PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 908–911 (1974)

On the Hepatic Sulphurylating Activity in Male Pseudohermaphroditic Rats[1] (37922)

JAN-ÅKE GUSTAFSSON, JAN CARLSTEDT-DUKE, AND ALLEN S. GOLDMAN[2]

Departments of Chemistry and Germfree Research, Karolinska Institutet, Solnavägen 1, S-104 01 Stockholm 60, Sweden, and Department of Pediatrics, University of Pennsylvania School of Medicine, Division of Experimental Pathology, The Children's Hospital of Philadelphia, 1740 Bainbridge Street, Philadelphia, Pennsylvania 19146

Hepatic sulphurylation of steroid hormones in rats is sex dependent (1–4); liver preparations from female rats generally sulphurylate various substrates 3–5 times more efficiently than corresponding preparations from male rats (2–4). Torday *et al.* have suggested that a stimulatory influence on the hepatic sulphurylating activity exerted by estrogens of ovarian origin could help to explain this sexual difference (3). On the other hand, Carlstedt-Duke and Gustafsson have indicated that hepatic sulphurylating activity in female rats is regulated by nongonadal factors and that the lower activity in male rats is due to a suppressive effect exerted by testicular androgens (4). It was therefore of interest to investigate the sulphurylating activity in the liver of male pseudohermaphroditic rats. These genotypically male rats have an inherited defect leading to lack of androgen-dependent differentiation with absence of reproductive organs other than inguinal testes (5). Bardin *et al.* have demonstrated that the failure of the rats to virilize is due to end-organ insensitivity to testosterone (6). Since the pseudohermaphroditic rats lack ovarian tissue, and since they are insensitive to physiological doses of androgens, measurement of their hepatic sulphurylating activity should contribute to the understandnig of the regulation of this enzyme activity.

Materials and Methods. Reference compounds. [1,2-^{3}H]Deoxycorticosterone (sp act 50 mCi/μmole) was obtained from New England Nuclear (Boston, MA). The purity of this radioactive steroid was assayed by radio-gas chromatography prior to use. Unlabeled deoxycorticosterone was kindly donated by Dr. J. Babcock.

Animal experiments. The sulphurylating activity was studied in 6 male pseudohermaphroditic, 9 normal female, and 11 normal male rats of the King-X Holtzmann strain weighing about 250 g. Furthermore, one group of castrated male pseudohermaphroditic (5 animals), normal female (4 animals), and normal male (6 animals) rats received daily im injections of 1 mg of testosterone propionate in 0.5 ml of propylene glycol for 14 days starting 1 day after operation. A control group consisting of 5 male pseudohermaphroditic, 5 normal female, and 5 normal male rats was castrated and injected for 14 days with propylene glycol only. In a separate experiment, five castrated male pseudohermaphroditic

[1] Aided by research grants from the U.S.P.H.S. (HD-4683), the Rockefeller Foundation, the Swedish Medical Research Council (13X-2819), and Carl-Bertel Nathhorsts Vetenskapliga Stiftelse.

[2] Recipient of Career Development Award (HD-13,682) from the U.S.P.H.S.

rats were given 0.1 mg of estradiol benzoate in 0.5 ml of propylene glycol for 14 days.

Preparation of homogenates. Liver homogenates, 20% (w/v), were prepared in a modified Bucher medium (7), pH 7.4, with a Potter–Elvehjem homogenizer equipped with a loosely fitting Teflon pestle. The homogenate was centrifuged at 20,000*g* for 15 min. The 20,000*g* supernatant fluid was centrifuged at 105,000*g* for 70 min and the obtained supernatant was the cytosol equivalent used for incubation. The protein concentration of the 105,000*g* supernatant fluid was determined according to Lowry *et al.* (8).

Incubations with [1,2-^{3}H]Deoxycorticosterone. The method used for the incubations was a modification of the method used by Torday *et al.* (3) and is described in detail in Ref. (4). Usually, 0.2 ml of the cytosol equivalent was edded to 4.8 ml of modified Krebs–Ringer bicarbonate medium, pH 7.4 (9). ATP disodium salt, 15 μmoles, Sigma grade (Sigma Chemical Co.), was added as solid. The mixture was preincubated at 37° for 30 min in order to generate PAPS. The substrate—400,000 cpm of [1,2-^{3}H]deoxycorticosterone diluted with 50 μg unlabled steroid—was added in 100 μl of acetone immediately after the preincubation period and the incubation was continued for 30 min. The incubation was stopped and the radioactivity was extracted with chloroform–methanol, 2:1 (v/v), as described previously (4). The extract was chromatographed on a Sephadex LH-20 column using the solvent system chloroform–methanol, 1:1 (v/v), 0.01 *M* with respect to NaCl. This chromatography allows the separation of free steroids, steroid monosulphates, and steroid disulphates (10). These three fractions were eluted from the column and measured for radioactivity in a Packard Liquid Scintillation Counter (Model 2425). The sulphurylating activity was expressed as the amount of mono- and disulphurylated metabolites of deoxycorticosterone formed. The conditions of incubations given above were chosen as optimum after kinetic studies had shown that production of products by the cytosol equivalent was proportional to time as well as to the amount of cytosol equivalent. Student's *t* test was used for statistical evaluation of the results and the significance level was set at 0.05.

Results. Rat liver sulphurylating activity was comparable in pseudohermaphroditic male rats and normal female rats, but this activity was significantly greater than that of normal male rats ($P < 0.005$ and 0.001, respectively) (Fig. 1). Gonad removal did

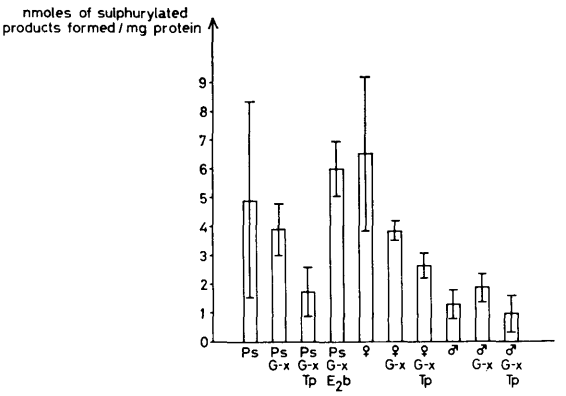

FIG. 1. The sulphurylating activity of rat liver (expressed as nmoles of sulphurylated metabolites of [1,3-^{3}H]deoxycorticosterone formed/mg protein) in intact, castrated, and treated male pseudohermaphroditic (Ps), female (♀), and male (♂) rats. G-x = gonadectomized; Tp = testosterone propionate, E_2b = estradiol benzoate. The vertical lines on the bars represent ±1 SD. In these experiments 50 μg of substrate was used.

not negate this sex difference in sulphurylase despite some decline in activity.

Administration of testosterone propionate decreased the sulphurylase activities. Sulphurylation in castrated male pseudohermaphroditic rats treated with testosterone propionate was 55% lower than in castrated male pseudohermaphroditic rats which had not been treated with steroid ($P < 0.05$). Testosterone propionate treatment of gonadectomized female rats decreased the sulphurylase activity by 30% ($P < 0.02$).

When castrated male pseudohermaphroditic rats were given estradiol benzoate, a significant increase in sulphurylating activity (about 60%; $P < 0.01$) was observed.

Discussion. Recently, we have shown that liver microsomal preparations from male pseudohermaphroditic and normal female rats metabolize 4-androstene-3,17-dione and 4-pregnene-3,20-dione in an identical manner and in quite a different way from corresponding preparations from normal male rats (11). In the present investigation, we have shown that male pseudohermaphroditic and normal female rats also have similar hepatic sulphurylating activities which are greater than the sulphurylating activity in liver cytosol preparations from normal male rats. Since male pseudohermaphroditic rats lack ovaries, and since they are insensitive to the action of testicular androgens (6), this finding gives strong support to the suggestion that the hepatic sulphurylating activity is regulated by nonovarian factors (4).

In agreement with previous results, the hepatic sulphurylating activity was suppressed in castrated female rats by testosterone propionate treatment. This treatment also resulted in an unexpected, similar effect in castrated male pseudohermaphroditic rats. Usually, these rats have been found to be insensitive to testosterone in the doses used in the present study and doses up to 100 mg of testosterone propionate per day have generally been needed to obtain androgenic effects on enzyme activities in hepatic and other tissues (6). Our finding reflects the fact that the androgen unresponsiveness of the male pseudohermaphroditic rat is relative rather than absolute (6).

In contrast to our previous findings on hormonal regulation of hepatic sulphurylating activity in Sprague–Dawley rats of the Swedish strain (4), male pseudohermaphroditic rats of the King-X Holtzmann strain have been found to respond to estradiol benzoate treatment by increased sulphurylating activity. The physiological significance of this finding is difficult to evaluate. In view of the results presented above, it seems adequate to presume that the sulphurylating activity in normal female rats is normally regulated mainly by nongonadal factors and that the sulphurylating activity in normal male rats is suppressed by testicular androgens. In certain strains of rats, estrogens may also play a minor role in the regulation of hepatic sulphurylation.

Summary. The sulphurylation of [1,2-^{3}H]-deoxycorticosterone was studied in the 105,000*g* supernatant fluid obtained from liver homogenates of intact and castrated male pseudohermaphroditic, normal female, and normal male rats with and without treatment with steroid hormones. Male pseudohermaphroditic and normal female rats had similar sulphurylating activities which were higher than that of normal male rats. It was shown that the sulphurylating activity in male pseudohermaphroditic and normal female rats is normally regulated mainly by nongonadal factors and that the sulphurylating activity in normal male rats is suppressed by testicular androgens.

1. Roy, A. B., Biochem. J. **63**, 294 (1956).

2. Wengle, B., Acta Soc. Med. Upsal. **68**, 154 (1963).

3. Torday, J. S., Klein, G. P., and Giroud, C. J. P., Can. J. Biochem. **49**, 437 (1971).

4. Carlstedt-Duke, J., and Gustafsson, J.-Å., Eur. J. Biochem. **36**, 172 (1973).

5. Allison, J. E., Stanley, A. J., and Gumbreck, L. F., Anat. Rec. **153**, 85 (1965).

6. Bardin, C. W., Bullock, L., Blackburn, W. R., Sherias, R. J., and Vanha-Perttula, T., Birth Defects: Original Article Series **7**, 185 (1971).

7. Bergström, S., and Gloor, U., Acta Chem. Scand. **9**, 34 (1955).

8. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

9. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., *in* "Manometric Techniques," p. 194. Burgess Publishing Co., Minneapolis, MN (1959).

10. Sjövall, J., and Vihko, R., Acta Chem. Scand. **20**, 1419 (1966).

11. Einarsson, K., Gustafsson, J.-Å., and Goldman, A. S., Eur. J. Biochem. **31**, 345 (1972).

Received July 19, 1973. P.S.E.B.M., 1974, Vol. 145.