Different Mechanisms of Regulation of Nuclear Reduced Nicotinamide–Adenine Dinucleotide Phosphate-Dependent 3-Oxo Steroid 5 α -Reductase Activity in Rat Liver, Kidney and Prostate

By JAN-ÅKE GUSTAFSSON and ÅKE POUSETTE

Department of Chemistry and Department of Germfree Research, Karolinska Institutet, Stockholm, Sweden

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The regulatory mechanisms involved in the control of the nuclear NADPH-dependent 3-ketosteroid 5 α -reductase (5 α -reductase) activity were studied in liver, kidney and prostate. The substrate used was [1,2-³H]androst-4-ene-3,17-dione (androstenedione) (for liver and kidney) or [4-¹⁴C]androstenedione (for prostate). The hepatic nuclear 5 α -reductase activity was greater in female than in male rats, was greater in adult than in prepubertal female rats, increased after castration of male rats, but was not affected by treatment with testosterone propionate or oestradiol benzoate. These regulatory characteristics are in part different from those previously described for the hepatic microsomal 5 α -reductase. The renal nuclear metabolism of androstenedione, i.e. 5 α reduction and 17 β -hydroxy steroid reduction, was relatively unaffected by sex, age, castration and treatment with testosterone propionate. However, treatment of castrated male rats with oestradiol benzoate led to a significant increase in the 5 α -reductase activity and a significant decrease in the 17 β -hydroxy steroid reductase activity. Finally, the nuclear 5 α -reductase activity in prostate was androgen-dependent, decreasing after castration and increasing after treatment with testosterone propionate. In conclusion, the nuclear 5 α -reductase activities in liver, kidney and prostate seem to be under the control of distinctly different regulatory mechanisms. The hypothesis is presented that whereas the prostatic nuclear 5 α -reductase participates in the formation of a physiologically active androgen, 5 α -dihydrotestosterone, this may not be the true function of the nuclear 5 α -reductase in liver and kidney. These enzymes might rather serve to protect the androgen target sites in the chromatin from active androgens (e.g. testosterone) by transforming them into less active androgens (e.g. 5 α -androstane-3,17-dione and/or 5 α -dihydrotestosterone).

The intranuclear formation of the physiologically active androgen 17 β -hydroxy-5 α -androstane-3-one (5 α -dihydrotestosterone) from testosterone, first shown in the prostate (Anderson & Liao, 1968; Bruchovsky & Wilson, 1968), was originally considered to be a typical feature of the accessory sex organs in the male rat (Bruchovsky & Wilson, 1968). Recent studies, however, have shown the presence of nuclear NADPH-dependent 3-oxo steroid 5 α -reductases (5 α -reductases) in tissues other than those classically regarded as androgen target organs. Verhoeven & De Moor (1971, 1972) described a nuclear 5 α -reductase in rat kidney and we characterized a 5 α -reductase enzyme in nuclei from rat liver (Gustafsson & Pousette, 1974). The general properties and substrate specificity of the hepatic nuclear 5 α -reductase resembled those described for the nuclear 5 α -reductases in prostate and kidney and seemed to be different from hepatic microsomal 5 α -reductase. Nuclear 5 α -reductase activity is higher in female than in male rat liver, which suggests a different type of regulation mechanism for the

hepatic enzyme than for the prostatic enzyme, the activity of which has been found to be androgen-dependent (Shimazaki *et al.*, 1969, 1972; Moore & Wilson, 1973). In view of the limited information available about the factors regulating nuclear 5 α -reductase activity in organs other than the prostate we decided to investigate the influence on the hepatic and renal nuclear enzymes of certain factors known to affect liver microsomal 5 α -reductase activity. Knowledge of factors regulating the intranuclear metabolism of androgens might help to clarify the physiological role of the participating enzymes. In the present work we studied the influence of age, sex, castration and treatment with androgens and oestrogens on the nuclear metabolism of androst-4-ene-3,17-dione (androstenedione) in liver and kidney. For comparison some experiments were also carried out with prostate nuclei. Androstenedione was chosen as substrate since it permitted measurement of the activity of nuclear 17 β -hydroxy steroid reductase at the same time as the activity of nuclear 5 α -reductase.

Materials and Methods

Materials

Androstenedione and testosterone were generously supplied by Dr. J. Babcock, Upjohn Co., Kalamazoo, Mich., U.S.A. 5α -Androstane-3,17-dione was obtained from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.

[1,2- ^3H]Androstenedione (sp. radioactivity 45.9 Ci/mmol) was purchased from New England Nuclear Corp., Boston, Mass., U.S.A., and [4- ^{14}C]androstenedione (sp. radioactivity 60 mCi/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K.

The steroids were purified by t.l.c. in the solvent system chloroform-ethyl acetate (4:1, v/v). The radioactive compounds were checked for purity by t.l.c. and by radio-g.l.c. (on an SE-30 column) and were more than 98% pure.

Dithiothreitol, NADP^+ , DL-isocitric acid (tri-sodium salt) and isocitrate dehydrogenase (type IV; 36 units/ml) were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A.

Animal experiments

Rats of the Sprague-Dawley strain were used. In one series of experiments male and female rats were castrated at the age of 56 days. At 1 day after the operation daily injections of 0.5 ml of propylene glycol (control group), 400 μg of testosterone propionate in 0.5 ml of propylene glycol or 100 μg of oestradiol benzoate in 0.5 ml of propylene glycol were commenced on the male rats and were continued for 7 days. After this period the animals were killed and studied with respect to the nuclear metabolism of androstenedione in liver and kidney. The castrated female rats that received no treatment were also killed 7 days after the operation and the nuclear enzyme activities in liver and kidney investigated. In a separate experiment these parameters were studied in male and female rats of 28 and 56 days of age. The hepatic nuclear 5α -reductase activity was also studied in male rats that had been castrated on their first day of life (Einarsson *et al.*, 1973; Gustafsson & Stenberg, 1974a). Finally, the nuclear 5α -reductase activity in prostate was investigated in intact 56-day-old rats and in rats castrated at 56 days of age and treated for 4 consecutive days with propylene glycol (control rats), testosterone propionate or oestradiol benzoate at the doses described above.

Preparation of nuclei

The method for preparation of nuclei from liver, kidney and prostate was a modification of that described for liver nuclei by Gustafsson & Pousette (1974). The animals were killed by cervical

dislocation, the liver was perfused with cold 0.9% NaCl and the liver, one kidney and the ventral prostate were quickly removed, rinsed in cold 0.9% NaCl and cut into small pieces in 10 ml of medium A (0.88 M-sucrose-1.5 mM- CaCl_2 -1 mM- MgSO_4 -0.01 M-Tris-HCl buffer, pH 7.4) (Verhoeven & De Moor, 1972) and homogenized. Homogenate corresponding to 1 g of liver tissue and homogenates of kidney (1.0 g) and prostate (0.5 g) were centrifuged at 20000g for 30 min. The sediments were resuspended in 2.2 M-sucrose and centrifuged at 58000g for 90 min in a SW 50.1 rotor. The pellets obtained were resuspended and homogenized in 2.5 ml of medium A and layered over 2.5 ml of 2.2 M-sucrose and centrifuged again at 58000g for 30 min in an SW 50.1 rotor. The sediment was suspended in 2 ml of medium B (50 mM-NaCl-5 mM- MgSO_4 -50 μM -EDTA-0.01 M-Tris-HCl buffer, pH 7.4) and homogenized. A sample of the nuclear preparation was always taken for control of purity and for calculation of concentration of nuclei by using phase-contrast microscopy and a Burkner counting chamber. In a previous study a similar purification method was shown to give a pure hepatic nuclear preparation as judged by electron microscopy (Gustafsson & Pousette, 1974). A sample of the nuclear preparation was also taken for determination of DNA (Burton, 1956) with calf thymus DNA (Sigma Chemical Co.) as standard.

Conditions of incubation

Incubations with nuclear preparations were always started 6 h after the rats were killed and never later than 30 min after the nuclear preparation was ready. The incubation conditions were tested and were designed to give conversions linear with respect to time and enzyme concentrations.

In all incubations a total incubation volume of 1 ml of medium B was used; this incubation mixture also contained 1×10^6 d.p.m. of [1,2- ^3H]androstenedione (for liver and kidney nuclei) diluted with unlabelled androstenedione (final substrate concentration 1.75-17.5 μM) or 1×10^5 d.p.m. of [4- ^{14}C]androstenedione (for prostate nuclei) (giving a substrate concentration of 0.76 μM), and an NADPH-regenerating system consisting of NADP^+ (0.4 mM), DL-isocitric acid (3.87 mM), isocitrate dehydrogenase (0.4 unit/ml) and MnCl_2 (9.2 μM).

In the incubations with liver nuclei the amount of nuclei added and the length of the incubation time were varied according to the type of metabolism characterizing the nuclear preparation in question. For a female type of metabolism (high 5α -reductase activity) about 2×10^6 nuclei (corresponding to about 21.4 μg of DNA)/ml were added and an incubation time of 10 min was used. For a male type of metabolism (low 5α -reductase activity) a concen-

tration of about 6×10^6 nuclei (corresponding to about $64.2 \mu\text{g}$ of DNA)/ml and an incubation time of 20 min were used.

Incubations with kidney and prostate nuclei were carried out in the presence of dithiothreitol (0.06 M). The concentration of dithiothreitol was chosen to give an optimum activity of 5α -reductase. The concentration of nuclei generally corresponded to about 200 and $65 \mu\text{g}$ of DNA for kidney and prostate nuclei respectively. Incubations with kidney nuclei were carried out for 20 min, whereas those with prostate nuclei were for 60 min.

The incubations were terminated by the addition of 5 ml of chloroform-methanol (2:1, v/v). The mixture was shaken well and then left for 12–16 h. The chloroform phase was transferred to another tube, evaporated to dryness under N_2 and the dry extract was redissolved in about $500 \mu\text{l}$ of methanol.

Analysis of incubation extracts

The extracts from the incubations were analysed by t.l.c. in the solvent system chloroform-ethyl acetate (4:1, v/v). The t.l.c. plates were scanned for radioactivity with a Berthold Model II thin-layer scanner (Berthold, Wildbad, Germany). This technique allowed a reliable quantification of formed metabolites and residual substrate. In selected cases the radioactive zones were scraped off the t.l.c. plates, eluted with methanol and subjected to radiog.l.c.-mass spectrometry (Gustafsson & Pousette, 1974). A compound was considered identified if it had the same mass spectrum and g.l.c. behaviour as the reference compound. Retention times (t_R : s) were calculated relative to that of 5α -cholestan-3-one ($t_R = 1.00$).

For the statistical analysis Student's t test was used and the significance level was set at 0.05.

Results

Regulation of hepatic nuclear 5α -reductase activity

As described by Gustafsson & Pousette (1974), only 5α -androstane-3,17-dione is formed from androstenedione after incubation with liver nuclei from female rats under the standard conditions given above. When incubations were performed with liver nuclei from male rats 5α -androstane-3,17-dione still constituted a major metabolite, but 3β -hydroxy- 5α -androstane-17-one and testosterone (small amounts) were also formed. The 5α -reductase activity in male liver nuclei was measured as the sum of the amounts of 5α -androstane-3,17-dione and 3β -hydroxy- 5α -androstane-17-one formed. Testosterone was only formed in normal adult male rats.

In agreement with our earlier observations (Gustafsson & Pousette, 1974) normal adult female rats showed about 30 times more nuclear

Fig. 1. Activity of hepatic nuclear 5α -reductase active on androstenedione in male and female rats after castration and treatment with steroid hormones

28d and 56d, 28- and 56-day-old rats; C, castrated male and female rats; CTp and CE2b, castrated male rats treated with testosterone propionate and oestradiol benzoate, respectively; NC, neonatally castrated male rats. The number of animals is given in parentheses and 1 s.d. is indicated by vertical lines on the top of the bars.

5α -reductase activity than normal adult male rats (see Fig. 1). The enzyme activity in 28-day-old female rats was only about 12% of that in adult female rats, whereas the 5α -reductase activity in 28-day-old male rats was similar to that in adult male rats. Castration of male rats, either neonatally or postpubertally, resulted in an almost tenfold stimulation of the nuclear 5α -reductase activity. Castration of adult female rats did not result in any change in the activity of the 5α -reductase. No effects on the enzyme activity were seen after the treatment of castrated male rats with testosterone propionate or oestradiol benzoate.

Regulation of renal nuclear metabolism

Incubation of renal nuclear preparations from male and female rats with 4-androstene-3,17-dione resulted in the formation of two metabolites. One was identified as 5α -androstane-3,17-dione (t_R on SE-30, 0.45). The other metabolite was identified as testosterone (t_R of the trimethylsilyl ether on SE-30, 0.66).

In contrast with the hepatic enzyme activity the renal nuclear 5α -reductase activity (measured as the amount of 5α -androstane-3,17-dione formed from androstenedione) was not characterized by any sexual differences, neither in adults nor in 28-day-old animals (see Fig. 2). The younger animals tended to have a slightly lower enzyme activity than the older animals, but no significant differences were observed. The enzyme activity was not influenced by castration. When castrated male rats were treated with testosterone propionate, no significant change in the renal nuclear 5α -reductase activity was observed.

Fig. 2. Activity of renal nuclear 5α -reductase active on androstenedione in male and female rats after castration and treatment with steroid hormones

For further details see the legend to Fig. 1. Significant effects obtained after hormone treatment when compared with control values (C) are indicated in the bars by * ($P < 0.05$), or *** ($P < 0.001$).

Fig. 3. Activity of renal nuclear 17β -hydroxy steroid reductase active on androstenedione in male and female rats after castration and treatment with steroid hormones

For further details, see the legends to Figs. 1 and 2.

However, oestradiol benzoate treatment of castrated male rats resulted in a more than fivefold stimulation ($P < 0.05$) of the enzyme activity.

The renal nuclear 17β -hydroxy steroid reductase activity (measured as the amount of testosterone formed from androstenedione) was also relatively uninfluenced by age, sex, castration and treatment with testosterone propionate (see Fig. 3). After treatment of castrated male rats with oestradiol benzoate, the enzyme activity was decreased by 40% ($P < 0.05$).

Regulation of nuclear 5α -reductase activity in prostate

When nuclear preparations from prostate were incubated with androstenedione only 5α -androstane-

Fig. 4. Activity of nuclear 5α -reductase in prostate active on androstenedione in rats after castration and treatment with steroid hormones

For further details see the legends to Figs. 1 and 2.

3,17-dione was formed. Castration resulted in a decrease in the 5α -reductase activity by about 60% ($P < 0.05$) (see Fig. 4). The enzyme activity was completely restored by treatment with testosterone propionate. Treatment of castrated male rats with oestradiol benzoate had no effect on the activity of the nuclear 5α -reductase.

Discussion

In the present study the prostatic nuclear 5α -reductase activity was found to be androgen-dependent. This agrees with the findings of Shimazaki *et al.* (1969, 1972) and Moore & Wilson (1973), who showed that the 5α -reductase activity in rat ventral prostate decreased after castration and was increased after testosterone administration. In contrast with the prostatic enzyme activity, the hepatic nuclear 5α -reductase activity in male rats was markedly increased after castration. This finding was not unexpected in view of the much more efficient 5α reduction in liver nuclei from female rats than from male rats, and this reaction also conforms to the regulation pattern characterizing the rat liver microsomal 5α -reductase enzyme (Yates *et al.*, 1958; Einarsson *et al.*, 1973). Similarly, the increase in nuclear 5α -reductase activity with age shown in female rats is paralleled by an age-dependent increase in microsomal 5α -reductase activity (Yates *et al.*, 1958). Quite unexpectedly, however, the higher activity of nuclear 5α -reductase in castrated male

rats was not affected by androgen or oestrogen treatment, which contrasts with the suppression and stimulation respectively of microsomal 5 α -reductase activity after similar treatment (Einarsson *et al.*, 1973). Likewise, neonatal castration of male rats did not completely alter the nuclear 5 α -reductase activity to the pattern found in female rats, whereas this treatment leads to complete alteration to the female pattern of the microsomal 5 α -reductase activity in male rats (Einarsson *et al.*, 1973; Gustafsson & Stenberg, 1974a).

A third type of regulation pattern was seen for the renal nuclear 5 α -reductase activity. In this case the enzyme activity was practically unaffected by age, sex, castration and treatment with testosterone but markedly changed after treatment with oestradiol. A similar type of regulation also characterized the other renal enzyme studied, 17 β -hydroxy steroid reductase.

The present study gives further evidence that the hepatic microsomal and nuclear 5 α -reductase enzyme activities represent two separate enzyme systems subjected to different regulatory mechanisms. As Gustafsson & Pousette (1974) pointed out, it is difficult to consider hepatic nuclear 5 α -reductase as an enzyme activating androgens during their transport from cytoplasm to nuclei, since female rats, which respond much less well to androgen treatment than male rats with respect to androgen-regulated hepatic enzymes (Berg & Gustafsson, 1973; Gustafsson & Stenberg, 1974b), have a higher nuclear 5 α -reductase activity than the male counterparts. It is possible that some other metabolite of testosterone than 5 α -dihydrotestosterone is the physiologically active androgen in liver tissue and that the high nuclear 5 α -reductase activity in female nuclei protects the nuclear androgen target sites from active androgens. Possibly the nuclear 5 α -reductase in kidney might serve a similar function. Recent findings seem to indicate that testosterone rather than 5 α -dihydrotestosterone is the physiologically active androgen in kidney tissue (Bardin *et al.*, 1973). The marked increase in renal nuclear 5 α -reductase activity and significant decrease in 17 β -hydroxy steroid reductase activity after treatment with oestradiol benzoate may be interpreted as an anti-androgen-induced change in the nuclear androgen metabolism, from pathways leading to testosterone towards pathways leading to less active 5 α -reduced 17-oxo steroids.

In conclusion, the present work indicates that the nuclear 5 α -reductase activities in rat liver, kidney and prostate are subject to distinctly different regulatory control mechanisms. These results are difficult to reconcile with the hypothesis that androgen action in peripheral target organs generally is mediated by 5 α -dihydrotestosterone, formed by androgen-dependent nuclear and/or cytoplasmic 5 α -reductases. The general applicability of the findings on the mechanism of action of androgens in the prostate should be judged with caution until more information is available about regulation of androgen metabolism in other target organs.

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