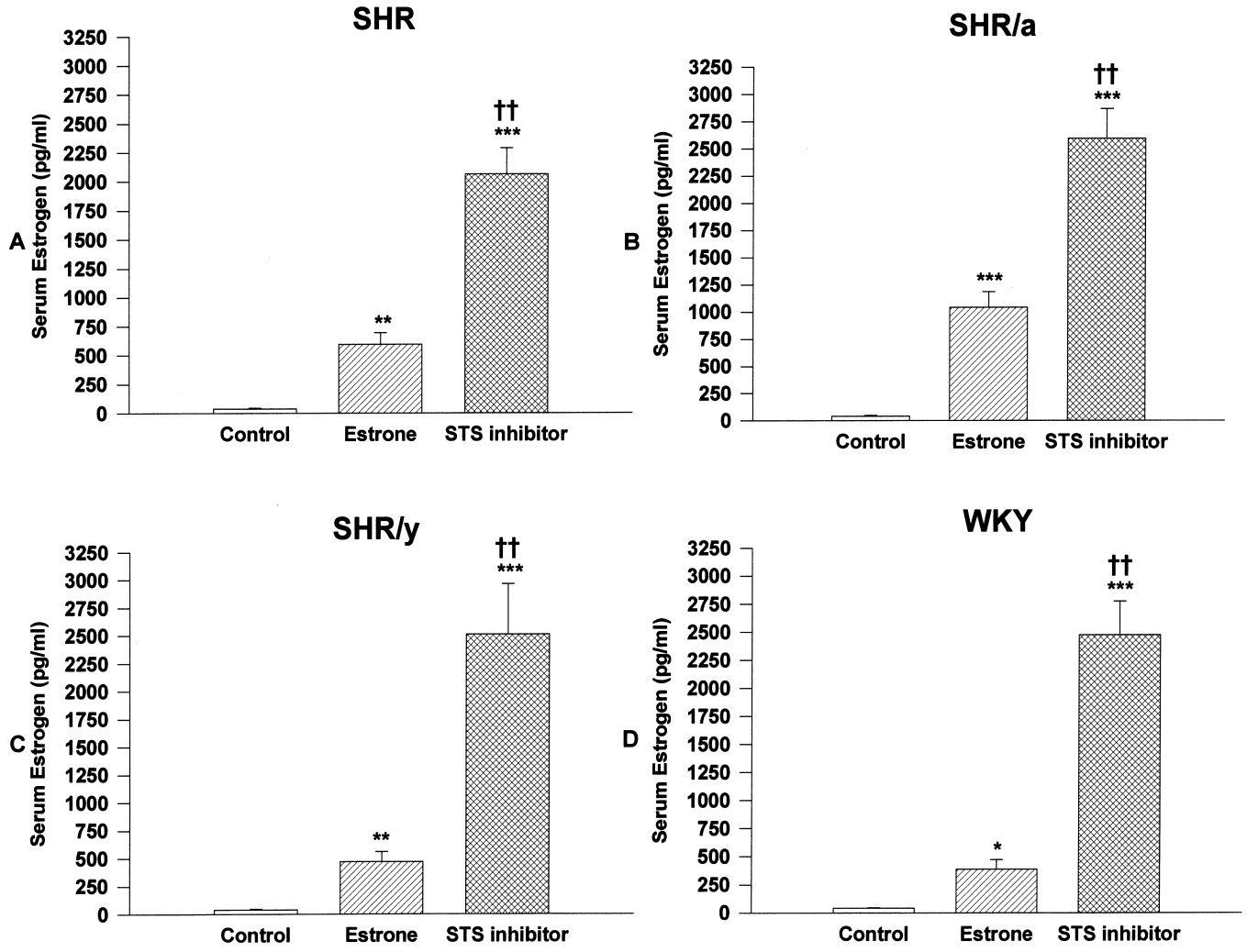

Fig. 4. Average serum estrogen for entire period by strain and treatment (means, ±SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to respective control, †† is $p < 0.01$ compared to estrone group).

BP was not altered in the WKY strain and yet corticosterone also decreased significantly. Just the opposite was found in SHR and SHR/a strains where BP decreased but there was no decrease in corticosterone as a result of the STS inhibitor. Indeed, a correlation coefficient for BP vs. corticosterone was run on the estrone and inhibitor groups, individually and together, and there was no significance. Therefore, it is not likely that in this model of hypertension, these small changes in corticosterone play a role in BP modulation. We did not measure plasma aldosterone which could have also been suppressed in the inhibitor group. However, in the SHR model of hypertension aldosterone is not a major factor like it is in several forms of mineralcorticoid hypertension.

Corticosterone increases blood glucose concentration by acting on fat, protein and carbohydrate metabolism. Since the STS inhibitor groups from the hypertensive strains had lower corticosterone levels than the estrone groups, this could have contributed to the lower body weights which could also indirectly lower BP [23–25]. Another important mechanism contributing to decreased body weight is that the males in both treatment groups had severely depressed testosterone levels. During this rapid developmental growth period (5–15 weeks) the anabolic effect of testosterone was markedly reduced. In addition, estrogen contributes to reduced body weight acting centrally as well as peripherally. Experimental evidence suggests that the initial effects of estrogen on body weight are mediated by the brain [26–28]. Brewster et al. [29] showed that serum estrogen levels of 50 pg/ml were required to retard weight gain and maximal effects were generated in the 2000 pg/ml range which is the range of estrogen in the STS inhibitor group. Consistent with this analysis, body weights in STS inhibitor and estrogen groups were comparable to female rats of that age.

With regard to both the testosterone and estrogen

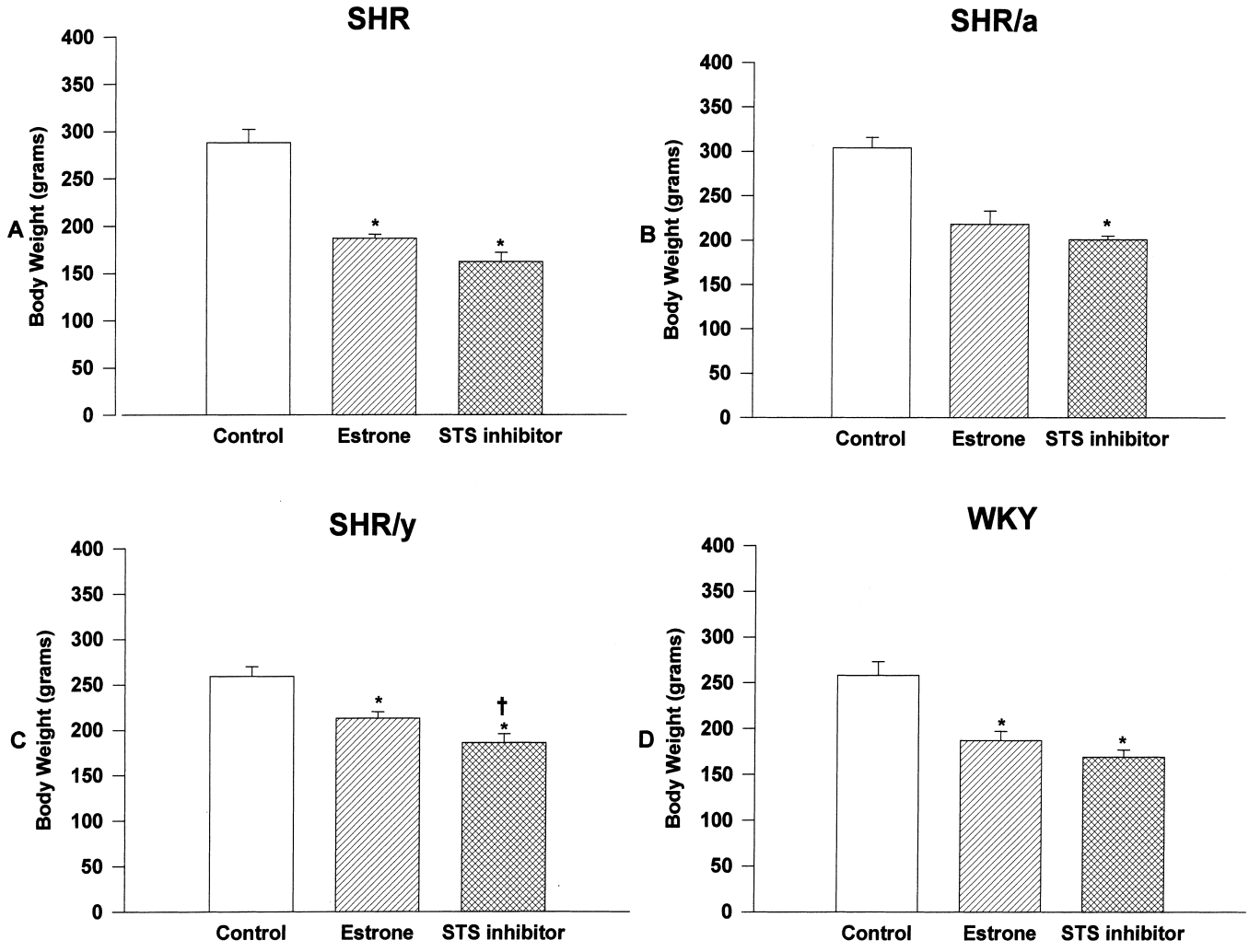

Fig. 5. Final body weight by strain and treatment (means, ±SEM, * $p < 0.05$ compared to respective control, † is $p < 0.05$ compared to estrone group).

levels there was evidence of steroid negative feedback and a reciprocal relationship of the steroids. First, all four strains of both estrone and inhibitor groups showed extremely low testosterone levels for intact, non-castrated males and the control male groups showed low estrogen in the range reported for adult male rats (35–45 ng/ml) [29]. So, estrone could have been converted to E_2 through the enzyme 17*B*-hydroxysteroid dehydrogenase and through negative feedback acting on the hypothalamus, shut down GnRH release and testosterone production. Indeed, all four strains of the estrone and inhibitor groups showed remarkably high levels of estrogens although it was much higher in the STS inhibitor group. When taken off, the inhibitor at week 16, the SHR/y and WKY estrogen levels fell dramatically and testosterone increased. The STS inhibitor, acts to maintain steroids (i.e. estrone-sulfate) in their inactive sulfonated forms. Usually, these inactive forms of steroids are cleared from the blood but, E_2 has been known to stimulate steroid binding proteins. So, estrone-sulfate may be maintained in the blood by a steroid binding protein. Urabe et al. [30] found that estrone-sulfate can be converted to E_2 which could potentiate the effect of the STS inhibitor in decreasing testosterone levels and BP. Indeed, E_2 at low blood levels, in a dose dependent manner can rapidly suppress testosterone levels in male rats [23]. Normal plasma levels of testosterone in adult male rats is about 1.2–2.3 ng/ml. At high E_2 levels, such as, in our study, the levels of testosterone were suppressed to 0.1 ng/ml which was similar to previous results in rats [23]. E_2 can suppress testosterone by several routes in the male [27,31,32]. For instance, E_2 can inhibit LHRH at both hypothalamic and pituitary levels. Also E_2 can directly effect the testis to reduce testosterone biosynthesis and release [23]. Therefore, this leads us to conclude that the main role of the STS inhibitor is acting on testosterone to directly and indirectly suppress the free circulating levels through E_2. We have shown previously [2,5] that testosterone is a key player in the development of hypertension in the male SHR. We are still trying to understand the mechanism but there appear to be several ones. Acutely, testosterone can facilitate norepinephrine release, a potent peripheral vasoconstrictor [16]. Chronically, testosterone can increase resistance vessel and coronary collagen deposition which stiffens the vessel and increases peripheral resistance [17,18].

The regression analysis supported the concept that BP was partially dependent upon final body weight and blood levels of corticosterone, testosterone and estrogen. The three hypertensive strains showed significant predictive values for BP, but the WKY did not. This was expected since the WKY BP is normotensive and they are not as responsive to external or internal stimuli as the SHR strains.

The hypertensive effect of the SHR Y chromosome is illustrated by the BP of the parental strains (SHR and WKY) and the backcross consomic strains (SHR/a and SHR/y). The BP between the SHR and SHR/a strains is the result of loss of the SHR Y chromosome in SHR/a and the increase from WKY to SHR/y is the result of the addition of the SHR Y chromosome to SHR/y [32]. With the STS inhibitor or estrone treatment, all strains had similar BP, serum testosterone, serum estrogen, and body weight, regardless of the presence or absence of the SHR Y chromosome and other hypertensive loci. The STS inhibitor and estrone treatments, could disrupt or mask physiological pathways necessary for the differences between the strains. Therefore, there does not appear to be a Y chromosome effect caused by the STS inhibitor on blood pressure, body weight, testosterone and estrogen. There may be an autosomal effect on corticosterone levels since the comparison strains SHR/y and WKY are only different in the Y chromosome and the controls were similar for hormonal levels and body weight. The response of plasma corticosterone between treatments was different in SHR and SHR/a strains with corticosterone levels 2–3 times that of the SHR/y and WKY strains. The genetic origin of the corticosterone difference between strains is the result of a SHR autosomal or X linked locus rather than the SHR Y chromosome.

In the rat, the *Sts* gene is located on the X chromosome with no known homologous Y chromosome locus. This is different from many rodents including the mouse in which a Y chromosome locus is present and active. Whether the rat *Sts* locus undergoes X inactivation in females is not known [33–35]. Evidence from other mammals is mixed, such as the root vole in which *Sts* does undergo X inactivation [36,37] and whereas the mouse and human *Sts* genes do not [24,38,39]. Without X inactivation males would only have one active gene copy and females would have two. This could produce higher E_2 levels in females, thus lowering BP; and raise testosterone levels in males, thereby raising BP. Therefore the sexual dimorphism in BP in mammals may be partially explained by different STS levels between males and females.

Clinical use of STS inhibitors started with research on estrogen-dependent breast cancers. Estrone sulfate pools and steroid sulfatase are important in regulating the supply of active estrogens to cancer cells [12]. Reports have shown that estrone may be released during the inactivation of steroid sulfatase by the estrone-3-*O*-sulfamate and render the inhibitor to be estrogenic [9,40,41]. Based on our results showing an estrone effect on BP and breast cancer sensitivity to estrone, there is a need for a potent non-steroidal sulfatase inhibitor that lacks estrogenic traits. Indeed, a

series of (*p*-*O*-sulfamoyl)-*N*-alkanoyl tyramines have been synthesized and proven to be potent estrone sulfatase inhibitors [9] which may be beneficial both in some types of hypertension as well as cancer treatment. Also a deletion of the STS locus produces ichthyosis in humans and although the effect on BP is not known, there are side effects which can cause low blood calcium and phosphorus levels which could elevate BP [42,43]. In male patients with ichthyosis the testosterone production after human chorionic gonadotropin stimulation is normal suggesting that alternate paths regulating active testosterone may be involved [21].

In conclusion, the inhibition of steroid sulfatase in hypertensive rat strains consistently lowered BP, body weight, and serum testosterone and raised serum estrogen. The effects may be partially due to an estrogenic effect since estrone injections had similar effects on the parameters studied and the inhibitor can be converted to E_2. Further studies will separate these effects.

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References

[1] D.L. Ely, M. Turner, Hypertension in the spontaneously hypertensive rat is linked to the Y chromosome, Hyper. 16 (1990) 27–281.
[2] D.L. Ely, H. Daneshvar, M.E. Turner, M.L. Johnson, R.L. Salisbury, The hypertensive Y chromosome elevates blood pressure in F_{11} normotensive rats, Hyper. 21 (1993) 1071–1075.
[3] P. Hilbert, K. Lindpaintner, J.S. Beckmann, T. Serikawa, F. Soubrier, C. Dubay, P. Cartwright, B. DeGouyon, C. Julien, S. Takahashi, M. Vincent, D. Ganten, M. Georges, G.M. Lathrop, Chromosomal mapping of two genetic loci associated with blood pressure regulation in hereditary hypertensive rats, Nature 353 (1991) 521–529.
[4] H.J. Jacob, K. Lindpaintner, S.E. Lincoln, K. Kasumi, R.K. Bunker, Y.-P. Mao, D. Ganten, V. Dzau, E. Lander, Genetic mapping of a gene causing hypertension in the stroke-prone SHR, Cell 67 (1991) 213–224.
[5] D.L. Ely, J. Falvo, G. Dunphy, A. Caplea, R. Salisbury, M. Turner, The SHR Y chromosome produces an early testosterone rise in normotensive rats, J. Hyper. 12 (1994) 769–774.
[6] E.C. Salido, X.M. Li, P.H. Yen, N. Martin, T.K. Mohandas, L.J. Shapiro, Cloning and expression of the mouse pseudoautosomal steroid sulphatase gene (*Sts*), Nat. Gene. 13 (1996) 83–86.
[7] M.L. Johnson, D.L. Ely, M. Turner, Steroid sulfatase and the Y chromosome hypertensive locus of the spontaneously hypertensive rat, Steroids 60 (1995) 681–685.
[8] S. Singer, The properties and the endocrine control of the production of the steroid sulfotransferases, in: G. Litwack (Ed.), Biochemical Actions of Hormones, Academic Press, New York, 1982, pp. 271–303.
[9] P.K. Li, S. Milano, L. Kluth, M.E. Rhodes, Synthesis and sulfatase inhibitory activities of non-steroidal estrone sulfatase inhibitors, J. Steroid. Biochem. Mole. Biol. 59 (1996) 41–48.
[10] O.B. Holland, C. Gomez-Sanchez, Mineralcorticoids and hypertension, Nephrol. 3 (1983) 156–163.
[11] E.G. Biglieri, J.R. Stockiat, M. Schambelan, Adrenal mineralcorticoid hormones causing hypertension, in: J.H. Laragh (Ed.), Hypertension Manual, York Medical Books, New York, 1974, pp. 461–483.
[12] W. Elger, S. Schwarz, A. Hedden, G. Reddersen, B. Schneider, Sulfamates of various estrogens — prodrugs with increased systemic and reduced hepatic estrogenicity at oral application, J. Steroid. Biochem. Mole. Biol. 55 (1995) 396–403.
[13] V. Riley, Adaptation of orbital bleeding technique to rapid serial blood studies, Proc. Soc. Exper. Biol. Med. 104 (1960) 751–754.
[14] M.A. Runge-Morris, Regulation of expression of the rodent cytosolic sulfotransferases, FASEB J. 11 (1997) 109–117.
[15] A. Purohit, G.J. Williams, C.J. Robers, B.V.L. Potter, M.J. Reed, In vivo inhibition of oestrone sulfatase and dehydroepiandrosterone sulfatase by oestrone-3-*O*-sulphamate, Internatl. J. Cancer 63 (1995) 106–111.
[16] D. Ely, A. Caplea, G. Dunphy, H. Daneshvar, M. Turner, A. Milsted, M. Takiyyuddin, Spontaneously hypertensive rat Y chromosome increases indexes of sympathetic nervous system activity, Hyper. 29 (1997) 613–618.
[17] H. Wolinsky, Response of the rat aortic media to hypertension, Circ. Res. 62 (1970) 507–522.
[18] C.N. Martyn, S.E. Greenwald, Impaired synthesis of elastin in walls of aorta and large conduit arteries during early development as an initiating event in pathogenesis of systemic hypertension, Lancet. 350 (1997) 953–955.
[19] C.P. Weiner, I. Lizasoain, S.A. Baylis, R.G. Knowles, I.G. Charles, S. Moncada, Induction of calcium-dependent nitric oxide synthases by sex hormones, Proc. Natl. Acad. Sci. 91 (1994) 5212–5216.
[20] F. Amin, N. Niederhoffer, R. Tatchum-Talom, T. Makki, J. Guillou, P. Tankosic, J. Atkinson, A new technique for study of impact of arterial elasticity on left ventricular mass in rats, Amer. J. Physiol. 270 (1996) H981–987.
[21] A. Ruokonen, A. Oikarinen, R. Vihko, Regulation of serum testosterone in men with steroid sulfatase deficiency: response to human chorionic gonadotropin, J. Ster. Biochem. 25 (1986) 113–119.
[22] B.R. Don, M. Schambelan, Pathophysiology of adrenal cortical hypertension, in: J. Izzo, H.R. Black (Eds.), Hypertension Primer, 2nd ed., Lippincott, Williams and Wilkens, Baltimore, MD, 1999, pp. 138–140.
[23] M.A. Ferguson-Smith, N.A. Affara, E. Boyd, A.C. Cooke, D.A. Aitken, L. Florentin, J. Tolmie, Analysis of variable X–Y interchange in XX males, Amer. J. Hum. Gene. 37 (1985) A451.
[24] R.J. Garrison, W.B. Kannel, J.S. Stokes III, W.P. Castelli, Incidence and precursors of hypertension in young adults: the Framingham Offspring Study, Prev. Med. 16 (1987) 235.
[25] J. Staessen, R. Fagard, A. Amery, Obesity and hypertension, Acta Cardiol. Suppl. 29 (1988) 37–44.
[26] M.E. Brewster, M.S.M. Bartruff, W.F. Anderson, P.J. Druzgala, N. Bodor, E. Pop, Effect of molecular manipulation of the estrogenic activity of a brain-targeting estradiol chemical delivery system, J. Med. Chem. 37 (1994) 4237–4244.
[27] A. Nunez, J.M. Gray, G.N. Wade, Food intake and adipose tissue lipoprotein lipase activity after hypothalamic estradiol benzoate implants in rats, Physiol. Behav. 25 (1980) 595–598.

[28] K. Palmer, J.M. Gray, Central vs. peripheral effects of estrogen on food intake and lipoprotein lipase activity in ovariectomized rats, Physiol. Behav. 37 (1986) 187–189.
[29] M.E. Brewster, W.R. Anderson, E. Pop, Effect of sustained estradiol release in the intact male rat: correlation of estradiol serum levels with actions on body weight, serum testosterone and peripheral androgen-dependent tissues, Physiol. Behav. 61 (1997) 225–229.
[30] M. Urabe, G. Chetrite, J.R. Pasqualini, Transformation of estrone, estradiol, and estrone sulfate in uterine and vaginal isolated cells of fetal guinea pig. Effect of various antiestrogens in the conversion of estrone sulfate to estradiol, Steroids 58 (1993) 209–214.
[31] W.R. Anderson, J.W. Simpkins, M.E. Brewster, N. Bodor, Evidence for the reestablishment of copulatory behavior in castrate male rats with a brain enhanced estradiol chemical delivery system, Pharmacol. Biochem. Behav. 27 (1987) 265–271.
[32] M.E. Turner, M.L. Johnson, D.L. Ely, Separate sex-influenced and genetic components in spontaneously hypertensive rat hypertension, Hyper. 17 (1991) 1097–1103.
[33] F.W. Keeley, L.A. Bartoszewicz, Elastin in systemic and pulmonary hypertension, Ciba Found. Symposium 192 (1995) 259–273.
[34] M.F. Lyon, Gene action in the X-chromosome of the mouse (*Mus musculus*, L.), Nature 190 (1961) 372–373.
[35] S.M. Gartler, M. Rivest, Evidence for X-linkage of steroid sulfatase in the mouse: steroid sulfatase levels in oocytes of XX and XO mice, Gene 103 (1983) 137–141.
[36] E. Keitges, M. Rivest, M. Siniscalco, S.M. Gartler, X-linkage of steroid sulfatase in the mouse is evidence for a functional Y-linked allele, Nature 315 (1985) 226–227.
[37] M. Crocker, I. Craig, Variation in regulation of steroid sulfatase locus in mammals, Nature 303 (1983) 721–722.
[38] L.J. Shapiro, Steroid sulfatase deficiency and the genetics of the short arm of the human X chromosome, Advan. Hum. Gene. 14 (1985) 331–389.
[39] X.M. Li, E.S. Alperin, E. Salido, Y. Gong, P. Yen, L.J. Shapiro, Characterization of the promoter region of human steroid sulfatase: a gene which escapes X inactivation, Som. Cell. Mole. Gene. 22 (1996) 105–117.
[40] B. Robaire, I.L. Ewing, D.C. Irby, C. Deshardins, Interactions of testosterone and estradiol-17 on the reproductive tract in the male rat, Biol. Repro. 21 (1979) 455–463.
[41] N.M. Howarth, A. Purohit, M.J. Reed, B.V.L. Potter, Estrone sulfamates: potent inhibitors of estrone sulfatase with therapeutic potential, J. Med. Chem. 37 (1979) 219–221.
[42] T. Sugawara, M. Iwaki, S. Fujimoto, Diagnosis of a deletion of steroid sulfatase by polymerase chain reaction and high-performance liquid chromatography, Clin. Chem. Acta 263 (1997) 25–32.
[43] A.I. el Hag, Z.A. Karrar, Nutritional vitamin D deficiency rickets in Sudanese children, Ann. Trop. Pediat. 15 (1995) 69–76.