Biochemical Pharmacology, Vol. 33, No. 21, pp. 3485–3490, 1984.
Printed in Great Britain.

0006–2952/84 $3.00 + 0.00

ENZYMATIC SULFATION OF STEROIDS—XX

EFFECTS OF TEN DRUGS ON THE HEPATIC GLUCOCORTICOID SULFOTRANSFERASE ACTIVITY OF RATS *IN VITRO* AND *IN VIVO*

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(*Received* 15 *November* 1983; *accepted* 10 *March* 1984)

Abstract—The effects of ten drugs on hepatic glucocorticoid sulfotransferase activity (HGSTA) were examined in male rats. The enzyme activity per 100 g body weight was elevated 152, 94.9, 140, 140, 73.1, 63.9, 76.9, and 140% after administration of daily i.p. doses of 111 mg spironolactone/kg (6–10 days), 66.7 mg WIN-24540/kg (6–10 days), 150 mg metyrapone/kg (19–31 days), 33.3 mg pentachlorophenol/kg (9–16 days), 16.5 mg aspirin/kg (10–16 days), 90.5 mg alloxan/kg (23–27 days), 104 mg aminoglutethimide/kg (12–20 days), and 16.8 mg propranolol/kg (21–27 days). Shorter experimental periods or lower drug doses caused smaller effects on HGSTA. Most notably, spironolactone (111 mg/kg) and WIN-24540 (66.7 mg/kg) caused 50–75% elevation of HGSTA in 2 days. Effects of WIN-24540, aspirin and pentachlorophenol were due mostly to elevation of hepatic levels of sulfotransferase III (STIII), the glucocorticoid-preferring sulfotransferase of rat liver. Effects of the other test drugs were due to elevation of hepatic levels of sulfotransferases I and II (STI and STII), which much prefer dehydroepiandrosterone as substrate, but also catalyze glucocorticoid sulfation. Enzyme inhibition studies showed that the test drugs interacted with the HGSTA *in vitro* in a fashion that appeared to be related to the *in vivo* effects already described. None of the drugs interacted exclusively with STI, STII or STIII *in vitro*. However, some differences of the strengths of individual drug–sulfotransferase interactions were observed. The drug effects are discussed in relation to drug and glucocorticoid actions.

During the last 8 years, we have extensively examined and purified sulfotransferases I, II and III (STI, STII and STIII), the rat liver enzymes that catalyze glucocorticoid sulfation [1, 2]. These research efforts are reviewed elsewhere [3–5]. The results of our studies have included demonstration of relationships between the enzymes and both hypertension [6] and ageing [7]. Our observations have interfaced well with other reports proposing involvement of glucocorticoid sulfates in control of corticosteroid metabolism [8, 9], hypertension [10–12], cancer [13, 14], diabetes [15], and enzyme induction [16, 17].

Our examination of effects of hormones and several drugs on the glucocorticoid sulfotransferases [5–7, 18, 19] supported these involvements of the enzymes. We therefore believed that additional evidence for such roles could come from examination of interactions between STI, STII and STIII and drugs related to the phenomena mentioned above. Accordingly, we chose ten drugs for testing: antihypertensive propranolol† and lopressor; inhibitors of corticosteroid biosynthesis (aminoglutethimide, metyrapone, and spironolactone); drugs with glucocorticoid-like properties (WIN-24540, aspirin, and theophylline); diabetogenic alloxan; and the sulfotransferase inhibitor/suspect carcinogen, pentachlorophenol. This paper describes the *in vivo* effects of the drugs on STI, STII and STIII and points out *in vitro* drug–sulfotransferase interactions that may be related to them.

MATERIALS AND METHODS

Animals, injections and chemicals. Male and female Fisher-344 rats (151–170 g) were purchased from the Charles River Laboratories, maintained here on tap water and Purina chow, *ad lib.*, and used at indicated body weights. Males were used for injection studies. Drug injections were i.p. at indicated intervals, in 1 ml of 1% NaCl/100 g body weight. Control animals were uninjected because the vehicle [20] had no significant effect on rat liver glucocorticoid sulfotransferase levels. Preparation of 3′-phosphoadenosine-5′-phosphosulfate, the reaction coenzyme, was as we reported it elsewhere [21]. [1,2-^{3}H]Cortisol (48 Ci/mmole) came from New England Nuclear (Boston, MA). Its purity was tested periodically as already described [1]. Nonradioactive cortisol, propranolol, aspirin, aminoglutethimide, alloxan, spironolactone and theophylline were purchased from the Sigma Chemical Co. (St. Louis, MO). Pentachlorophenol came from the Fluka Chemical Co. (Hauppage, NY). Lopressor and metyrapone were gifts from CIBA-Geigy (Summit,

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† Abbreviations: propranolol, 1-(isopropylamino)-2-(1-napthoxyl)-2-propanol; lopressor, (±)-1-[4-(2-methoxyethyl)phenoxyl] - 3 - [(1-methylethyl)amino] - 2 - propanol; aminoglutethimide, 3-(4-aminophenyl)-2-ethylglutarimide; metyrapone, 2-methyl-1,2-di-3-pyridyl-1-propanone; spironolactone, 7α-(acetylthio)-17α-hydroxy-3-oxopregn-4-ene-21-carboxylic acid-γ-lactone; and WIN-24540(trilostane), 2β-cyano-4α,5α-epoxy-androstane-17β-ol-3-one.

NJ). WIN-24540 was a gift from the Sterling-Winthrop Research Institute (Rensselaer, NY). All other chemicals used were the finest quality, obtained from standard suppliers.

Preparation and fractionation of liver cytosol. Rats were decapitated and their livers were rapidly removed, chilled, trimmed, and homogenized in 1 vol. of ice-cold 50 mM Tris–250 mM sucrose–3.00 mM mercaptoethanol (pH 7.5), Buffer 1. Then homogenates were centrifuged at 105,000 *g* (1 hr, Beckman L5-65B centrifuge). The resultant supernatant fraction, cytosol, was assayed for glucocorticoid sulfotransferase content (next section) or fractionated on 2.0 × 50 cm columns of DEAE-Sephadex A-50. The columns were prepared and eluted as we described elsewhere [18]. The protein content of selected effluent fractions was estimated from their absorbance at 280 nm. Salt gradients were measured by the conductance method we reported earlier [22].

Assay of glucocorticoid sulfotransferase activity. The pH 6.8 assay of the enzyme activity used 40 μM cortisol and 220 μM 3′-phosphoadenosine-5′-phosphosulfate in 500-μl reaction mixtures prepared and treated as we described earlier [1]. The enzyme-catalyzed reaction was terminated immediately after addition of the last reaction component—or after 60 min of incubation at 37.5°—by boiling for 1 min in a water bath. This was followed by addition of 500 μl of deionized water. Unreacted [^{3}H]cortisol was removed from diluted reaction mixtures by CH_2Cl_2 extraction [1]. The reaction product cortisol-21-sulfate—in the final aqueous extracts [1]—was counted in Aquasol-2 (New England Nuclear). The channels-ratio method [1] was used to convert counts observed in an Intertechniques SL-30 scintillation counter to disintegrations per minute. The enzyme activity, determined from the radioactivity measurements, is given in nanomoles cortisol sulfated per hour. Statistical significance was ascertained by use of Student's *t*-test [23].

RESULTS

In vivo *effects of test drugs on hepatic glucocorticoid sulfotransferase levels.* Male rats were used here because their low hepatic glucocorticoid sulfotransferase content, compared to females [1, 2], made them sensitive indicators of changes of the enzyme levels after experimental manipulation [1, 6, 16–20]. Our study showed that eight of ten test drugs (WIN-24540, spironolactone, metyrapone, pentachlorophenol, aspirin, alloxan, aminoglutethimide, and propranolol) caused significant elevation of hepatic glucocorticoid sulfotransferase levels. The maximum effects observed are given in Table 1. In each case, the enzyme-activity data indicated similar responses whether expressed per g liver or per 100 g body weight. No effect was observed with the highest test dose of theophylline (50 mg/kg). Lopressor was lethal within 24 hr at doses above 40 mg/kg. At that dose the drug had no effect on hepatic glucocorticoid sulfotransferase levels.

All of the drugs shown in Table 1 caused smaller

Table 1. *In vivo* effects of drugs on hepatic glucocorticoid sulfotransferase activity in male rats*

Drug and daily dose	Days injected and (expts.)	Body wt (g)	Liver wt (g)	GST activity (nmoles/hr) per: g liver	100 g body wt
(a) None	6–10 (6)	217 ± 13	8.31 ± 0.61	33.7 ± 6.9	130 ± 25
Spironolactone (111 mg/kg)		190 ± 11	8.48 ± 0.80	70.2 ± 7.3† (108%)‡	327 ± 24† (152%)
(b) None	6–10 (6)	218 ± 13	8.31 ± 0.62	33.7 ± 6.8	130 ± 23
WIN-24540 (66.7 mg/kg)		197 ± 17	7.81 ± 1.0	64.2 ± 9.2† (91.1%)	254 ± 38† (94.9%)
(c) None	19–31 (6)	242 ± 6.9	9.03 ± 0.54	23.1 ± 6.1	86.8 ± 25
Metyrapone (150 mg/kg)		213 ± 12	9.20 ± 0.36	47.6 ± 9.3† (110%)	206 ± 45† (140%)
(d) None	9–16 (6)	212 ± 13	8.89 ± 0.18	19.5 ± 4.3	79.7 ± 13
Pentachlorophenol (33.3 mg/kg)		212 ± 12	8.92 ± 0.61	40.2 ± 20† (110%)	196 ± 61† (140%)
(e) None	10–16 (5)	224 ± 15	9.08 ± 0.99	24.9 ± 1.5	96.8 ± 11
Aspirin (16.5 mg/kg)		223 ± 20	9.39 ± 0.88	43.9 ± 3.5† (75.9%)	168 ± 13† (73.1%)
(f) None	23–27 (4)	284 ± 16	9.90 ± 1.4	28.8 ± 2.2	97 ± 11
Alloxan (90.5 mg/kg)		263 ± 17	9.24 ± 0.96	45.6 ± 5.6† (58.3%)	159 ± 18† (63.9%)
(g) None	12–20 (7)	233 ± 17	9.27 ± 0.55	32.1 ± 8.1	126 ± 33
Aminoglutethimide (104 mg/kg)		201 ± 13	8.97 ± 0.88	49.6 ± 8.0† (55.1%)	223 ± 45† (76.9%)
(h) None	21–27 (7)	239 ± 12	9.65 ± 0.80	21.0 ± 7.0	87.1 ± 33
Propranolol (16.8 mg/kg)		223 ± 16	8.22 ± 0.69	54.1 ± 11† (150%)	211 ± 51† (140%)

* Studies began with 160–181 g rats. Drug injections were i.p. in 1% NaCl (1.00 ml/100 g body wt). Controls were uninjected as the vehicle had no effect on the enzyme activity [20]. Injections were daily in experiments a–f and every other day in experiments g and h (due to drug toxicity). Values are means ± S.D.

† Statistically significant difference between treated and control groups ($P < 0.02$).

‡ Average percent increase in glucocorticoid sulfotransferase (GST) activity.

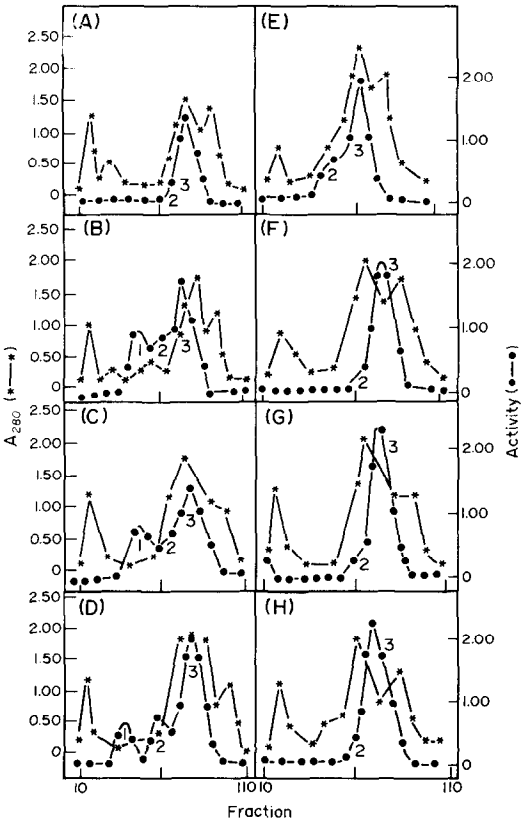

Fig. 1. DEAE-Sephadex fractionation of cytoplasmic glucocorticoid sulfotransferase activity in livers of control and drug-treated rats. Paired, 2.0 × 50 cm columns of DEAE-Sephadex A-50 (see Materials and Methods) were used in each experiment. One column of each pair was loaded with 3.00 ml of cytosol from a control rat and the other one was loaded with an equal amount of cytosol from a drug-treated rat. Each column was then eluted with a linear gradient composed of 300 ml each of Buffer 1 and Buffer 1–300 mM KCl. Elutions were carried out overnight, at 21–29 ml/hr. The glucocorticoid sulfotransferase activity (Activity) of indicated effluent fractions is given as nmoles cortisol sulfated/hr per ml fraction. A_{280} is the 280 nm absorbance—protein content—of indicated fractions. Numerals 1, 2, and 3 represent sulfotransferases I, II and III [1], denoting their positions in chromatograms. Panel A is a control chromatogram. Panels B–H are chromatograms obtained with rats given spironolactone (10 days), aminoglutethimide (20 days), metyrapone (25 days), propranolol (23 days), WIN-24540 (10 days), aspirin (12 days), and pentachlorophenol (14 days). The data shown came from one of three similar experiments carried out with each drug. Cytosol samples were from rats described in Table 1.

elevations of the glucocorticoid sulfotransferase levels (not shown) at doses between 25 and 33% of those indicated in the table. When the drugs were tested for several other time periods, they were found to elevate the enzyme levels sooner than shown. The effects chosen for Table 1 were increases of glucocorticoid sulfation large enough for use in comparison of STI, STII, and STIII levels present in liver before and after drug administration. Short-term drug effects meriting individual mention were 50–75% increases of the enzyme levels observed when WIN-24540 (66.7 mg/kg) or spironolactone (111 mg/kg) was injected at 0 and 24 hr and rats were killed 24 hr after the second injection.

Enzymatic basis for the in vivo *effects of the test drugs on the glucocorticoid sulfotransferase activity.* The results of this study are given in Fig. 1. As indicated, cytosol samples from control and experimental animals were fractionated on paired DEAE-Sephadex A-50 columns. Comparison of the data obtained with drug-treated rats and controls (e.g. Fig. 1A) indicated that two types of enzyme response occurred. First (Fig. 1, panels B–E), elevation of the glucocorticoid sulfotransferase activity due to spironolactone, aminoglutethimide, metyrapone, and propranolol was due mostly to elevation of STI and/or STII levels. Contrastingly, WIN-24540, aspirin and pentachlorophenol (Fig. 1, panels F–H) effects were due mostly to elevated STIII levels. The effect of alloxan on the individual sulfotransferases was not examined.

In vitro *effects of the test drugs on the cytoplasmic glucocorticoid sulfotransferase activity.* This study used liver cytosol from female rats, which was deemed especially suitable because it contained roughly equal amounts of STI, STII, and STIII [1]. We assumed that drug–sulfotransferase interactions (inhibition, activation, and/or stabilization) could help to explain the *in vivo* elevation of the hepatic enzyme levels just described. This assumption was partly due to the induction of the hepatic glucocorticoid sulfotransferase activity by steroids that inhibited STI, STII, and/or STIII *in vitro* (see Refs. 17–20). Furthermore, many reports (e.g. Refs. 24 and 25) indicate that chemicals that stabilize and/or inhibit enzymes elevate their *in vivo* levels.

Figure 2 shows that each of the test drugs inhibited the glucocorticoid sulfotransferase activity. It also allows us to rank the relative effectiveness of the drugs from the concentrations estimated as needed for 50% inhibition of the enzyme activity (Table 2). As shown, WIN-24540 was the most potent inhibitor. Alloxan was least potent. Aminoglutethimide, alloxan, and WIN-24540 were noncompetitive inhibitors (Table 2). The other drugs were competitive inhibitors.

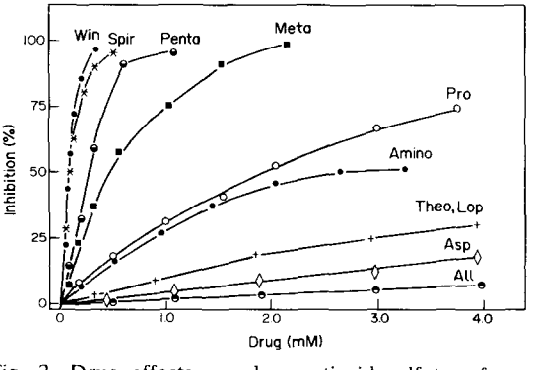

Fig. 2. Drug effects on glucocorticoid sulfotransferase activity in liver cytosol from female rats. Cytosol samples were assayed for their glucocorticoid sulfotransferase content (see Materials and Methods) with and without indicated amounts of each drug. The uninhibited enzyme activity was 231–304 nmoles cortisol sulfated/hr per ml cytosol. Three experiments gave similar results.

Table 2. Properties of drug-mediated inhibitions of cytoplasmic glucocorticoid sulfotransferase activity in livers from female rats*

Drug and No. of experiments	Inhibitor IC_{50} (mM)	Type of inhibition
WIN-24540 (3)	0.0100	Noncompetitive
Spironolactone (3)	0.0250	Competitive
Pentachlorophenol (3)	0.300	Competitive
Metyrapone (4)	0.400	Competitive
Propranolol (4)	2.30	Competitive
Aminoglutethimide (3)	4.10	Noncompetitive
Theophylline (3)	6.05	Competitive
Lopressor (3)	7.90	Competitive
Aspirin (3)	29.1	Competitive
Alloxan (2)	100	Noncompetitive

* Enzyme assays were carried out as indicated in the legend of Fig. 1. The drug concentrations giving 50% inhibition were estimated from experiments like those in Fig. 1. The type of inhibition obtained with each drug was determined from kinetic studies where the cortisol concentration was varied with or without enough inhibitor to give 20–50% inhibition, and double-reciprocal plots were prepared. Those plots were evaluated as described by Plowman [26] to allow assignment of the type of inhibition observed.

In vitro *effects of the test drugs on STI, STII, and STIII.* Figure 2 also points out that, although several of the drugs (e.g. WIN-24540) could completely inactivate the enzyme activity in cytosol from female rats, others (e.g. aminoglutethimide) caused maximum inhibitions of 50% or less. This suggested that the drugs might have varying abilities to interact with (and inhibit) STI, STII, and STIII.

To test this possibility, we separated the three enzymes in cytosol from female rats by DEAE-Sephadex chromatography (see Ref. 1) and examined the effects of seven of the drugs on the STI, STII and STIII pools isolated. The other drugs were not tested because they were not soluble enough for use at concentrations needed for reliable data. As shown in Table 3, drug concentrations that caused 40–50% inhibition of the cytoplasmic enzyme activity (Fig. 2 and Table 2) were used. Most of the drugs exhibited some differences of their abilities to inactivate STI, STII, and STIII. The largest of these was observed with aminoglutethimide, which inhibited STI and STII twice as effectively as it inhibited STIII. However, no highly specific inhibitor of any one of the three enzymes was identified. Study of similar STIII preparations from males (not shown) gave comparable results.

DISCUSSION

Rat liver STIII prefers glucocorticoid substrates [5, 19], while dehydroepiandrosterone is the best substrate for STI and STII [5, 27]. Thus, endocrine-mediated differences of the amounts of the three enzymes [1–3, 18, 20, 22] in rat liver may indicate modified abilities to produce glucocorticoid sulfates. This is supported by sexual dimorphism of anionic (sulfated) cortisol metabolites in rats given tracer doses of [^{3}H]cortisol [16]. Furthermore, corticosteroid sulfates and analogous unconjugated steroids are metabolized differently [8, 9] and variations

Table 3. Effects of drugs on individual glucocorticoid sulfotransferases from livers of female rats*

Drug and concentration	No. of Expts.	Percent inhibition of: STI	STII	STIII
Aminoglutethimide (5.00 mM)	4	70.2 ± 12	83.1 ± 17	42.3 ± 20
Lopressor (9.10 mM)	4	43.3 ± 8.0	63.5 ± 8.5	58.3 ± 4.8
Metapyrone (250 μM)	6	27.7 ± 10	40.9 ± 11	30.3 ± 10
Pentachlorophenol (330 μM)	4	38.9 ± 3.6	39.1 ± 3.8	23.6 ± 5.1
Propranolol (3.30 mM)	4	28.5 ± 13	46.4 ± 13	45.3 ± 11
Spironolactone (20.0 μM)	5	34.4 ± 4.2	44.7 ± 4.1	44.1 ± 8.7
WIN-24540 (10.0 μM)	5	51.9± 7.6	58.8 ± 12	54.0 ± 15

* For these experiments, cytosols were prepared from livers of female rats and fractionated on DEAE-Sephadex A-50 columns (see Materials and Methods). After assay of column fractions for cortisol sulfotransferase activity, fractions that contained sulfotransferases I, II and III were pooled separately, as described elsewhere [1]. The pools, STI, STII and STIII, were each incubated with or without indicated amounts of inhibitors. The inhibition data are given as the means ± S.D. The enzyme activity in STI, STII and STIII pools averaged 3.5 to 5.1 nmoles·h^{-1}·(ml pool)$^{-1}$.

of glucocorticoid sulfation can cause additional changes of *in vivo* corticosteroid metabolism [9, 28]. These changes could lead to modification of glucocorticoid actions.

Occurrence of such modifications is suggested by the fact that livers of guinea pigs, which respond poorly to glucocorticoids [29, 30], contain mostly steroid sulfotransferases with very high K_m values for cortisol [31] and produce only small amounts of glucocorticoid sulfates *in vivo* [29]. In addition, decreases of STIII levels and basal levels of glucocorticoid-inducible enzymes occur in livers of adrenalectomized rats [20, 32]. Moreover, decreased relative amounts of STIII in old rats, due to increased STI and STII levels [7], may be linked to impaired responses to cortisol [33] apparently unrelated to ACTH or glucocorticoid receptor [7]. Furthermore, increased glucocorticoid sulfotransferase [34]—STIII [6]—levels in several types of hypertensive male rats appear to be relevant to the understanding of adrenal cortical involvement in the disease, and to increased glucocorticoid sulfate levels and excretion in human hypertensives [10, 11, 15].

Against this background, it is of considerable interest that, despite great differences of their chemical structures, eight of ten drugs tested *in vivo* (Table 1) elevated rat liver glucocorticoid sulfotransferase levels quite extensively. Moreover, statistically significant elevations of the enzyme levels occurred within dose ranges used for pharmacological study of animals (e.g. Ref. 35–37) and for therapeutic purposes in humans (e.g. Refs. 38–41). Consequently, the elevated glucocorticoid sulfotransferase levels observed may be related to the therapeutic actions of the test drugs and/or to their side effects.

The *in vivo* effects of the drugs on the hepatic levels of the individual sulfotransferases (Fig. 1) fit into two classes. First, the drugs with various glucocorticoid-like properties (aspirin, WIN-24540, and pentachlorophenol), like glucocorticoids [6, 18, 20], elevated STIII levels almost exclusively. In contrast, the drugs which exhibited various antiglucocorticoid properties (spironolactone, metyrapone, aminoglutethimide, and propranolol) elevated STI and STII levels mostly. Such elevations of those two sulfotransferases are consistent effects of a number of hormonal steroids [18, 22] which are classed as antiglucocorticoids.

Several alternative explanations exist for the basis of the *in vivo* drug effects on the sulfotransferases. One of these, which was tested, is that direct drug–sulfotransferase interactions, like those that occur in actions of "substrate inducers" [24, 25, 42], are responsible. In support of this possibility, our *in vitro* inhibition study (Fig. 2 and Table 2) pointed out that uptake of 1% of injected doses of most of our test drugs by liver would yield hepatic concentrations that could cause the proposed drug–sulfotransferase interactions. We have not yet carried out drug uptake studies. However, proposed uptake values are consistent with those reported for drugs and hormones (e.g. Refs. 16 and 17).

Aspirin, pentachlorophenol, and propranolol were much more potent *in vivo* than expected on the basis of the *in vitro* study. It appears possible that this could have been due to their metabolism to compounds with higher affinities for the sulfotransferases. Alternatively, these drugs may have caused their effects via interaction with specific endocrines, as reported with other enzymes [43, 44]. Although we will ultimately assess the extent of endocrine contributions to the elevation of STI, STII and STIII levels by the drugs, we believe that the studies reported here provide a useful tool that may facilitate elucidation of links between drugs and glucocorticoid actions.

Our examination of the *in vitro* effects of the test drugs on STI, STII, and STIII (Table 3) showed that none of them interacted exclusively with any one of the sulfotransferases. Yet some differences of relative drug–sulfotransferase interactions occurred (e.g. 5 mM aminoglutethimide caused 70.2 ± 12, 83.1 ± 17 and 42.3 ± 20% inhibitions of STI, STII, and STIII). Furthermore, in several instances, like elevation of STI and STII levels by aminoglutethimide injection (Fig. 1C), the study provided a rationale for the *in vivo* sulfotransferase elevations. In other cases, however, additional explanations must be sought if complete understanding of the drug effects is to be obtained.

Acknowledgements—This study was supported by a research grant from the National Institutes of Health (AM-19669) and funds from the University of Dayton Research Council.

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