Journal of Steroid Biochemistry, Vol. 9, pp. 585–593

0022-4731/78/0701-0585$02.00/0

ANDROGENIC REGULATION OF A TISSUE SPECIFIC ISOENZYME OF ACID PHOSPHATASE IN RAT VENTRAL PROSTATE

PAUL S. RENNIE*, NICHOLAS BRUCHOVSKY and SHELLY L. HOOK
Department of Medicine, University of Alberta, Edmonton, Alberta, Canada T6G 2G3

(*Received* 8 *November* 1977)

SUMMARY

Acid phosphatase of rat ventral prostate is resolved by polyacrylamide gel electrophoresis, into a major isoenzyme (A), and 2 minor ones (B1 and B2), essentially as described by Tenniswood and co-workers [text, ref. 10]. Tissue specific B1 enzyme disappears within 2 days after castration. Both high and low doses (respectively, 400 μg and 40 μg/100 g BW) of dihydrotestosterone and 3β-androstanediol are able to maintain B1 enzyme if treatment is started at the time of castration. However, only a high dose of dihydrotestosterone prevents the involution of prostate, indicating a differential response for the maintenance of B1 enzyme and glandular size. If treatment commences 7 days after castration and consists of a daily high dose of dihydrotestosterone, cell proliferation in the prostate begins on the 2nd day and is sharply curtailed on the 5th day, but 12 days elapse before B1 enzyme is restored to the tissue. In contrast, if treatment consists of a daily low dose of dihydrotestosterone or a daily high dose of 3β-androstanediol, B1 enzyme is restored to the tissue on the 4th day in the absence of any significant change in DNA content or weight of prostate. Finally, if B1 enzyme is induced in regressed prostate by the daily administration of a high dose of 3β-androstanediol for 4 days, it is not maintained by the subsequent daily administration of a high dose of dihydrotestosterone. Two conclusions follow from these results. First, since the induction of B1 enzyme by high doses of 3β-androstanediol is mimicked by low doses of dihydrotestosterone, and since 3β-androstanediol is partly metabolized to dihydrotestosterone, it is extremely unlikely that the effects of 3β-androstanediol are independent of those of dihydrotestosterone. Second, the specific type of prostatic response, elicited by the administration of dihydrotestosterone, depends on concentration of hormone. At a low dose, dihydrotestosterone induces B1 enzyme; at a high dose, B1 enzyme is not induced but the prostate undergoes extensive growth.

INTRODUCTION

Androgens secreted by the gonads and the adrenal glands are metabolized by peripheral hormone-sensitive tissues to dihydrotestosterone† in amounts roughly proportional to biological strength [1–6]. Consequently, it seems reasonable to infer that dihydrotestosterone alone accounts for the action of a variety of natural androgens [2]. But evidence supporting this view is not complete enough to exclude the possibility that some androgens act entirely on their own. For example, from observation of the effects of androgens on organ cultures of rat ventral prostate, it has been suggested that certain features of cellular differentiation, such as the maintenance of epithelial height and the stimulation of secretion, are fostered by 3β-androstanediol, whereas cell proliferation is controlled by dihydrotestosterone [7–9]. In the present study, a similar approach was taken to examine the relationship between androgens and the *in vivo* regulation of prostatic secretion and growth. This entailed a comparison of the effects of 3β-androstanediol and dihydrotestosterone on the induction and maintenance of the secretory form of acid phosphatase recently described by Tenniswood *et al.*[10], in relation to regression and regeneration of prostatic tissue.

MATERIALS AND METHODS

Experimental animals. Male Wistar rats, weighing 250–300 g, were castrated via the scrotal route while under ether anaesthesia. The animals received daily subcutaneous injections of dihydrotestosterone, testosterone, 3β-androstanediol or 3α-androstanediol, in approximately 0.6 ml of an aqueous solution consisting of 10% (vol./vol.) ethanol and 10% (vol./vol.) polyoxyethylene sorbitan monopalmitate (Tween 40, Sigma Chemical Co., St. Louis, MO). The dose administered was in the range of 4-400 μg/100 g BW. Details of the treatment are given in the text.

Preparation of tissue extracts. All procedures were performed at 0–4°C. The pooled ventral prostates from 3–10 rats were minced finely with scissors and homogenized in 3–5 ml of 0.05 M acetate buffer, pH 4.8, with 25 strokes of a large clearance and 10 strokes of a small clearance ball-type Dounce apparatus. After centrifugation at 1000 *g* for 10 min (Sorvall

* Recipient of a National Cancer Institute of Canada Scholarship.

† The trivial names used are: 3β-androstanediol, 5α-androstan-3β, 17β-diol; 3α-androstanediol, 5α-androstan-3α, 17β-diol; dihydrotestosterone, 5α-androstan-17β-ol-3-one; testosterone, 4-ene-androsten-17β-ol-3-one.

GLC-1; HL-4 rotor), the supernatant, containing both intracellular and intraluminal secretions, was decanted, subjected to 3 cycles of freezing and thawing according to the method of Harding and Samuels[11], and forced under positive nitrogen pressure through a Metricel (0.45 mμ) filter (Gelman Instruments, Ann Arbor, MI). The filtrates were made to 33% glycerol (vol./vol.) and then either assayed for acid phosphatase activity immediately or stored at −20°C. There was no appreciable change in acid phosphatase activity in the extracts during a storage period of 3–4 weeks. In the present experiments the assays were performed within 1 week after preparation of the filtrates. To estimate the DNA content of tissue, DNA determinations were performed on the 1000 *g* crude nuclear pellet.

Gel electrophoresis of acid phosphatase. Prostatic acid phosphatase isoenzymes were separated using cationic polyacrylamide disc gels described by Williams and Reisfeld[12] and Tenniswood *et al.*[10]. The gels were composed of a 7.5% polyacrylamide separating gel, pH 4.3, and a 2.5% polyacrylamide stacking gel, pH 6.7. The separating gel was polymerized by the addition of 0.35% ammonium persulphate. The ammonium persulphate was subsequently removed by running the gels at 3 milliamps/gel for 2 h prior to the loading of the stacking gel. Riboflavin was used to polymerize the stacking gel. The running buffer consisted of β-alanine (3.12 g/l) and acetic acid (0.8 ml/l) at pH 4.5. Samples of tissue filtrate containing 50–400 μg of protein in 25–150 μl of 0.05 M acetate buffer with 33% glycerol, pH 4.8, were layered onto the stacking gels. These were carefully overlayed with the running buffer, and electrophoresed for 2.5 h at 4°C with a current of 4 milliamps/gel.

Staining of specific acid phosphatase bands in the gels was accomplished by the technique of Pais *et al.*[13]. The gels were first incubated for 30 min at 25°C in 0.05 M acetate buffer, pH 4.8, and then for 1.5 h at 25°C with 10 ml of the same buffer containing fast garnet GBC (1 mg/ml) and α-naphthyl phosphate (1 mg/ml). At the conclusion of the staining interval, the gels were rinsed several times with 7% acetic acid and stored in acetic acid solution at 0–4°C. No appreciable fading of the gel bands was observed during a period of 8 weeks.

Acid phosphatase determinations. The procedure used routinely was as follows: 1 ml of 0.2% (wt/vol.) *p*-nitrophenyl disodium phosphate in 0.05 M acetate buffer, pH 4.8, was incubated at 37°C for 10 min. Tissue filtrate, (0.2 ml), was then added, and the mixture was incubated at 37°C for 30 min. The reaction was stopped by the addition of 3.8 ml of 0.1 N NaOH. Measurement of the absorbance at 415 nm of the reaction mixture yielded an estimate of the amount of *p*-nitrophenol formed. The enzyme activity was expressed as moles of *p*-nitrophenol formed per h and normalized on the basis of tissue protein or DNA.

Other analytical procedures. Protein was measured by the method of Lowry *et al.*[14] using bovine serum albumin as the reference standard. DNA determinations were done by the diphenylamine method [15] with calf thymus DNA as the standard.

RESULTS

Effects of castration on acid phosphatase

Ventral prostate of normal rats contains 3 forms of acid phosphatase as shown by the results in Fig. 1a. The isoenzymes are resolved by polyacrylamide gel electrophoresis into a major slow moving component (A) and two fast moving components (B1 and B2). These results differ slightly from the findings of Tenniswood *et al.*[10] who observed only one fast moving component equivalent to B1 enzyme. This difference is probably explained by our observation that the recovery of B2 enzyme is variable. On the other hand, B2 enzyme does appear to be dependent on androgens since it is never found in prostates from castrated untreated rats (Fig. 1b). Although Tenniswood *et al.* allowed a castration interval of 2 weeks to effect the disappearance of B1 enzyme, in the present study it was found that a short interval of only 2 days is sufficient to produce the same result (Fig. 1c). On the other hand, A enzyme is present at all times even when the period of castration is 7 days or longer. Furthermore, A enzyme is present in kidney and liver (Fig. 1e, f), whereas B1 enzyme is only found in the ventral prostate. Comparison of the levels of total acid phosphatase in prostatic extracts from normal and castrated rats affords a means of estimating the amount of B1 enzyme relative to the total. The results in Table 1 indicate that the specific activity of total acid phosphatase is unchanged 2 days after castration but increases almost threefold by 7 days. However, when the results are normalized on the basis of DNA content there is no significant change in specific activity of total enzyme after castration. Since the loss of B1 enzyme from the prostate is not reflected in any significant alteration in overall specific activity, B1 enzyme is clearly a minor component of the total acid phosphatase in the prostate.

Maintenance of acid phosphatase

Since the B1 enzyme disappears upon androgen withdrawal, experiments were carried out to determine whether therapy with androgens preserves this activity. Rats were castrated and immediately treated with a high (400 μg/100 g BW) and a low (40 μg/100 g BW) dose of dihydrotestosterone or 3β-androstanediol administered daily by subcutaneous injection. After 7 days of treatment, the ventral prostates were removed and examined for acid phosphatase activity. The results presented in Fig. 2 indicate that with such therapy it is possible to maintain the B1 form of acid phosphatase regardless of whether dihydrotestosterone or 3β-androstanediol is given. Testosterone is equally potent in this respect (data not shown). No relationship exists between the maintenance of B1

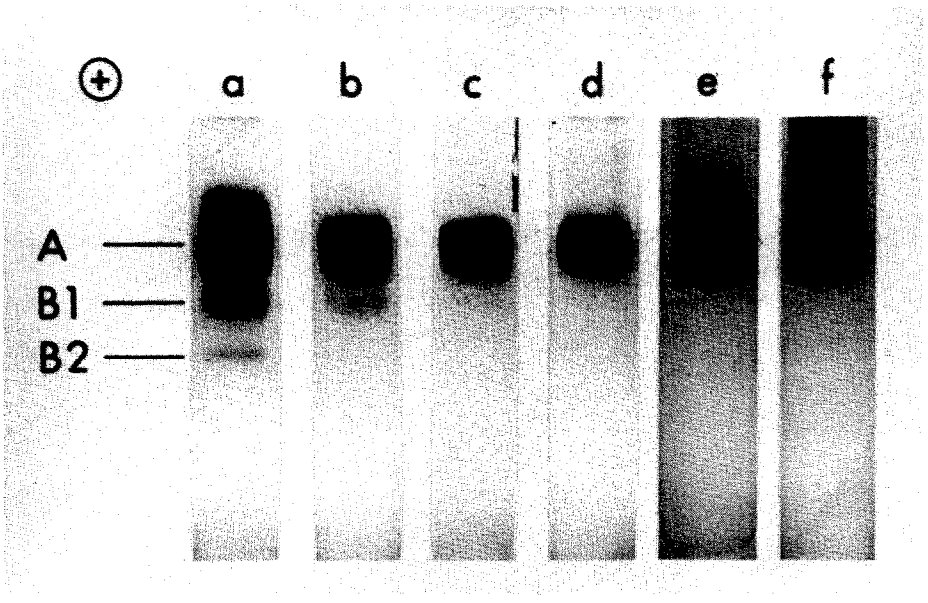

Fig. 1. Gel electrophoresis of prostatic acid phosphatase from normal and castrated rats. Samples consisting of 150 μg of prostatic extract protein, prepared as described in the "Materials and Methods" section, were fractionated in 7.5% (wt/vol.) cationic polyacrylamide gels by applying a current of 4 milliamp/gel for 2.5 h. Specific acid phosphatase bands were stained with a solution of fast garnet GBC (1 mg/ml) and α-naphthyl phosphate (1 mg/ml) in 0.05 M acetate buffer, pH 4.8. Prostatic extracts from (a) normal rats and rats (b) 1 day, (c) 2 days, and (d) 7 days after castration. Kidney (e) and liver (f) extracts prepared from normal rats.

enzyme and the maintenance of prostatic weight and DNA content (Table 2). Each parameter is maintained at the corresponding normal level by dihydrotestosterone at a dose of 400 μg/100 g BW) and at 50% of the normal level at a dose of 40 μg/100 g BW. In contrast, neither parameter is maintained by 3β-androstanediol and regression of prostate is observed. Assuming that 3β-androstanediol is converted in part to dihydrotestosterone [2–5], these findings strongly suggest that less dihydrotestosterone is required for the preservation of B1 enzyme than is necessary for the maintanance of organs weight and DNA content.

Regeneration of prostate: dihydrotestosterone versus 3β-androstanediol

When the number of cells in the ventral prostrate is below normal, the administration of dihydrostestosterone or testosterone to castrated animals stimulates the initiation of DNA synthesis and cell-proliferation [16–18]. On the other hand, 3β-androstanediol is without effect on DNA synthesis when added to organ cultures of rat ventral prostate [7–9]. To extend these observations, we checked for a stimulatory action of 3β-androstanediol *in vivo*, expecting that such might be observed in a dose-response or a time-course experiment.

(1) *Dose-response.* Dihydrotestosterone and 3β-androstanediol were administered daily by subcutaneous injection to animals castrated 7 days previously in doses ranging from 10 to 400 μg/100 g BW. On the 4th day, prostatic tissue was recovered, weighed and analyzed for DNA content. From the results presented in Fig. 3, it is clear that 3β-androstanediol has no effect on prostatic weight even at the highest dose tested. In contrast, at all doses of dihydrotestosterone above 40 μg/100 g BW, a significant increase in prostatic weight is observed. Analysis of the DNA content of regenerating tissue also yields results in keeping with the difference in the potency of the two agents (data not shown).

(2) *Time-course.* Dihydrotestosterone and 3β-androstanediol at a dose of 400 μg/100 g BW were administered daily by subcutaneous injection to animals castrated 7 days previously. The changes in weight and DNA content over a period of 14 days are shown in Fig. 4. As expected [17] dihydrotestosterone stimulates a marked hypertrophic and hyperplastic response such that increased levels of prostatic DNA and weight are seen after 4, 7, and 12 days of treatment. In contrast, 3β-androstanediol produces little

Table 1. Effects of castration on acid phosphatase activity

Time after castration (days)	Activity (μmol h^{-1}) per mg protein	per mg DNA
2	1.77 ± 0.20	47.0 ± 5.0
7	4.42 ± 0.43	53.0 ± 9.0
Control (non-castrated)	1.64 ± 0.12	53.0 ± 6.0

Prostatic tissue was recovered from groups of 3–6 male rats (250–300 g) castrated 2 and 7 days previously, and also from control non-castrated rats. Tissue extracts were analyzed for total acid phosphatase using ρ-nitrophenyl phosphate as substrate. The values shown represent the mean (±S.E.) of the results of at least 3 separate experiments.

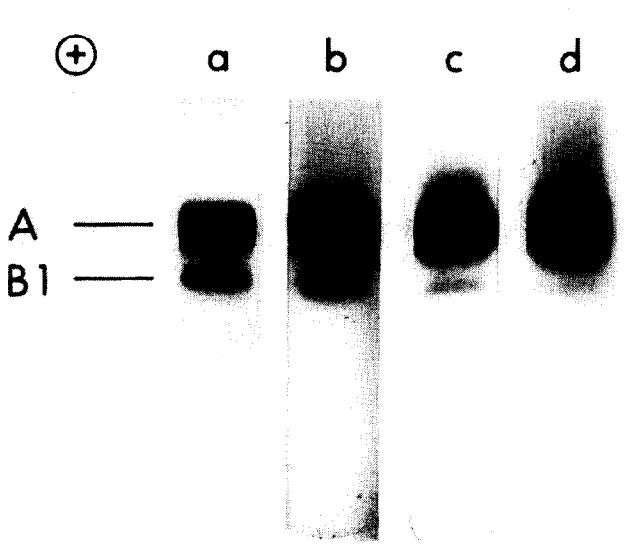

Fig. 2. Gel electrophoresis of prostatic acid phosphatase from castrated rats maintained by androgens. Groups of 3–6 male rats (250–300 g) were castrated and immediately started on a course of daily injections of androgens. After 7 days the ventral prostates were removed, homogenized, and analyzed for acid phosphatase using the electrophoresis technique described in the legend to Fig. 1. Samples consisting of 150 μg of prostatic extract protein from rats treated with dihydrotestosterone at doses of (a) 400 μg and (b) 40 μg/100 g BW and with 3β-androstanediol at doses of (c) 400 μg and (b) 40 μg/100 g BW.

change in DNA content and organ weight over control levels after 4 or 7 days of treatment, but after 12 days of treatment slight increases in both parameters are observed. These results confirm that 3β-androstanediol behaves strictly as a weak androgen when assessed on the basis of its ability to stimulate prostatic regeneration.

Appearance of acid phosphatase in regenerating prostate

(1) *Effect of dihydrotestosterone.* In order to determine whether B1 enzyme is restored in androgen stimulated prostate, animals castrated 7 days previously were treated daily for 14 days with dihydrotestosterone and 3β-androstanediol at a dose of 400 μg/100 g BW. The effect of dihydrotestosterone on the electrophoretic pattern of acid phosphatase activity is shown in Fig. 5. B1 enzyme is detected on the 12th day of treatment, corresponding to a time when DNA synthesis is reduced to a low level [19]. During this period of treatment, the acid phosphatase activity, expressed on the basis of DNA content, remains constant; however, the activity, on the basis of protein content, declines to 1.74 mol h^{-1} mg^{-1} by the 12th day, an essentially normal level (Table 1). If animals are treated with testosterone or with 3α-androstanediol then, as with dihydrotestosterone, 12 days elapse before B1 enzyme is detected in prostate (data not shown).

(2) *Effect of 3β-androstanediol.* In contrast to the foregoing results, 3β-androstanediol stimulates the return of B1 enzyme within 4 days (Fig. 6). Thus, under *in vivo* conditions, 3β-androstanediol restores the B1 form of acid phosphatase earlier than the more potent androgens, dihydrotestosterone, testosterone,

Table 2. Maintenance of prostatic weight and DNA by androgens

Treatment	Dose (μg/100 g BW)	Weight* (% of control)	DNA*
Dihydrotestosterone	400	103	104
	40	51	54
3β-Androstanediol	400	31	60
	40	20	32
None	—	17	21
Control (non-castrated)		100 (300 ± 17)	100 (282 ± 28)

* Per prostate.

Groups of 3–6 male rats (250–300 g) were castrated and started immediately on daily doses for 7 days of either dihydrotestosterone or 3β-androstanediol in the amounts indicated. Following treatment, the ventral prostates were removed, weighed and analyzed for DNA content. The results are expressed as per cent of non-castrated control values (shown in brackets as mean ± S.E. in mg and μg for weight and DNA respectively).

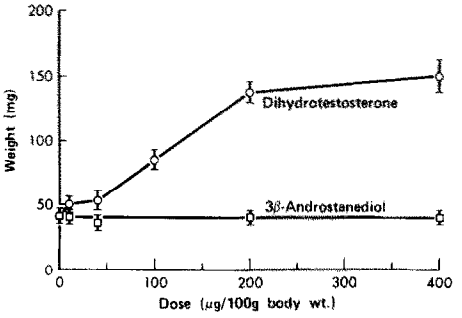

Fig. 3. Effects of dose of androgen on the weight of ventral prostate. Male rats (250–300 g) castrated 7 days previously were treated daily with various doses of dihydrotestosterone or 3β-androstanediol. After 4 days of treatment, the animals were sacrificed and ventral prostates weighed. Results are shown as mean ± S.E. of 8–15 determinations. Treatment: ○, dihydrotestosterone; □, 3β-androstanediol.

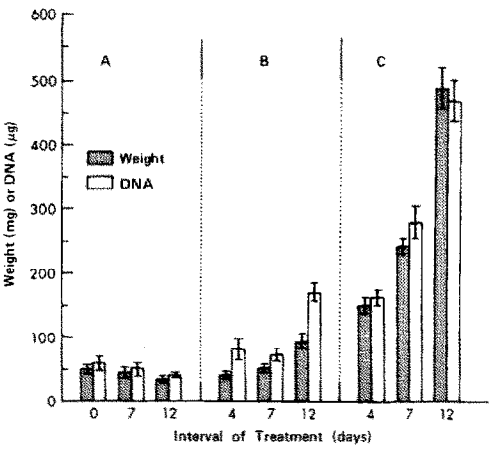

Fig. 4. Time course of androgen effects on the weight and DNA content of ventral prostate. Male rats (250–300 g) castrated 7 days previously were treated with daily injections of vehicle alone (castrated controls), or 400 μg/100 g BW of either 3β-androstanediol or dihydrotestosterone. After 4, 7, or 12 days of treatment the ventral prostates from groups of 3–7 animals were removed, weighed and analyzed for DNA content. The results are expressed as mean ± S.E. of at least 3 separate determination. Treatment: A, vehicle alone; B, 3β-androstanediol; C, dihydrotestosterone.

and 3α-androstanediol. Also the B1 enzyme continues to be present in prostate after 7, 12, and 14 days of treatment with 3β-androstanediol, even though the specific activity of total acid phosphatase (approximately 4 mol h^{-1} mg^{-1}) is not different than the specific activity in unstimulated prostate of animals castrated 7 days previously (Table 1). These results indicate that the appearance of B1 enzyme is not conditional on cell proliferation; in addition, they raise the possibility that 3β-androstanediol is a more potent inducer of B1 enzyme than dihydrotestosterone.

Early induction of B1 enzyme by dihydrotestosterone

Since *in vivo* there is a slight but significant conversion of 3β-androstanediol to dihydrotestosterone [2–5], we assumed that the unusual effect of 3β-androstanediol on the induction of B1 enzyme might be reproduced by low dose of dihydrotestosterone. Accordingly, animals castrated 7 days previously were treated daily with dihydrotestosterone in doses ranging from 4 to 400 μg/100 g BW. After 4 days of treatment, the prostatic tissue was analyzed for acid phosphatase activity. The results are shown in Fig. 7. At doses of less than 10 μg or greater than 100 μg/100 g BW, B1 enzyme is not detected, but at a dose of 40 μg/100 g BW its presence is clear. Furthermore, loading up to 500 μg of protein failed to

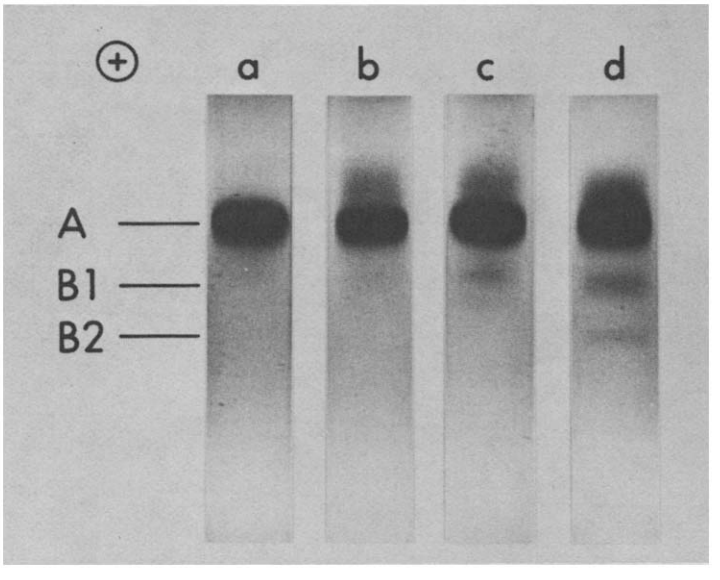

Fig. 5. Gel electrophoresis of prostatic acid phosphatase from castrated rats treated with dihydrotestosterone. Samples consisting of 180 μg of prostatic extract protein, obtained from 7-day castrated rats treated with dihydrotestosterone (400 μg/100 g BW) for (a) 4 days, (b) 8 days, (c) 12 days, or (d) 14 days, were analyzed for acid phosphatase activity by gel electrophoresis.

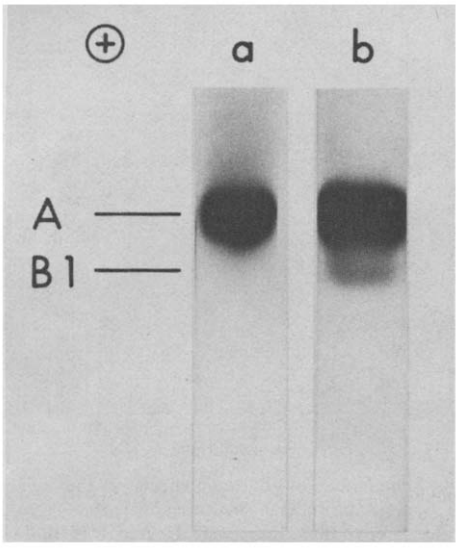

Fig. 6. Gel electrophoresis of prostatic acid phosphatase from castrated rats treated with 3β-androstanediol. The experimental conditions were identical to those described in the legend to Fig. 5, except that the animals were injected with 3β-androstanediol (400 μg/100 g BW) for either (a) 2 or (b) 4 days.

reveal any B1 enzyme activity in the negative samples. In parallel experiments using 3β-androstanediol, B1 enzyme is only detected when the amount of steroid injected is greater than 200 μg/100 g BW. Finally, in all instances the levels of total acid phosphatase, whether expressed on the basis of protein content, or on the basis of DNA content, resemble those of unstimulated prostate from animals castrated 7 days previously.

Thus, the specific effect of 3β-androstanediol on the induction of B1 enzyme is mimicked by dihydrotestosterone at a low concentration. About 10 times less dihydrotestosterone is required to elicit a comparable response.

Treatment with combined androgens

The possibility that 3β-androstanediol acts independently to induce B1 enzyme was further investigated in experiments using combinations of 3β-androstanediol and dihydrotestosterone for therapy. We assumed that if 3β-androstanediol is able to induce B1 enzyme without being converted to dihydrotestosterone, then concurrent injections of dihydrotestosterone at high doses should have no effect on enzyme induction. Outlines of the experimental approaches are shown in Table 3, and the results are summarized in Fig. 8. In the first experimental approach (Table 3A) animals castrated 7 days previously were treated with dihydrotestosterone alone (experimental group A.1), or with combinations of 3β-androstanediol and dihydrotestosterone (experimental groups A.2 and A.3) in 1 cycle of therapy lasting 7 days. At the end of this cycle, no B1 enzyme is detectable in prostate from any of the treatment groups (data not shown). Furthermore, as indicated by the results in Fig. 8A, the DNA content of prostate in experimental groups A.2 and A.3 is similar to that in experimental group A.1. The mean prostatic weight, however, is slightly lower in experimental groups A.2 and A.3 compared to that in experimental group A.1. These results imply that 3β-androstanediol is neither able to induce B1 enzyme in the presence of high concentrations of dihydrotestosterone, nor is it able to significantly inhibit prostatic growth induced by dihydrotestosterone.

In the second experimental approach, we studied

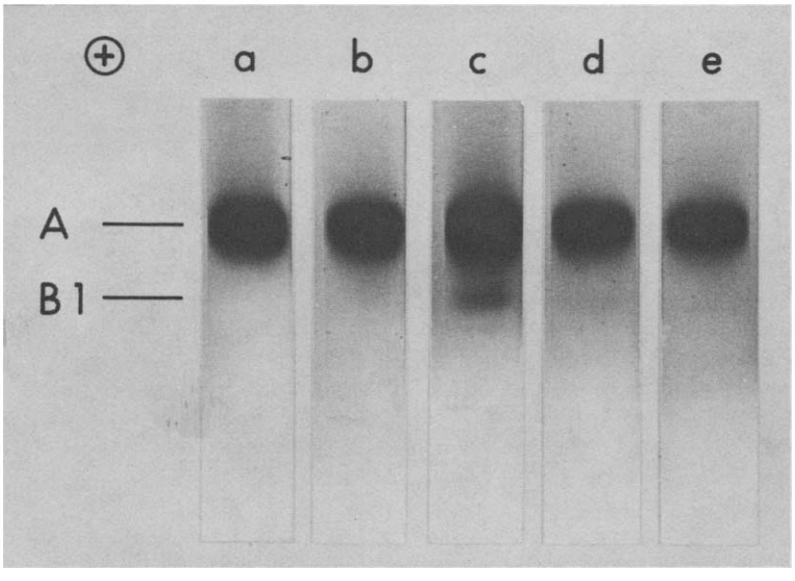

Fig. 7. Early induction of B1 enzyme by dihydrotestosterone. Male rats (250–300 g) castrated 7 days previously were treated for 4 days with daily injections of dihydrotestosterone at doses ranging from 4–200 μg/100 g BW. Samples consisting of 150 μg of prostatic extract protein were analyzed for acid phosphatase by gel electrophoresis. Dihydrotestosterone administered: (a) 4 μg, (b) 10 μg, (c) 40 μg, (d) 100 μg, (e) 200 μg/100 g BW.

Table 3. Schedule of treatment with combined androgens

Experiment	Group	Cycle 1 Treatment	Cycle 1 Dose (μg/100 g BW)	Cycle 1 Interval (days)	Cycle 2 Treatment	Cycle 2 Dose (μg/100 g BW)	Cycle 2 Interval (days)
A.	1	dihydrotestosterone	400	7			
	2	3β-androstanediol	400	7			
		+dihydrotestosterone	400				
	3	3β-androstanediol	800	7			
		+dihydrotestosterone	400				
B.	1	vehicle	—	4	dihydrotestosterone	400	7
	2	3β-androstanediol	400	4	dihydrotestosterone	400	7
	3	3β-androstanediol	400	4	3β-androstanediol	400	7
					+dihydrotestosterone	400	
	4	3β-androstanediol	400	4	3β-androstanediol	800	7
					+dihydrotestosterone	400	

In experiments employing only 1 cycle of therapy (A), animals castrated 7 days previously were treated with dihydrotestosterone alone (group A.1), or with combinations of 3β-androstanediol and dihydrotestosterone (groups A.2 and A.3). The animals were injected daily and the therapy was continued for 7 days.

In experiments employing 2 cycles of therapy (B), animals castrated 7 days previously were treated in the 1st cycle, daily for 4 days, with 3β-androstanediol. During the 2nd cycle lasting 7 days, experimental group B.1 received dihydrotestosterone, whereas experimental groups B.2, B.3, and B.4 received combinations of 3β-androstanediol and dihydrotestosterone as indicated.

At the end of each course of therapy, ventral prostates were recovered, weighed and analyzed for DNA content and acid phosphatase.

the fate of B1 enzyme induced by 3β-androstanediol during a subsequent round of cell proliferation induced by dihydrotestosterone. Animals castrated 7 days previously were treated in 2 cycles as described in Table 3B. During the 1st cycle lasting 4 days, each group received 3β-androstanediol in order to induce B1 enzyme. During the 2nd cycle lasting 7 days, experimental group B.1 received dihydrotestosterone, whereas experimental groups B.2, B.3, and B.4 received combinations of 3β-androstanediol and dihydrotestosterone. At the end of the 2nd cycle no B1 enzyme is detectable in prostate from any of the treatment groups (data not shown). Also, the results presented in Fig. 8B demonstrate that the DNA content and mean weight of prostate in experimental groups B.2, B.3, and B.4, receiving both 3β-androstanediol and dihydrotestosterone, are about identical to those in experimental group B.1, receiving only dihydrotestosterone during the 2nd cycle of therapy. Two conclusions can be drawn from these findings: first, B1 enzyme activity is suppressed in prostatic cells stimulated to grow by high doses of dihydrotestosterone; second, the initial presence of B1 enzyme does not imply a degree of tissue differentiation sufficient to retard or prevent the growth of prostate in response to dihydrotestosterone.

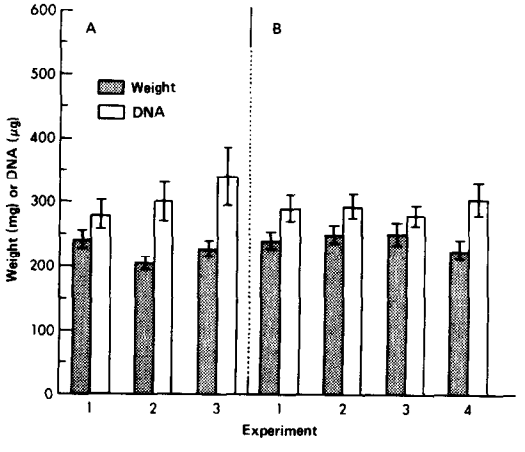

Fig. 8. Treatment with combined androgens. Groups of 3–6 male rats (250–300 g) castrated 7 days previously were treated with combinations of androgens according to the schedule in Table 3. Following the treatments, the ventral prostates were removed, weighed, and analyzed for DNA content. The results are expressed as mean ± S.E. of at least 4 determinations. The numbers of the abscissa correspond to the experiments listed in Table 3.

DISCUSSION

Although it was originally believed that the acid phosphatase of rat prostate is unaffected by castration [20, 21], it is now known that only the lysosomal form of the enzyme is insensitive to hormones [22], while the secretory form is hormone-dependent and declines after castration [10, 23]. Tenniswood *et al.*[10], were able to distinguish between the two types of acid phosphatase on the basis of sensitivity to inhibition by tartrate and formaldehyde, and of relative mobility in cationic polyacrylamide gels. In the present investigation we used the latter method to follow changes in acid phosphatase activity during prostatic involution and regeneration.

Our results confirm the findings of Tenniswood *et al.*[10] in that we observe 2 major forms of acid phosphatase in ventral prostate of normal rats (Fig. 1a); occasionally, a 3rd form not previously described, is also observed (Fig. 1a). The B1 form of acid phosphatase is hormone dependent (Fig. 1c), tissue specific (Fig. 1e, f), and is maintained by androgens if treat-

ment is started immediately after castration (Fig. 2). However, B1 enzyme is a minor component of the total acid phosphatase in prostate, since its disappearance is not reflected in a significant decrease in the specific activity of the total enzyme (Table 1).

The disappearance of B1 enzyme from prostate 2 days after castration is not associated with a decrease in the number of cells per gland nor in the weight. Although one can preserve both the B1 enzyme and the size of the prostate with high doses of dihydrotestosterone, treatment with 3β-androstanediol or with low doses of dihydrotestosterone, maintains the enzyme without maintaining either weight or DNA content (Table 2). Thus, the hormonal mechanisms responsible for involution or maintenance of prostate are probably different from those which regulate B1 enzyme.

The induction of B1 enzyme in castrated animals occurs only when there is little or no glandular growth. This condition is fulfilled when animals castrated 7 days previously are treated with high doses of 3β-androstanediol (Fig. 6) or low doses of dihydrotestosterone (Fig. 7). Both types of therapy induce B1 enzyme within 4 days without causing enlargement of the prostate. On the other hand, if growth is induced with high doses of dihydrotestosterone, the appearance of B1 enzyme is delayed until the 12th day. The reciprocal relationship between enzyme induction and cell proliferation is further demonstrated by the results of experiments in which castrated animals are treated with combinations of 3β-androstanediol and dihydrotestosterone (Table 3; Fig. 8). These results indicate that B1 enzyme is not maintained in glandular tissue undergoing rapid growth. It follows that even in the presence of B1 enzyme, the state of differentiation of prostatic tissue, may not be sufficiently advanced to be incompatible with further rounds of cell division.

Results of *in vitro* studies with organ cultures of rat prostate indicate that 3β-androstanediol maintains epithelial height and secretory activity but has no stimulatory effect upon DNA synthesis [7–9]. However, this androgen is partly metabolized to dihydrotestosterone [2–5], and since dihydrotestosterone itself is able to maintain the differentiation of cultured prostates, it is not entirely certain that 3β-androstanediol independently promotes cellular differentiation. In the present study we found that 3β-androstanediol, at a dose of 400 μg/100 g BW, restores B1 enzyme more rapidly than an equal dose of dihydrotestosterone (Figs. 5 and 6). However, at a dose of only 40 μg/100 g BW, dihydrotestosterone mimics the effect of 3β-androstanediol (Fig. 7). Relevant to this observation is our previous finding that when equal amounts of dihydrotestosterone and 3β-androstanediol are injected into castrated rats, the resultant intranuclear concentration of dihydrotestosterone is 10 times lower after the injection of 3β-androstanediol than after the injection of dihydrotestosterone [2, 24]. Therefore, it is reasonable that a 400 μg dose of 3β-androstanediol would be equivalent to a 40 μg dose of dihydrotestosterone. Taken together, our evidence is consistent with the conclusion that the induction of B1 enzyme by 3β-androstanediol is mediated by a low level of dihydrotestosterone formed as a result of the metabolism of 3β-androstanediol.

The finding that a small amount of dihydrotestosterone rapidly restores B1 enzyme, whereas a large amount stimulates prostatic hyperplasia and hypertrophy but inhibits the induction of B1 enzyme, indicates a differential control of hormonal responsiveness. Further evidence in support of such a mechanism is drawn from the observations on the androgen requirements for the maintenance of B1 enzyme (Fig. 2), and the maintenance of prostatic weight and DNA content (Table 2). Thus, when the prostate is exposed to low levels of dihydrotestosterone it maintains or acquires the B1 form of acid phosphatase which is normally present in the mature gland. In the case of the involuted prostate, exposure to high concentrations of dihydrotestosterone both prevents the early induction of enzyme, and results in its suppression, if it has already been induced.

Although the cellular mechanisms controlling this differential response are unknown, one might speculate that it is related to the concentration of androgen receptors or acceptors present in the nucleus of the prostatic cell. Recently, it has been reported that a dose-dependent response also exists for the induction of ovalbumin and conalbumin by estrogen in the chick oviduct [25]. Thus, differential responses may be a general feature of steroid hormone action.

Acknowledgements—This work was supported by grants from the National Cancer Institute of Canada and the Medical Research Council of Canada (MT 3729). We thank Miss A. Koat for technical assistance and Dr. E. E. Baulieu and Dr. A. Clark for several helpful discussions.

REFERENCES

1. Bruchovsky N.: Comparison of the metabolites formed in rat prostate following the *in vivo* administration of seven natural androgens. *Endocrinology* **89** (1971) 1212–1222.
2. Bruchovsky N., Lesser B., Van Doorn E. and Craven S.: Hormonal effects on cell proliferation in rat prostate. *Vitamin. Horm.* **33** (1975) 61–102.
3. Becker H., Grabosch E., Hoffmann C. and Voigt K. D.: Metabolism and mode of action of androgens in target tissues of male rats. 3. metabolism of 5α-androstane-3,17-dione, of 5α-androstane-3α,17-diol and of 5α-androstane-3β, 17β-diol in target organs and peripheral tissues. *Acta endocr., Copenh.* **73** (1975) 407–416.
4. Horst H. J., Dennis M., Kaufmann J. and Voigt K. D.: *In vivo* uptake and metabolism of [^{3}H]-5α-androstane-3α, 17β-diol and of [^{3}H]-5α-androstane-3β, 17β-diol by human prostatic hypertrophy. *Acta endocr., Copenh.* **79** (1975) 394–402.
5. Krieg M., Horst H. J. and Sterba M. L.: Binding and metabolism of 5α-androstane-3α, 17β-diol and of 5α-androstane-3β, 17β-diol in the prostate, seminal vesicles and plasma of male rats: studies *in vivo* and *in vitro*. *J. Endocr.* **64** (1975) 529–538.

6. Baker H. W. G., Sonstein F. M., Eichner G. J., Sante R. J., Jefferson L. S. and Bardin C. W.: Perfusion of rat testes and accessory sex organs: a new method. *Endocrinology* **100** (1977) 699–708.
7. Baulieu E. E., Lasnitzki I. and Robel P.: Metabolism of testosterone and action of metabolites on prostate glands grown in organ culture, *Nature* **219** (1968) 1155–1156.
8. Lasnitzki I.: Some aspects of the aetiology and biochemistry of prostatic cancer. In *Third Tenovus Workshop* (Edited by K. Griffiths and C. G. Pierrepoint). Alpha Omega Alpha, Cardiff (1970) pp. 68–73.
9. Robel P., Lasnitzki I. and Baulieu E. E.: Hormone metabolism and action: testosterone and metabolites in prostate organ culture. *Biochemie* **53** (1971) 81–96.
10. Tenniswood M., Bird C. E. and Clark A. F.: Acid phosphatases: androgen dependent markers of rat prostate. *Can. J. Biochem.* **54** (1976) 350–357.
11. Harding B. W. and Samuels L. T.: A tissue fractionation study of rat ventral prostate: subcellular distribution of nucleic acids, succinate oxidizing systems, cytochrome c reductases, cytochrome oxidase and acid phosphatase. *Biochim. biophys. Acta* **54** (1961) 42–51.
12. Williams D. E. and Reisfeld R. A.: Disc electrophoresis in polyacrylamide gels: extension to new conditions of pH and buffer, *Ann. N.Y. Acad. Sci.* **121** (1964) 373–381.
13. Pais W. M., Mangold A. W. and Mahoney S. A.: Fractionation and purification of prostatic acid phosphatase. *Invest. Urol.* **12** (1974) 13–16.
14. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: Protein measurement with the folin phenol reagent, *J. biol. Chem.* **193** (1951) 265–275.
15. Burton K.: A study of the conditions and mechanism of the dephenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* **62** (1956) 315–323.
16. Coffey D. S., Shimazaki J. and Williams-Ashman H. G.: Polymerization of deoxyribonucleotides in relation to androgen-induced prostatic growth. *Archs biochem. Biophys.* **124** (1968) 184–198.
17. Lesser B. and Bruchovsky N.: Effect of duration of the period after castration on the response of the rat ventral prostate to androgens. *Biochem. J.* **142** (1974) 429–431.
18. Rennie P. S., Symes E. K. and Mainwaring W. I. P.: The androgenic regulation of the activities of enzymes engaged in the synthesis of deoxyribonucleic acid in rat ventral prostate gland. *Biochem. J.* **152** (1975) 1–16.
19. Lesser B. and Bruchovsky N.: The effects of testosterone, 5α-dihydrotestosterone and adenosine 3',5'-monophosphate on cell proliferation and differentiation in rat prostate. *Biochim. biophys. Acta* **308** (1973) 426–437.
20. Butler W. W. S. III and Schade A. L.: The effects of castration and androgen replacement on the nucleic acid composition, metabolism, and enzymatic capacities of the rat ventral prostate. *Endocrinology* **63** (1958) 271–279.
21. Bialy G. and Pincus G.: Hormonal influences on phosphatase activity of rat accessory sexual glands. *Endocrinology* **81** (1967) 1125–1131.
22. Vanha-Perttula T., Niemi R. and Helminen H. J.: Separate lysosomal and secretory acid phosphatases in the rat ventral prostate. *Invest. Urol.* **9** (1972) 345–352.
23. Helminen H. J., Ericsson J. L. E. and Arborgh B.: Differing patterns of acid phosphatase and cathepsin d activities in the rat ventral prostate gland during castration-induced prostatic involution. *Acta Endocr., Copenh.* **69** (1972) 747–761.
24. Bruchovsky N., Lesser B. and Rennie P.: Control of the concentration and distribution of dihydrotestosterone in prostatic cells. In *Normal and Abnormal Growth of the Prostate* (Edited by M. Goland). Charles C. Thomas, Springfield (1975), pp. 125–143.
25. Mulvihill E. R. and Palmiter R. D.: Relationship of nuclear estrogen receptor levels to induction of ovalbumin and conalbumin mRNA in chick oviduct. *J. biol. Chem.* **252** (1977) 2060–2068.