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HEPATIC CORTISOL SULFOTRANSFERASE ACTIVITY IN SEVERAL TYPES OF EXPERIMENTAL HYPERTENSION IN MALE RATS

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Abstract—The relationship between hepatic cortisol sulfotransferase activity (HCSA) and several types of hypertension was examined in male rats. It was found that development of Grollman hypertension was paralleled by increased HCSA. The maximum blood pressure differences observed between experimental animals and controls, in individual experiments, were 83 and 95 mm, accompanied by 138 and 124% HCSA increases. HCSA was also elevated significantly, compared to controls, in spontaneously hypertensive Okamoto rats (SHR) and in hypertension elicited in intact or Grollman operated rats by daily, intramuscular injection of 0.6 mg of FLHC (9α-fluoro-11β, 17α, 21-trihydroxy-pregn-4-ene-3,20-dione) for 30–45 days. HCSA increased 47 and 152% in SHR and in FLHC treated animals respectively. Blood pressures were elevated 40–60 mm in both cases. Grollman operated animals given FLHC exhibited higher blood pressures than did intact animals. Administration of cortisol (6 mg) and corticosterone (3 mg) also caused hypertension in intact rats. This was accompanied by elevated HCSA. Deoxycorticosterone (3 mg) caused hypertension only in animals restricted to drinking 1% saline. This hypertension was also accompanied by elevated HCSA. Conditions or steroid concentrations that did not result in hypertension did not elevate HCSA significantly. The increased HCSA in all the types of hypertension studied was largely due to STIII, the major glucocorticoid sulfotransferase of livers from male rats.

The adrenal cortex has been implicated in hypertension for many years. Its role has most often been attributed to the interaction of mineralocorticoids, renin and angiotensin. However, several mechanisms may be involved in adrenal cortical control of blood pressure. Mineralocorticoids, as deoxycorticosterone, require sodium replete diets for maximal hypertensive effects [1, 2] and their action appears to be dependent on plasma angiotensin II levels [3, 4]. In contrast, cortisol and other natural and synthetic glucocorticoids [5–8] cause hypertension which is unaffected by dietary sodium levels [5, 6] and may be accompanied by depressed renin and angiotensin levels [8].

The first evidence suggesting a relation between hypertension and a glucocorticoid metabolite came from studies by Kornel and coworkers [9, 10]. They reported that 17-hydroxycorticosteroid sulfate levels in blood and urine from human essential hypertensives were elevated in parallel with increased blood pressure. More recently [11] the Kornel group showed that after injection of tracer doses of [^{14}C]cortisol, glucocorticoid sulfates were among the metabolites present at significantly higher levels in hypertensives than in normotensives. One possible explanation for this elevation of glucocorticoid sulfates could be increased glucocorticoid sulfotransferase activity. This possibility was first examined by Turcotte and Silah [12] who linked elevated "corticosterone sulfotransferase" activity with Grollman hypertension in male rats.

The studies described in this manuscript demonstrate that increased glucocorticoid sulfation accompanies elevated blood pressure in several types of experimental hypertension in male rats. The glucocorticoid used as test substrate was cortisol. It was chosen because we have shown [13] that it is an excellent substrate for all three glucocorticoid sulfotransferases (STI, STII and STIII) of rat liver. Our studies also demonstrate that most of the observed elevation of the enzyme activity in the various types of hypertension tested is due to elevation of STIII, the major glucocorticoid sulfotransferase of livers from male rats.

MATERIALS AND METHODS

Animals and chemicals. Animals were purchased from Charles River Breeding Labs (Wilmington, MA) or bred in our laboratory from selected Charles River rats. They were fed Purina rat/mouse chow and water *ad lib.* Thirteen-week-old male CFSHR rats (Okamoto-derived spontaneously hypertensive rats, SHR) and normotensive CFN controls were purchased for studies of spontaneous hypertension. Male CDR Fisher rats, weighing 150–170 g were purchased for study of Grollman [14]. FLHC (9α-fluoro-11β, 17α, 21-trihydroxy-pregn-4-ene-3, 20-dione), and other steroid mediated hypertension. Tritiated cortisol, [1,2-^{3}H]cortisol (44 Ci/m-mole), was obtained from the New England Nuclear Corporation (Boston, MA). The steroid was checked for purity, periodically, as described earlier [15]. Non-radioactive steroids were obtained from Sigma (St. Louis, MO). All other supplies and chemicals were obtained from standard suppliers.

Blood pressure determination. Systolic blood pressures were obtained weekly and on the day of sacrifice, by the tail cuff method. Animals were anaesthesized lightly with ether before the measurements were made. The blood pressure module consisted of an electrosphygmograph coupler (No. 7211), a channel

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amplifier (No. 7000), a pneumatic pulse transducer and a beeper (BPR 4) purchased from Narco Biosystems (Houston, TX).

Enzyme assays. Suitably diluted enzyme samples were assayed as described earlier [13]. Assays were started by adding 0.20 ml of 1.2 mM PAPS (3'-phosphoadenosine-5'-phosphosulfate)* to 0.80 ml of a solution (pH 6.8) containing enzyme, 300 μmoles of KH_2PO_4, 5 μmoles of $MgCl_2$, 3 μmoles of mercaptoethanol and 0.040 μmoles of [^{3}H]cortisol (1.25 mCi/m-mole). Reaction mixtures were incubated for 0 and 60 min at 37.5° in a Dubnoff shaker. Then the reaction was terminated by immersion, for 2 min, in a boiling water bath and addition of 1 ml of distilled water. The diluted mixtures were cooled on ice and extracted 3 times with 5 ml portions of dichloromethane. The extracts were discarded and the aqueous residues transferred to scintillation vials. Ten ml of cabosil [16] and 4–5 drops of 1 M NaOH were added to each vial. Vials were shaken to ensure homogeneity and counted in an Intertechnique SL-30 liquid scintillation spectrometer. Suitable sets of quenched standards were utilized to convert cpm to dis/min. Enzyme activity is given as μg cortisol sulfated hr^{-1} ml of 50% cytosol^{-1}. Statistical significance was determined using Students *t*-test [17].

Production of hypertension. Renal hypertension was produced, in 150–205 g rats by the Grollman method [14]† First, the renal parenchyma was compressed by drawing a suture tightly around the pole and body of the left kidney. Seven to 10 days later, the right kidney was removed. Between 15 and 20 per cent of the animals died in the immediate postoperative period. The remainder were maintained until blood pressures were significantly higher than those in unoperated controls (2.5–6 months). Then both groups were sacrificed and hepatic cortisol sulfotransferase levels determined. For studies of spontaneous hypertension, 13-week-old SHR and CFN controls were monitored until maximum blood pressures were obtained in SHR (3–4 weeks). The animals were then sacrificed and cortisol sulfotransferase studies carried out. Studies of FLHC hypertension were carried out with intact or Grollman-operated rats. The method used was a modification of that described by Hepp *et al.* [8]. Animals were injected i.m. with 0.10 ml of sesame oil or sesame oil containing 0.60 mg of FLHC. Maximum blood pressure differences were obtained within 30–45 days. Both groups were then sacrificed and their hepatic cortisol sulfotransferase levels compared. Studies of the hypertensive effects of other steroid hormones were carried out similarly. Indicated amounts of cortisol, corticosterone and deoxycorticosterone were injected daily, i.m., in 0.10 ml of sesame oil. After blood pressure differences of 40–60 mm had developed between hypertensives and oil injected controls (or a maximum of 80 days) animals were sacrificed and cortisol sulfotransferase activity levels compared. In the case of deoxycorticosterone, hormone and oil injected groups were each divided into two subgroups which received water or 1% saline to drink.

Preparation of cytosols. Rats were sacrificed by decapitation. Livers were removed rapidly, trimmed, chilled and homogenized in one vol. of TSM [13], 0.05 M tris-0.25 M sucrose-0.003 M mercaptoethanol, pH 7.5. This and all further preparative steps were carried out at 0–4°. Homogenates were centrifuged at 35,000 *g* (Sorval RC-2B) for 30 min and the pellets discarded. The supernatants were recentrifuged at 105,000 *g* (Beckman L2-65B) for 60 min. The final supernatant, 50% cytosol, contains most of the cortisol sufotransferase activity.

Fractionation of cytoplasmic cortisol sulfotransferase activity. Cytosol samples, 2.5–3.0 ml, were loaded on 2 × 50 cm columns of DEAE Sephadex A-50. The columns were then eluted with linear gradients consisting of 300 ml each of TSM and TSM containing 0.30 M KCl. Three ml fractions were collected. The protein content of the eluates was determined from the absorbance at 280 nm. Enzyme assays were carried out on 0.5 ml aliquots of the column fractions, as already described. Recoveries from columns were 75–95% of the enzyme activity originally applied. Paired columns were always run. One contained cytosol from a control animal, the other contained cytosol from an experimental animal. The sulfotransferases eluting from the columns were called STII and STIII as described earlier [13].

RESULTS

Hepatic cortisol sulfotransferase activity and development of Grollman hypertension in male rats. We have already described the enzyme assay for tissue preparations from intact rats and identified the reaction product as cortisol-21-sulfate [13]. We have also found that of eight tissues studied, liver contained by far the most cortisol sulfotransferase activity‡. The properties of the enzyme system are similar in Grollman hypertensives (data not shown). Figure 1 shows that development of high blood pressure in Grollman operated male rats is paralleled by elevation of hepatic cortisol sulfotransferase activity. The two largest blood pressure increases, 83 and 95 mm respectively, occurred 6 months after Grollman surgery. They were accompanied by 138 and 124 per cent increases of the enzyme activity. The insert in the figure shows that there is a statistically significant difference in blood pressure and in cortisol sulfotransferase activity between operated and intact rats. No significant difference in growth or liver weight was observed between the two groups. The Grollman technique was a poor model to use to study hypertension, despite the indicated relation between cortisol sulfotransferase activity and elevation of the blood pressure. Only 40–50% of the operated animals survived for extended periods of time. In addition (see Fig. 1) few animals developed blood pressures more than 40 mm above controls, and this required up to 6 months. Accordingly, we next attempted to find a better system to study and to extend the correlation between cortisol sulfotransferase activity and hypertension.

Study of spontaneous hypertension and FLHC hypertension. First, we tested the relation between cortisol

* PAPS was prepared by a rapid modification of the enzymatic method of the Lipmann group [19]. A manuscript is presently in preparation.

† The hypertension produced by this method is termed Grollman hypertension.

‡ Presently unpublished studies from our laboratory.

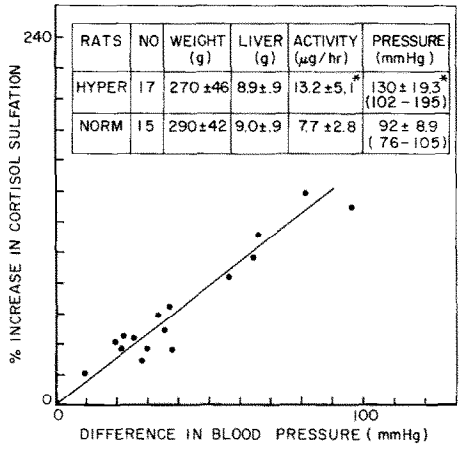

Fig. 1. The relation between cortisol sulfotransferase activity and blood pressure in Grollman operated male rats. Grollman operated rats and intact controls were sacrificed and liver cytosols were prepared (see Methods). Cortisol sulfotransferase activities were determined and the per cent increase of cortisol sulfation in hypertensives was plotted against the difference in blood pressure between control and hypertensive animals. Each point represents a single experiment. The insert summarizes the blood pressure, enzyme activity, liver weight and body weight data for the two groups of animals. The data are given as the mean ± the standard deviation. Cortisol sulfotransferase activity is given as μg cortisol sulfated hr^{-1} ml 50% $cytosol^{-1}$. The asterisks indicate statistically significant differences between control and experimental groups ($P < 0.01$).

sulfation and spontaneous hypertension in Okamoto derived rats (SHR) purchased from Charles River Breeding Labs. Table 1a shows that SHR exhibit an average 53 mm blood pressure increase and an average 47 per cent increase of hepatic cortisol sulfotransferase activity, compared to CFN controls. Both changes are statistically significant. We also examined FLHC hypertension in male CDR Fisher rats injected daily for 30–45 days with 0.6 mg of FLHC. Table 1b shows that FLHC injected rats developed statistically significant increases of blood pressure (average 46 mm) and of cortisol sulfotransferase activity (average 152 per cent), as compared to controls. Thus it appears that there is a relation between cortisol sulfation and blood pressure in SHR and in FLHC treated rats, as well as in Grollman hypertension.

We also tested the effect of administering FLHC to Grollman operated rats, in the hope of developing an even faster more extensive hypertensive response. These studies (Table 2) suggest that the hypertensive effect of FLHC may be more extensive in Grollman operated animals than in intact animals (78 ± 7 mm and 53 ± 12 mm respectively), although the final levels of cortisol sulfotransferase activity (26.5 ± 5.4 and 23.5 ± 4.3 μg cortisol sulfated hr^{-1} ml 50% $cytosol^{-1}$) did not differ significantly.

Fractionation of individual cortisol sulfotransferases of cytosols from normal and hypertensive rats. These studies were carried out to determine the enzymatic basis for increased cortisol sulfotransferase activity in hypertensive rats. Mixing cytosol samples from control animals and the various experimental groups gave simple additive enzyme activity. This suggested that differences in enzyme activity were not due to activators or inhibitors of sulfotransferase activity or changes in coenzyme utilization. Cytosol samples were fractionated on DEAE Sephadex A-50 columns. Figure 2 compares fractionation of cytosol samples from intact rats given oil or 0.6 mg of FLHC for 40 days. Their blood pressures were 90 mm and 165 mm, respectively. As indicated, most of the increased enzyme activity appears to be due to elevated STIII levels. Similar chromatograms were obtained with cytosol samples from Grollman rats and SHR (not shown).

The effects of other steroid hormones on cortisol sulfotransferase activity and blood pressure in intact rats. Although FLHC is a potent mineralocorticoid as well as a glucocorticoid, Hepp *et al.* [8] showed that it elevates the blood pressure as a glucocorticoid. We next examined the effects of two natural glucocorticoids (cortisol and corticosterone) and of the mineralocorticoid deoxycorticosterone on blood pressure and hepatic cortisol sulfotransferase activity in intact rats. These studies were carried out to determine whether the hypertensive response, if present, was separable from the elevation of the enzyme activity

Table 1. Blood pressure and hepatic cortisol sulfotransferase activity in spontaneously hypertensive rats (SHR) and FLHC treated rats

Experiment		No.	Blood pressure (mm Hg)	Sulfotransferase activity*	Average % increase†
(a)	CFN	9	97 ± 6	4.7 ± 1.0	
	SHR	9	151 ± 4‡	6.9 ± 1.2‡	47
(b)	Oil	9	100 ± 7	9.5 ± 1.4	
	FLHC	9	146 ± 9‡	23.9 ± 3.4‡	152

16–17-week-old SHR and CFN rats were used for study of spontaneous hypertension. For studies of FLHC hypertension, CDR Fisher rats (180–200 g) were injected daily, i.m., for 30–45 days with 0.1 ml of sesame oil or oil containing 0.6 mg of FLHC. Blood pressures were monitored until differences between experimental and control rats were 40–60 mm. Then animals were sacrificed and hepatic cortisol sulfotransferase levels were determined (see Methods).

* Cortisol sulfotransferase activity is given as μg cortisol sulfated hr^{-1} ml 50% $cytosol^{-1}$ ± the standard deviation.

† The average % increase in cortisol sulfotransferase activity is given by

$$[\text{Experimental Activity}/\text{Control Activity} \times 100] - 100.$$

‡ Statistically significant difference between experimental animals and controls ($P < 0.01$).

Table 2. The effect of FLHC on blood pressure and cortisol sulfotransferase activity of livers from intact and Grollman operated rats

Experiment	No.	Blood pressure (mm Hg)	Sulfotransferase activity*	Average % increase†
Intact-Oil	4	97 ± 5	9.9 ± 1.8	
Intact-FLHC	4	150 ± 11‡	23.5 ± 4.3‡	137
Grollman-Oil	5	122 ± 4	13.1 ± 4.3	
Grollman-FLHC	3	200 ± 10‡	26.5 ± 5.4‡	100

One week after completion of Grollman surgery, operated rats were divided into two groups. These groups were given 0.1 ml of sesame oil or oil containing 0.6 mg of FLHC daily, i.m., for 34 45 days. Two groups of intact animals from the same shipment were treated similarly. Blood pressures of all rats were taken weekly and on the day of sacrifice. After sacrifice hepatic cortisol sulfotransferase levels were determined as described in Methods.

* Cortisol sulfotransferase activity is given as μg cortisol sulfated hr^{-1} ml 50% cytosol^{-1} ± the standard deviation.

† The average % increase in cortisol sulfotransferase activity is given by [Experimental Activity/Control Activity × 100] −100.

‡ Statistically significant difference between experimental animals and controls ($P < 0.01$).

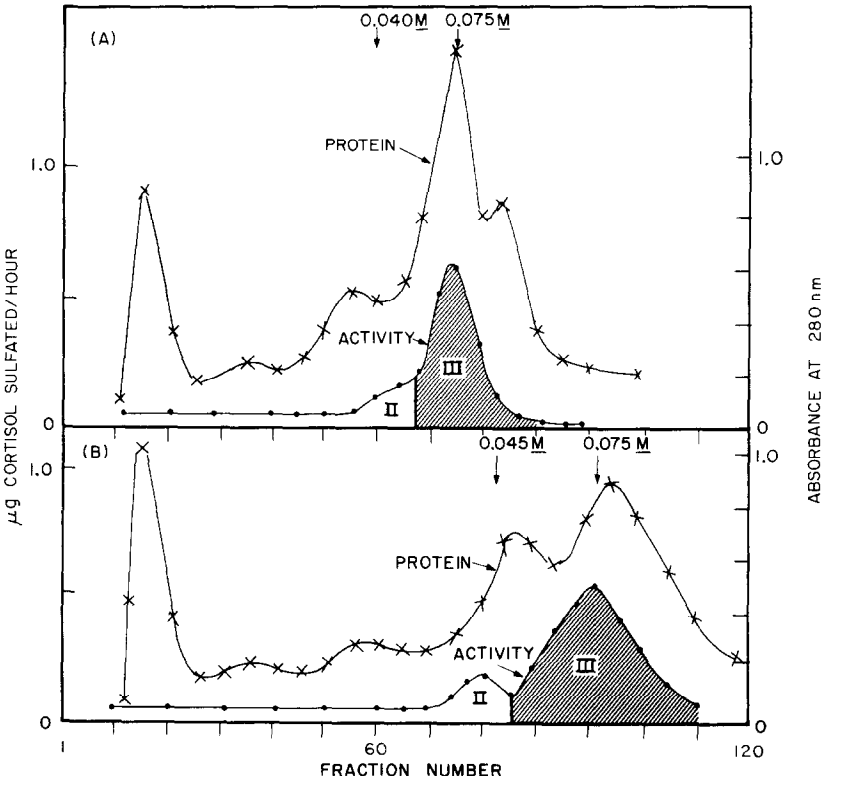

Fig. 2. DEAE Sephadex A-50 chromatography of cytoplasmic cortisol sulfotransferase activity from livers of intact and FLHC treated male rats. The figure depicts a representative paired experiment where cytosol samples from (A) oil and (B) FLHC treated animals described in Table 2 were used. Blood pressures were 90 and 165 mm, respectively. Two 2 × 50 cm columns of DEAE Sephadex A-50 were prepared. Each column was loaded with 2.5 ml of cytosol. They were then eluted with linear gradients consisting of 300 ml each of 0.05 M tris-0.25 M sucrose-0.003 M mercaptoethanol, pH 7.5 (TSM) and TSM containing 0.30 M KCl. Three ml fractions were collected. Protein was estimated from the absorbance at 280 nm. Aliquots, 0.50 ml, of indicated fractions were assayed for cortisol sulfotransferase activity (see Methods). Enzyme activity is expressed as μg cortisol sulfated hr^{-1} aliquot^{-1}. Roman numerals represent sulfotransferases II and III (STII and STIII). The STIII peaks are shaded for better comparison. Molar concentrations represent peak KCl concentrations for elution of the individual enzymes. Enzyme recoveries were 75 per cent. The data were from one of four very similar experiments.

Table 3. Blood pressure and hepatic cortisol sulfotransferase activity in rats given cortisol, corticosterone, deoxycorticosterone or fludrocortisol

Experiment		Days of injection	Dose (mg)	Blood pressure (mm Hg)	Sulfotransferase activity*
(a)	Oil (5)	33–40	—	103 ± 8	8.9 ± 1.5
	FLHC (5)	33–40	0.2	112 ± 8	11.6 ± 4.0
(b)	Oil (8)	35–53	—	101 ± 9	17.7 ± 6.6
	HC (5)	35–53	6.0	148 ± 12‡	37.1 ± 5.3‡
	DOC (5)	35–53	3.0	112 ± 11	19.9 ± 6.3
	COR (4)	35–53	3.0	129 ± 5‡	55.9 ± 15‡
(c)	Oil (5)†	64–80	—	108 ± 10	15.1 ± 1.1
	DOC (4)	64–80	3.0	118 ± 15	16.9 ± 1.9
	DOC′ (4)	64–80	3.0	142 ± 10‡	39.5 ± 4.6‡

Rats were injected daily, i.m., as indicated with cortisol (HC), corticosterone (COR), deoxycorticosterone (DOC) and fludrocortisol (FLHC). Blood pressures were monitored until experimental values stabilized or were 40–50 mm above controls. Animals were sacrificed and hepatic cortisol sulfotransferase levels determined (see Methods). Numbers in parentheses are numbers of individual determinations.

* Cortisol sulfotransferase activity is given as μg cortisol sulfated hr^{-1} ml 50% $cytosol^{-1}$ ± the standard deviation.

† DOC and DOC′ rats were given H_2O and 1% NaCl to drink, respectively. Oil data represents pooled values from controls given NaCl and water.

‡ Statistically significant difference between experimental animals and controls ($P < 0.01$).

and whether the cortisol sulfotransferase response could be used as additional differentiation between glucocorticoid and mineralocorticoid hypertension.

The hypertensive effects of the hormones tested (Table 3) were accompanied by increased cortisol sulfotransferase activity. Doses that did not elevate the enzyme activity did not increase the blood pressure significantly. For example, the results with 0.2 mg of FLHC and 3.0 mg of DOC are shown in Table 3a and 3b. All of the test compounds except corticosterone caused extensive weight loss compared to controls. Liver weight did not decrease markedly. Three mg doses of deoxycorticosterone caused hypertension only in the DOC′ group, which were maintained on 1% saline (Table 3c). Cortisol sulfotransferase activity was elevated only in the hypertensive group.

Fractionation of cytoplasmic cortisol sulfotransferase activity on DEAE Sephadex A-50 columns showed that compared to controls (Fig. 3A), increased enzyme activity in response to glucocorticoids was due mostly to elevation of STIII (Figs. 3B and 3C). Fractionation of cytosol from deoxycorticosterone treated hypertensives (Fig. 4B) showed a skewed enzyme peak which suggested that STII is present at a much higher relative concentration than in controls (Fig. 4A). Although clear resolution of STII and STIII was not obtained, STIII appears to be the major cortisol sulfotransferase of livers from deoxycorticosterone treated hypertensives.

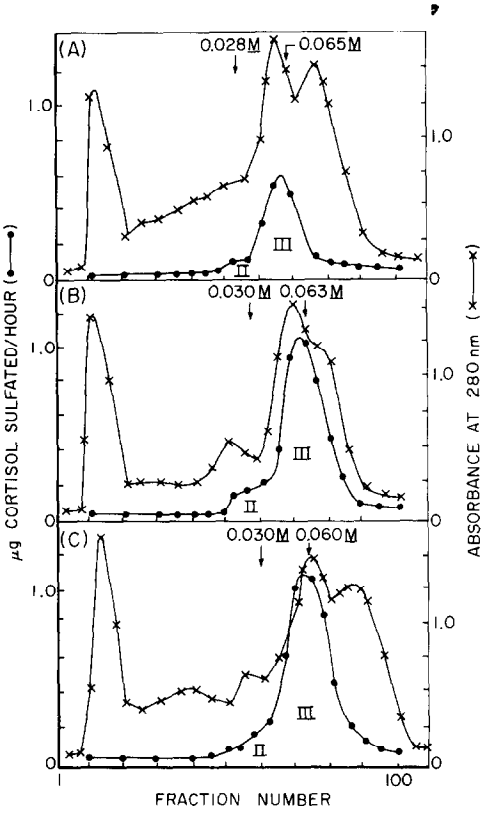

Fig. 3. Fractionation of cytoplasmic cortisol sulfotransferase activity from livers of intact rats given oil, corticosterone or cortisol. The data represent one of three very similar experiments. Cytosols were from animals described in Table 3b. The rats used in this experiment were injected, daily, with oil (A), 6.0 mg of cortisol (B), and 3.0 mg of corticosterone (C) for 42 days. Their blood pressures were 99, 152, and 136 mm respectively. Cytosols were prepared and treated as in Fig. 2. All symbols are described in Fig. 2.

DISCUSSION

Much remains to be done before the role of glucocorticoid sulfates in hypertension can be ascertained. However, we feel that the studies reported herein suggest the potential importance of these compounds in the disease. Our studies of the relation between cortisol sulfation and Grollman hypertension in male rats confirm and extend an earlier report by Turcotte and Silah [12] that glucocorticoid sulfation is elevated in

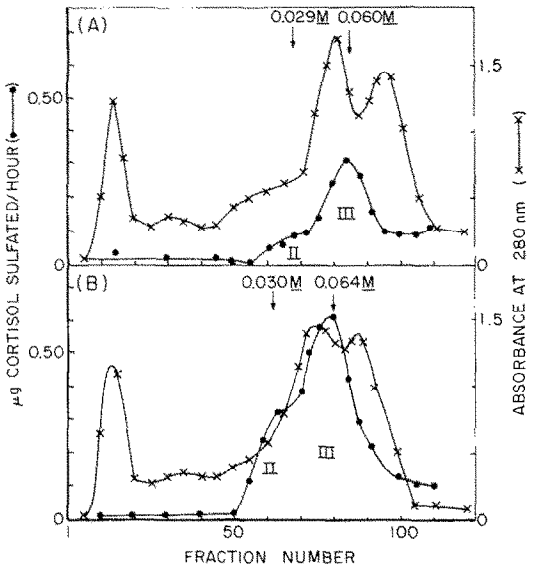

Fig. 4. Fractionation of cytoplasmic cortisol sulfotransferase activity from oil and deoxycorticosterone treated rats. Cytosols were from (A) oil and (B) deoxycorticosterone treated animals (DOC' group) from Table 3c. Conditions are as described in Fig. 2. The animals were injected for 69 days. Blood pressures were 97 and 153 mm respectively. This was one of three experiments.

this experimental hypertension. In addition, we found that the elevation of the enzyme activity parallels the development of Grollman hypertension (Fig. 1). We have also demonstrated increased hepatic cortisol sulfation in male SHR (Table 1); in FLHC hypertension elicited in intact or Grollman operated males (Tables 1 and 2); and in hypertension elicited by natural glucocorticoids (Table 3). These data suggest that sulfated glucocorticoid metabolites could be involved in control of the blood pressure. This possibility relates well with observations from the Kornel group [9–11] that glucocorticoid sulfates are elevated significantly in blood and urine from human essential hypertensives.

Kornel *et al.* [11] suggest that elevated hepatic glucocorticoid sulfotransferase activity is a secondary effect of hypertension, due to increased available glucocorticoid levels. They propose that the increased hormone levels are a consequence of enhanced hepatic perfusion pressure and other "primary abnormalities" of glucocorticoid metabolism. We have found (Table 3) that the hypertensive effects of the steroid hormones we tested were not separable from elevation of cortisol sulfotransferase activity. In addition, 3 mg doses of deoxycorticosterone were effective inducers of cortisol sulfotransferase activity only in saline-loaded rats, which also exhibited a hypertensive response to the mineralocorticoid. These studies suggest the possibility that the relation between the steroid sulfotransferases and hypertension may be more than a side effect of the disease.

We have already described the steroid sulfotransferases of rat liver [13], STI, STII and STIII, which sulfate cortisol. Two of these are present in livers from males. They have very different substrate preferences [13]. The minor cortisol sulfotransferase of livers from males (STII) sulfates dehydroepiandrosterone much more extensively than cortisol. The major enzyme (STIII) has a much greater preference for the glucocorticoid. In this manuscript, we have shown that most of the elevated cortisol sulfotransferase activity in all the types of hypertension studied appears to be due to STIII. Such elevation of STIII might increase glucocorticoid sulfation markedly at the expense of sulfation of other types of steroid hormones present at physiological concentrations. Such elevation of glucocorticoid sulfation could be involved in the control of blood pressure. Suggestion that STIII elevation could be involved in other types of hypertension comes from the studies of Nowaczynski and coworkers [18], who reported diminished excretion of dehydroepiandrosterone sulfate in human essential hypertensives. We also wonder if the much slower development of hypertension we observed in deoxycorticosterone treated rats, compared to glucocorticoid treated animals (Table 3), could be related to the higher ratio of STII activity to STIII activity observed in the mineralocorticoid treated rats (compare Figs. 3 and 4).

We are presently carrying out additional studies to clarify the relation between glucocorticoid sulfates and hypertension and testing to determine whether there are additional similarities between mineralocorticoid and glucocorticoid hypertensions.

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