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ENZYMATIC SULFATION OF STEROIDS—X. PHARMACOLOGICAL PROGESTERONE EFFECTS ON RAT LIVER GLUCOCORTICOID SULFOTRANSFERASES AND BRIEF STUDY OF SHORT-TERM EFFECTS OF HORMONAL STEROIDS ON THE ENZYMES

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Abstract—Hepatic cortisol sulfotransferase activity (HCSA) was elevated 126, 258 or 342 per cent (average values) in intact male rats given daily doses of 2.5, 6.0 or 12 mg progesterone (P) i.m. for 30–56 days. Examination of the effects of 12 mg doses given for intervals between 1 week and 4 months showed that HCSA nearly doubled in 7–9 days and that maximum HCSA elevations (averaging 340 per cent) occurred after 30–56 days of treatment. The elevation of P-mediated HCSA in males increased after either adrenalectomy or castration. In females, 12 mg P doses for 28–38 days did not affect HCSA. After 102–121 days of treatment, however, their HCSA decreased to 70–80 per cent of control values. Individual glucocorticoid sulfotransferase (STI, STII, and STIII) concentrations were similar in untreated and P-treated females. In males, 6.0 mg P doses elevated all three enzyme concentrations. STIII was the major enzyme present. STI and STII concentrations increased more modestly. P doses of 12 mg caused little additional STIII production, but increased the sum of STI and STII activities considerably. P elevated STI and STII higher in male castrates than in intact males. P-treated adrenalectomized and intact males, however, exhibited similar glucocorticoid sulfotransferase profiles. Thus, it appeared that P, or a closely related metabolite, mediated the observed HCSA elevation. Short-term effects of P and other hormones on STI, STII, and STIII production and possible roles for P in endocrine effects on the enzymes are discussed.

We reported previously that much of the endocrine control of the production of rat liver sulfotransferases I, II, and III (STI, STII, and STIII)—the enzymes that sulfate glucocorticoids [1]— is localized in the gonads [2], the adrenals [3], and the pituitary [4]. Several facets of the control process however, were not clear on this basis. For example, although estrogen treatment elevated the activities of the sulfotransferases in intact and castrated males to levels found in females [2], ovariectomy had only a slight effect on the enzymes in females [1, 2]. This suggested that another control factor exists.

Other studies support potential roles for glucocorticoid sulfation in hypertension [5, 6], ageing [7], corticosteroid metabloism [8, 9], and hepatic enzyme induction [10–12]. We chose, therefore, to examine more extensively the control of glucocorticoid sulfotransferase production. Progesterone was used in this study because of its central role in steroid hormone biogenesis, its actions *in vivo* [13] and *in vitro* [14] as an antiglucocorticoid, and its role as a potent inhibitor of purified STI [15] and STIII [16].

This paper describes the short-term and long-term effects of pharmacological doses of progesterone on sulfotransferases in intact, castrated, and adrenalectomized male rats, and in intact females. The long-term studies indicated that gonadal and adrenal hormones produced from administered progestin did not play a role in its effects and support a direct role for progesterone, or a closely related metabolite, in the control of sulfotransferase production. The short-term studies show that effects of progesterone and other hormonal steroids on the sulfotransferases occur much more rapidly than previously believed.

MATERIALS AND METHODS

Animals and chemicals. Male and female CDR Fisher rats weighing 101–150 g were purchased from Charles River Laboratories (Wilmington, MA). In most cases they were allowed to recuperate for 1–2 days after arrival, before surgery was carried out or hormone injections were begun with intact animals. In some cases, however, studies were carried out with animals that weighed up to 260 g initially. These animals had been maintained in the laboratory until the desired weights were attained. Adrenalectomy and gonadectomy are described elsewhere [2, 3]. All rats that had not been adrenalectomized were given tap water to drink. Adrenalectomized animals were maintained on 1% saline. All rats were fed Purina chow. Food and water (or 1% saline) were given *ad lib.* For hormone studies, indicated doses of progesterone and other hormones were injected i.m. in 0.20 ml of sesame oil, alternating between right and left hind legs. Controls were not injected, for we had previously shown [3] that the vehicle does not significantly affect hepatic cortisol sulfotransfer-

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ase activity. The onset and duration of each injection series are given in the text. [1,2-^{3}H]Cortisol (49 Ci/mmole) was purchased from the New England Nuclear Corp. (Boston, MA). Nonradioactive steroids were from the Sigma Chemical Co. (St. Louis, MO). The purity of [^{3}H] cortisol was tested periodically, as already described [17]. All other chemicals and supplies came from standard suppliers.

Cytosol preparation. Single rats were decapitated; the livers were rapidly removed, trimmed, chilled, and homogenized in 1 vol. of ice-cold 50 mM Tris–250 mM sucrose–3.0 mM mercaptoethanol, pH 7.5 (TSM) [1]. This and all other preparative steps were carried out between 0 and 4°. The homogenates were centrifuged for 30 min at 35,000 *g* (Sorval RC-2B) and the pellets were discarded. The supernatant fractions were recentrifuged at 105,000 *g* for 60 min (Beckman L5-65). The final supernatant fraction, cytosol, contained 80–90 per cent of the hepatic cortisol sulfotransferase activity.

Enzyme assays and protein determinations. Suitably diluted enzyme samples were assayed at 37.5° in 1 ml reaction mixtures (pH 6.8) as described elsewhere [3]. Reaction mixtures were incubated for 0 to 60 min. Then the reaction was terminated by a 2-min immersion in boiling water and addition of 1 ml of deionized water. Diluted reaction mixtures were extracted with CH_2Cl_2 as described earlier [3]. The final aqueous residues, which continued the reaction product, cortisol-21-sulfate [1], were then transferred to scintillation vials and counted (see Ref. 3). The cortisol sulfotransferase activity from radioactivity measurements is given as nmoles steroid sulfated per hour. Statistical significance was determined by Student's *t*-test [18]. The protein content of cytosols was not determined for all test groups, because we had not observed significant variation among rats of different sex, age, and endocrine status [3]. This decision was supported by comparison of intact controls (N = 8); intact rats given 2.5 mg (N = 4), 6.0 mg (N = 4), or 12 mg (N = 8) of progesterone; uninjected castrates (N = 4); and castrates given 12 mg of progesterone (N = 4). These male rats yielded liver cytosols that contained 40.4 ± 4.6, 39.0 ± 1.3, 38.3 ± 1.7, 38.5 ± 2.0, 40.3 ± 2.2, and 40.3 ± 3.1 mg protein/ml, determined by the method of Bucher [19], which measures the turbidity of CCl_3COOH precipitated proteins.

Fractionation of cytoplasmic sulfotransferase activity. Aliquots (2.5 to 3.0 ml) of liver cytosol from control or experimental animals were chromatographed on 2 × 50 cm columns of DEAE Sephadex A-50. Columns were eluted with linear gradients consisting of 300 ml each of TSM and TSM–300 mM KCl. The effluent fractions (3 ml) were collected and assayed for cortisol sulfotransferase activity as described above. The recoveries of applied enzyme activity were 78–91 per cent. Salt gradients were measured as described elsewhere [2]. The protein in each enzyme fraction was estimated from the absorbance at 280 nm.

RESULTS

Effects of progesterone on hepatic cortisol sulfotransferase activity and glucocorticoid sulfotransferases in male rats. Daily 2.5 mg doses of progesterone (Table 1) elevated the hepatic cortisol sulfotransferase activity in male rats 110–150 per cent after 30–56 days of hormone administration. Increasing the daily dose of the progestin to 6.0 mg increased the enzyme activity 230–291 per cent, doubling the effect. Doubling this dose resulted in a much smaller additional increase of enzyme activity (to 305–370 per cent). This suggested that the response of the enzyme activity to 12-mg daily doses of progesterone might have been the maximum effect possible. It was not possible, however, to test for an absolute maximum, because rats given 20-mg doses died within 6–9 days.

All the effects of progesterone described above (Table 1) were similar whether the enzyme activity was expressed per g liver or 100 g body weight.

Table 1. Effect of progesterone dose on hepatic cortisol sulfotransferase (CS) activity in male rats*

Hormone dose (mg)	No. of experiments	Body wt (g)	Liver wt (g)	Hepatic CS activity per: g liver or ml cytosol†	Hepatic CS activity per: 100 g body wt
None	14	299 ± 55	10.4 ± 1.2	31.8 ± 12	110 ± 39
1.0	4	243 ± 26	8.93 ± .64	32.5 ± 5.5 (+ 3.00%)	121 ± 26 (+ 10.0%)
2.5	9	246 ± 18	8.76 ± .74	72.0 ± 34‡ (+ 126%)	249 ± 110‡ (+ 126%)
6.0	13	270 ± 45	9.18 ± 1.1	114 ± 39‡ (+ 258%)	401 ± 120‡ (+ 264%)
12	9	268 ± 30	9.10 ± 1.2	141 ± 28‡ (+ 342%)	468 ± 67‡ (+ 325%)

* Rats were injected with progesterone daily, i.m., for 30–56 days or not treated. Initial body weights were 101–130 g or 220–260 g. After killing the animals, liver cytosols were prepared and cortisol sulfotransferase activity was assayed with three different amounts of cytosol (see Materials and Methods). Mean cortisol sulfotransferase activity ± S.D. is given as nmoles cortisol sulfated/hr. The percentages in parentheses are the mean increases of CS activity observed compared to controls.

† Each g of liver yielded 1 ml of 50% cytosol.

‡ Significantly different from control ($P < 0.01$).

Table 2. Time course of the response of the hepatic cortisol sulfotransferase (CS) activity in male rats to 12 mg daily doses of progesterone

Days injected	No. of experiments	Body wt (g)	Liver wt (g)	Hepatic CS activity per: g liver or ml cytosol†	Hepatic CS activity per: 100 g body wt
None	5	231 ± 19	9.68 ± 0.89	32.6 ± 5.3	135 ± 19
7–9	5	224 ± 24	9.45 ± 0.66	59.5 ± 7.5‡ (+ 82.5%)	245 ± 14‡ (+ 81.5%)
None	5	336 ± 45	11.7 ± 1.4	42.7 ± 12	151 ± 38
14–16	5	276 ± 31	9.87 ± 5.5	107 ± 28‡ (+ 151%)	384 ± 97‡ (+ 154%)
None	14	299 ± 55	10.5 ± 1.2	31.8 ± 12	110 ± 39
30–56	9	268 ± 30	9.10 ± 1.2	141 ± 28‡ (+ 342%)	468 ± 67‡ (+325%)
None	4	342 ± 40	10.5 ± 1.3	24.3 ± 10	73.0 ± 29
101–120	4	343 ± 11	9.98 ± 1.0	104 ± 27‡ (+ 330%)	298 ± 64‡ (+ 308%)

* Rats weighing 180–230 g were divided into two groups. The first group was injected daily, i.m., with 12 mg progesterone for 7–9, 14–16, or 101–120 days. The second group was uninjected controls. The data for 30–56 day animals is from Table 1. At the times indicated, injected and control rats were killed and used to prepare liver cytosol; the hepatic CS activity was determined as described in Table 1. Conditions are described in Table 1. Mean cortisol sulfotransferase activity ± S.D. is given as nmoles cortisol sulfated/hr. The percentages in parentheses are the mean increases of CS activity observed compared to controls.

† Each g of liver yielded 1 ml of 50% cytosol.

‡ Significantly different from control ($P < 0.01$).

Furthermore, the dose response was similar whether the initial weights of the rats used were 101–130 g or 220–260 g. In fact, Table 1 contains data from approximately equal numbers of males in the two weight ranges.

Study of the effect of varying the injection period from 1 week to 101–120 days (Table 2) shows that the hepatic cortisol sulfotransferase activity nearly doubled after seven to eight daily 12-mg doses of progestin. The maximum increase of the enzyme activity (305–370 per cent) was observed with the 30–56 day group. No significant additional increase of the enzyme activity was obtained by extending the injection period to 101–120 days. It was also found that, when cytosol samples from controls and the various progesterone-treated groups were mixed and assayed (not shown), simple additive results were obtained. This indicates that neither cytosol contained activators or inhibitors of the sulfotransferase activity.

DEAE Sephadex A-50 chromatography of cytosol from progesterone-treated males showed that much of the cortisol sulfotransferase activity was due to STIII. In fact, short-term (1-week) studies yielded cytosol in which most of the hepatic enzyme activity was due to STIII (not shown). Increased amounts of STI and STII, however, were produced in rats treated with the progestin for 2 weeks, 30–56 days, and 101–120 days. Comparison of data from a male rat given, 6.0 mg of progesterone daily for 35 days with that from an untreated control is made in Fig. 1. In rats given 12-mg doses (as in Fig. 2A), the sum of the STI and the STII activities recovered was always greater (45.2 ± 7.4 per cent, N = 5) than that (28.1 ± 4.3 per cent, N = 7) in rats given 6.0-mg doses (as in Fig. 1B). Because the chromatograms did not resolve STI and STII well, the effects on the individual enzymes are not clear. The weights of the rats used (101–130 g or 220–260 g) for 30–56 or 101–120 day injection periods did not, apparently, affect the relative amounts of the sulfotransferases produced in response to 12-mg daily doses of the hormone.

Effect of progesterone on hepatic cortisol sulfotransferase activity and glucocorticoid sulfotransferases in female rats. Female rats were given daily doses of progesterone (12 mg) for 28–38 or 102–121 days. No significant change in the heptatic cortisol sulfotransferase activity (Table 3A) or in the "female" glucocorticoid sulfotransferase profile (large, roughly equal amounts of STI, STII and STIII) [1] on DEAE Sephadex A-50 chromatograms (not shown) was observed in the 28–38 day experiments. In the 102–121 day experiments, a statistically significant 20–30 per cent decrease in the enzyme activity compared to controls (Table 3B) was observed. Fractionation of the enzyme activity (not shown) from this group on DEAE Sephadex A-50 columns gave glucocorticoid sulfotransferase profiles similar to those of controls. The chromatograms, however, contained less of each enzyme than those from controls (three experiments). This suggests that prolonged exposure to progesterone decreased the tissue concentrations of all three sulfotransferases similarly.

Effect of castration or adrenalectomy on progesterone-mediated elevation of hepatic cortisol sulfotransferase activity and glucocorticoid sulfotransferases in male rats. The result of castration (Table 4A) was as we described earlier [2]. The effect of repeated daily 12-mg doses of progesterone on the enzyme activity in castrates was similar to that in intact males,

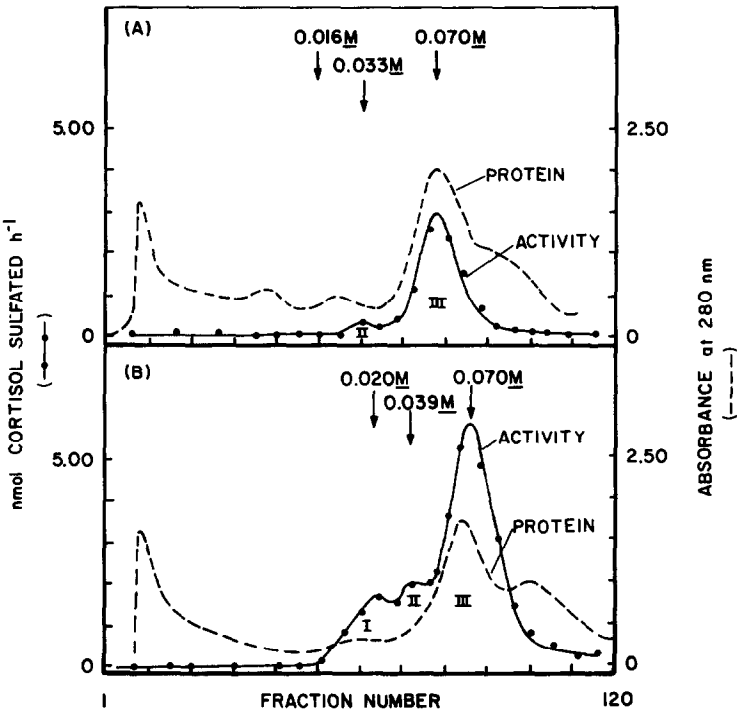

Fig. 1. Fractionation of glucocorticoid sulfotransferases of cytosol from untreated and progesterone-treated rats. Samples of (3 ml) cytosol from an untreated male (A) and a male injected daily, i.m. with 6.0 mg of progesterone for 35 days (B) were loaded on 2 × 50 cm columns of DEAE Sephadex A-50. Each column was eluted with a linear gradient consisting of 300 ml each of 50 mM Tris–250 mM sucrose–3.0 mM mercaptoethanol, pH 7.5 (TSM), and TSM–300 mM KCl. Elution was carried out at 60 ml/hr; 3-ml fractions were collected. Aliquots (0.50 ml) of indicated fractions were assayed for cortisol sulfotransferase activity (see Materials and Methods). Protein was estimated from 280 nm absorbance. The enzyme activity is given as nmoles cortisol sulfated per hr per ml enzyme fraction. Roman numerals represent STI, STII, and STIII [1]. Molar concentrations shown were the KCl concentrations [2] at which enzyme-peak tubes eluted. Recovery of applied enzyme activity was 85 and 86 per cent respectively.

Cytosols came from rats described in Table 1A. This was one of six similar experiments.

but more profound. DEAE Sephadex A-50 chromatography of cytosols (Fig. 2) show that the sum of the STI and STII activities (56 ± 4.1 per cent of recovered activity, N = 3) in progesterone-treated castrates was greater than that (39 ± 4.3 per cent, N = 3) in similarly treated intact males. Thus, it appears possible that the difference between the cortisol sulfotransferase activities of the two hormone-treated groups might be due to STI and STII elevation. The data also suggest that testicular secretions were not involved in the effect of progesterone on the sulfotransferases.

Table 3. Effect of progesterone administration on hepatic cortisol sulfotransferase (CS) activity in female rats*

Treatment	No. of experiments	Body wt (g)	Liver wt (g)	Hepatic CS activity per: g liver or ml cytosol†	Hepatic CS activity per: 100 g body wt
(A) None	4	180 ± 8.0	6.5 ± 1.1	438 ± 119	1,550 ± 390
Progesterone	5	195 ± 10	6.9 ± 1.3	469 ± 112 (+ 7.0%)	1,530 ± 291 (− 1.6%)
(B) None	5	194 ± 14	5.5 ± .32	445 ± 23	1,244 ± 78.5
Progesterone	5	213 ± 20	6.2 ± .81	337 ± 41‡ (− 24%)	987 ± 95.2‡ (− 24%)

* Rats were injected daily, i.m., with 12 mg progesterone or were untreated. Their initial weights were 140–160 g. Progesterone treatment was continued for (A) 28–38 days or (B) 102–121 days. Animals were then killed and hepatic CS activity was determined. Methodologic details are described in Table 1. Mean cortisol sulfotransferase activity ± S.D. is given as nmoles cortisol sulfated/hr. The percentages in parentheses are the mean changes of CS activity observed compared to controls.

† Each g of liver yielded 1 ml of 50% cytosol.

‡ Significantly different from the control ($P < 0.01$).

Table 4. Effect of castration or adrenalectomy on elevation by progesterone of hepatic cortisol sulfotransferase (CS) activity in male rats*

Treatment	No. of experiments	Body wt (g)	Liver wt (g)	Hepatic CS activity per: g liver or ml cytosol†	Hepatic CS activity per: 100 g body wt
(A) None	5	265 ± 23	9.57 ± 1.8	26.2 ± 6.2	93.1 ± 14
Progesterone (12 mg)	5	246 ± 25	8.94 ± 2.0	128 ± 38‡ (+ 378%)	522 ± 150‡ (+ 460%)
Castrated	5	255 ± 20	8.41 ± 2.1	86.8 ± 11‡ (+ 230%)	291 ± 45‡ (+ 212%)
Castrated + progesterone (12 mg)	5	249 ± 19	8.50 ± 19	159 ± 25‡ (+ 506%)	580 ± 91‡ (+ 522%)
(B) Adexed	4	193 ± 15	7.03 ± 0.20	12.4 ± 6.8	44.7 ± 23
Adexed + progesterone (12 mg)	4	235 ± 15	9.15 ± 0.61	121 ± 12¶ (+ 876%)	471 ± 29¶ (+ 975%)

* In (A), intact males or males castrated at 130–150 g were used. All were from a group born on the same day. Daily i.m., injections of 12 mg progesterone began 7 days after surgery and continued for 32–44 days; controls were not injected. In (B), all rats were adrenalectomized (Adexed) at 126–145 g; controls were not injected. Injection (i.m.) of the experimental group (12 mg progesterone every other day) began 3 days after surgery and continued for 21–31 days. Other experimental details are the same as in Table 1. Mean cortisol sulfotransferase activity ± S.D. is given as nmoles cortisol sulfated/hr. The percentages in parentheses are the mean increases of CS activity observed compared to controls.

† Each g of liver yielded 1 ml of 50% cytosol.

‡ Significantly different from control ($P < 0.01$).

¶ Statistically significant difference ($P < 0.029$) between Adexed groups.

Similar examination of the effect of adrenal ablation on the response of the cortisol sulfotransferase activity to progesterone was also carried out (Table 4B). Hormone-treated adrenalectomized rats were given 12-mg doses of progesterone every other day for 21–31 days. This was the largest permissible dose and most frequent injection regimen consistent with prolonged survival. As shown, the cortisol sulfotransferase activity in livers from the progesterone-treated adrenalectomized rats increased 9- to 12-fold compared to untreated adrenalectomized animals. The results, as with all other groups of rats tested, were similar whether the enzyme activity was expressed per g liver or per 100 g body weight. The absolute cortisol sulfotransferase activities in progesterone-treated adrenalectomized animals were very similar to those observed in intact rats given 12 mg of the progestin daily (Tables 1 and 4A), despite the fact that they were injected less often than the intact groups.

The very large difference in enzyme activity between progestin-treated and untreated adrenalectomized males was due partly to the decrease of the cortisol sulfotransferase activity after adrenalectomy, which we have already documented [3] in that sex [also compare untreated intact and adrenalectomized groups of Table 4 (A and B)]. Furthermore, fractionation of the cytoplasmic cortisol sulfotransferase activity from livers of adrenalectomized progesterone-treated males on DEAE Sephadex A-50 columns (not shown) yielded glucocorticoid sulfotransferase profiles similar to those obtained with intact rats given the same progestin dose. Therefore, it appears probable that adrenal secretions did not mediate the effect of progesterone on the enzymes.

Brief examination of the comparative effects of short-term administration of progesterone, corticosterone, and estradiol-17β on hepatic cortisol sulfotransferase activity in male rats. The effects of progesterone in 1 week experiments (Table 2) led us to reconsider the rates at which steroid hormones affect the *in vivo* concentrations of rat liver glucocorticoid sulfotransferases. It appeared important to ascertain whether a month, or more, of daily administration of other hormones was required to elevate the enzymes significantly, as suggested by studies carried out in this and other laboratories [2, 3, 5, 20]. Examination of the effects of progesterone, corticosterone, and estradiol-17β after daily administration for a week (Table 5) showed similar statistically significant elevation of sulfotransferase activity, to concentrations between 30 and 40 per cent of the maximum long-term effects, that we had reported earlier [2, 3, 5]. This suggested that the response of glucocorticoid sulfotransferases to hormones might be much more rapid than originally considered. The high elevation of the hepatic cortisol sulfotransferase activity, that was observed in 1-week experiments with estradiol, was due to elevation of the concentrations of all three glucocorticoid sulfotransferases compared to controls (compare panels A and B of Fig. 3). STIII was the most plentiful enzyme. STI

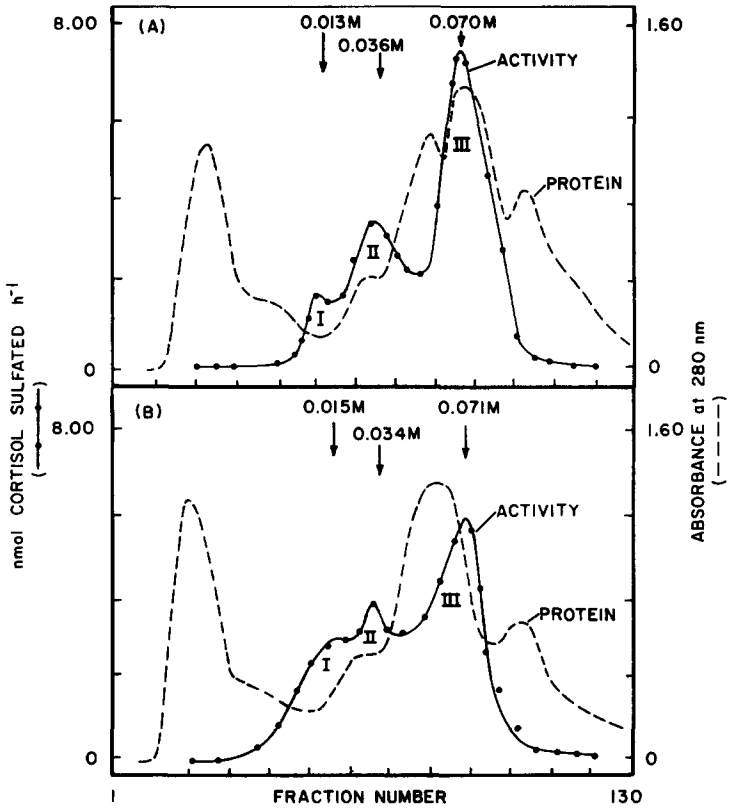

Fig. 2. Fractionation of glucocorticoid sulfotransterases of cytosol from progesterone-treated intact and castrated males. Samples (3 ml) of cytosol from (A), an intact male and (B), a male castrate, each described in Table 3A, were chromatographed on DEAE Sephadex A-50 columns. All conditions and symbols are described in Fig. 1. Both rats were given 12 mg progesterone daily for 39 days. The recoveries of applied enzyme activity were 81 and 84 per cent respectively. This was one of four similar experiments.

Table 5. Effect of short-term administration of corticosterone estradiol or progesterone on hepatic cortisol sulfotransferase (CS) activity in male rats*

Treatment	No. of experiments	Body wt (g)	Liver wt (g)	Hepatic CS activity per: g liver or ml cytosol†	Hepatic CS activity per: 100 g body wt
None	4	227 ± 11	9.20 ± 1.0	32.5 ± 6.1	131 ± 25
Corticosterone (3.0 mg)	4	210 ± 7.9	8.61 ± 0.56	58.0 ± 16‡ (+ 78%)	243 ± 77‡ (+ 86%)
Estradiol-17β (0.20 mg)	4	190 ± 19	7.92 ± 0.85	204 ± 21‡ (+ 528%)	852 ± 128‡ (+ 551%)
Progesterone (12 mg)	3	216 ± 23	9.13 ± 0.92	66.1 ± 17‡ (+ 103%)	284 ± 72‡ (+ 117%)

* Intact male rats, initially weighing 180–200 g, were either untreated controls or animals injected with indicated doses of corticosterone, estradiol-17β or progesterone. After 7–8 days of treatment the rats were killed, their livers were removed, and cytosol was prepared and assayed for CS activity. All conditions are as described in Table 1. Mean cortisol sulfotransferase activity ± S.D. is given as nmoles cortisol sulfated/hr. The percentages in parentheses are the mean increases of CS activity observed compared to control.

† Each g of liver yielded 1 ml of 50% cytosol.

‡ Significantly different from control ($P < 0.01$).

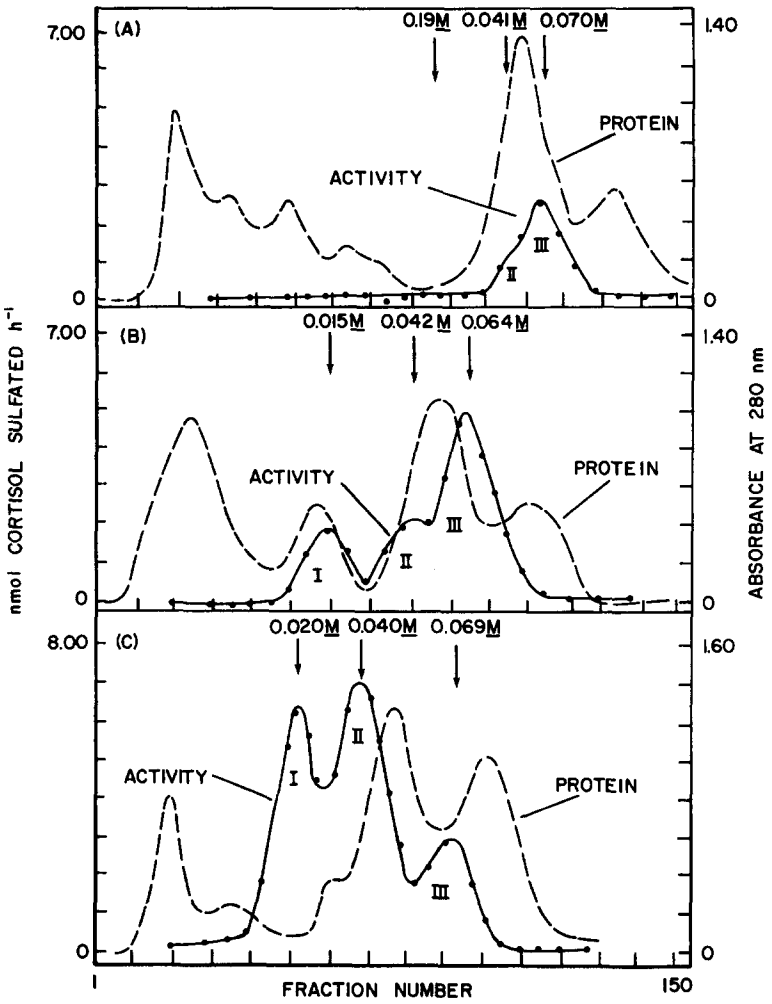

Fig. 3. Fractionation of cytosol glucocorticoid sulfotransferases from long-term and short-term estradiol-treated males. Cytosols from an untreated control (A) or from rats given 200 μg estradiol-17β daily for 2 days (B) or for 59 days (C) were used. All conditions are described in Fig. 1. Recoveries of eluted enzyme activity were 83–88 per cent of that applied to columns.

and STII were not elevated as high as in earlier [2] long-term experiments such as the 59-day experiment of Fig. 3C. This suggests a potentially significant change of the sulfotransferases in estrogen-treated rats with time that merits additional study. Ion exchange chromatography of cytosols from the short-term studies with corticosterone was not carried out.

DISCUSSION

Progesterone is centrally located in the schema for endocrine genesis of steroids, including gonadal hormones and corticosteroids. As pointed out by King and Mainwaring [20], it is well known that administered progesterone rapidly disappears from the circulation in rats and other mammals, and that its widespread metabolism in target tissues often obscures the elucidation of the mechanism of progestin action. Although it appeared possible at first that the effect of progesterone on STI, STII, and STIII could have been due to such metabolism, the study described here suggests the action of the progestin (or a very closely related metabolite) in the observed changes of hepatic glucocorticoid sulfotransferase activity. It also may provide plausible explanations for several responses of the enzymes to endocrine manipulation that were not clearly explained by earlier studies.

A role for estrogen in the process does not seem likely for several reasons. First, the effect of 12-mg doses of progesterone was much less profound than expected, even if 1–2 per cent of it had been converted to estrogen, because daily 200-μg doses of estradiol elevated the hepatic cortisol sulfotransferase activity 10-fold in 1–2 months [2] and 5–fold in 1 week Table 5). Furthermore, progesterone (Figs. 1 and 2) elevated STIII greatly and increased STI and STII concentrations more modestly, whereas estrogen (Fig. 3C and Ref. 2) elevated STI and STII the most in intact males. It also appears unlikely that

progesterone masked or prevented estrogen effects on the sulfotransferases, since 12-mg doses administered for 3.5 to 4 months (Table 3) affected the enzyme activity in female rats only slightly.

Roles for corticosteroid and androgen production from progesterone were also ruled out despite the ability of these hormones to modify hepatic glucocorticoid sulfotransferase activities in males [2, 3, 5]. Conversion of progesterone to testosterone appeared uninvolved in progestin-mediated sulfotransferase activity elevation, because this effect was augmented by castration of male rats (Table 4A). The augmentation appeared most likely to be due to additional STI and STII elevation that could have resulted from removal of the suppressive effect of androgen [2] on their production. Furthermore, adrenalectomy (Table 4B) did not change the final activities of hepatic glucocorticoid sulfotransferases obtained after administration of 12-mg doses of progesterone. Therefore, it also appeared that adrenal corticosteroid production from the progestin was not involved in the process.

The apparent direct pharmacologic action of progesterone on the sulfotransferases suggests a possible explanation for our hitherto puzzling observation that although estrogen elevated STI, STII, and STIII in castrated males to activities normally found in females, ovariectomy did not have a marked effect on the enzymes [2]. It appears possible, now, that physiological progesterone may facilitate the maintenance of high STI and STII activities in ovariectomized rats and act synergistically with estrogen to induce and maintain female sulfotransferase profiles. Such estrogen–progestin synergism could also explain why STI elevation without the progestin was not possible in males that had not been treated with estrogen [2]. The proposed role for progesterone replaces our earlier supposition [3] that corticosteroids are responsible. This explanation had never been comfortable, for it was not clear how the adrenal hormones could maintain STI and STII when their major effects [3] involved STIII.

The effects of estrogen, progestin, and corticosterone (Table 5) within 1 week suggest that steroid hormones affect the sulfotransferases much more rapidly than we [2, 3, 5] and others [21] had reported. This quite rapid response of the enzymes to endocrine manipulation suggests that they may play a more positive role in hormone actions than previously considered, a possibility that merits further investigation. It should also be noted that the 1-week induction of the enzymes reported here is the most rapid sulfotransferase elevation that we have observed. Such an effect may account for the rapid accumulation of small amounts of deoxycholate sulfate reported recently in liver-derived tumor tissue cultures 1 week after glucocorticoid administration [22].

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