Short Communications

Effect of Duration of the Period after Castration on the Response of the Rat Ventral Prostate to Androgens

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If 5 α -dihydrotestosterone (17 β -hydroxy-5 α -androstan-3-one) is administered to castrated rats before prostatic atrophy has commenced, secretory activity of the prostate is stimulated, but not proliferation. Once the number of cells has fallen below normal, hormone induces proliferation until the normal cellular complement has been restored; then proliferation ceases, but secretory activity is stimulated.

The stimulation by androgens of various biochemical activities in male sex accessory tissues has been and continues to be a focus of considerable research. Biosynthetic processes that have been shown to be stimulated by androgens include DNA synthesis (e.g. Coffey *et al.*, 1968; Lesser & Bruchovsky, 1973), RNA synthesis (e.g., Wicks & Kenney, 1964; Williams-Ashman *et al.*, 1964; Liao & Lin, 1967; Mangan *et al.*, 1968; Mainwaring *et al.*, 1971, 1974), protein synthesis (Williams-Ashman *et al.*, 1964; Chung & Coffey, 1971; Mainwaring & Wilce, 1972), mitochondrial activity (Doeg *et al.*, 1972) and various enzyme activities (Brandes & Bourne, 1963; Kirchheim & Scott, 1965; Singhal & Valadares, 1968). In addition, mechanisms involved in the uptake and metabolism of androgens by these tissues are receiving considerable attention (Baulieu & Robel, 1970; Williams-Ashman & Reddi, 1972; King & Mainwaring, 1973). These studies have in general been performed by injecting hormone into castrated animals; little attention seems to have been paid to the length of time between castration and initiation of hormone treatment, and various investigators have employed intervals ranging from hours to weeks. It has been known for many years that androgens can elicit two types of cellular responses in the sex accessory tissues of castrated animals, namely proliferation (Burkhart, 1942) and cytological changes characteristic of secretory activity (Moore *et al.*, 1930). We now report that the particular response observed depends on the duration of the interval between castration and initiation of hormone treatment, and point out that this finding must be considered when one is studying mechanisms of hormone action.

The response of the rat ventral prostate to hormonal manipulation has been monitored by measuring wet weight and number of nuclei as indices of functional capacity, and by using rate of incorporation of [*Me*-³H]thymidine into DNA by prostatic minces *in vitro* and changes in number of nuclei per prostate as indicators of proliferative

activity, as described previously (Lesser & Bruchovsky, 1973).

In order to determine, for regeneration studies, the kinetics of atrophy of the prostate after castration, prostatic weight, number of nuclei per prostate and rate of incorporation of labelled thymidine into DNA were determined at various times after castration of adult male rats. Three stages in the atrophic process can be defined (Fig. 1, closed circles): first, at days 1–3, the rate of DNA synthesis is significantly lowered from normal (Fig. 1*a*), but prostatic weight (Fig. 1*c*) and nuclear content (Fig. 1*b*) are still within the normal range; secondly, at days 4–7, prostatic weight and nuclear content decline rapidly; and thirdly, after day 7, prostatic weight and nuclear content are maintained at about 10–15% of normal and rate of incorporation of thymidine is about 2% of the normal value.

The responses of the prostate in normal animals (0-day castrated animals) and in castrated animals during the three stages of atrophy defined above to administration of 5 α -dihydrotestosterone (17 β -hydroxy-5 α -androstan-3-one) were determined. When normal animals are castrated and hormone treatment is immediately started (Fig. 1, closed squares), neither rate of DNA synthesis (Fig. 1*a*) nor number of nuclei (Fig. 1*b*) is elevated ($P > 0.05$), but wet weight (Fig. 1*c*) is stimulated to increase significantly ($P < 0.01$ by day 2). Similar results are observed when 1-day castrated animals are treated with daily doses of hormone (Fig. 1, open triangles). The number of nuclei remains within the normal range, but prostatic weight increases significantly. Incorporation of thymidine remains depressed for 2 days, then increases to a value not more than twice the normal rate of incorporation. Thus, in animals whose prostates have not yet started to atrophy, the failure to observe significant stimulation of the rate of DNA synthesis above normal or an increase in number of nuclei indicates that the hormone does not induce proliferation under these conditions. The results obtained when 4-day castrated animals, whose

Fig. 1. Response of rat prostate to 5 α -dihydrotestosterone

Groups of three to seven rats were castrated on day 0. Animals were left untreated (●—●), or treatment with daily doses of 400 μ g of 5 α -dihydrotestosterone/100 g body wt. was commenced immediately (■—■) or 1 day (△—△), 4 days (○—○) or 7 days (□—□) after castration. Values for normal rats are shown as the zero-time points. At the appropriate time, the animals were killed and prostates removed and weighed. Rate of DNA synthesis was assayed *in vitro* and nuclei were isolated and counted as described previously (Lesser & Bruchovsky, 1973). Error bars, where shown, indicate the means $\pm$ S.E.M. for at least three separate determinations; otherwise each point represents a single experiment. All values are expressed as percentages of the corresponding value for normal animals, and the hatched areas represent the means $\pm$ S.E.M. for normal animals. (a) Rate of incorporation of [Me^3H]thymidine (measured as d.p.m./ μ g of DNA in 20 min); (b) number of nuclei per prostate; (c) prostatic wet weight.

prostates are in the process of atrophy, are treated with 5α -dihydrotestosterone are shown in Fig 1 (open circles). Atrophy continues for 1 day, then DNA synthesis is stimulated (Fig. 1a) and the number of nuclei starts to increase (Fig. 1b). Rate of DNA synthesis reaches a maximum at day 4 after onset of treatment and then declines, and the number of nuclei has returned to normal by day 5 after onset of treatment. After this time the diminished rates of DNA synthesis and of production of nuclei indicate that proliferation has essentially stopped. Wet weight, on the other hand, continues to increase beyond its normal value (Fig. 1c), and by day 7 after onset of treatment is 70% higher than normal. Finally, Fig. 1 (open squares) shows the response of 7-day castrated animals to daily administration of hormone. After a lag period of 24–36h, rate of incorporation of thymidine increases rapidly to a maximum, by day 3 of treatment, of about 10 times the normal value (Fig. 1a). Then, despite continued administration of hormone, the rate of incorporation declines, until by day 10 it has fallen to below the normal value and by day 14 to the value observed in control untreated castrated animals. Numbers of nuclei (Fig. 1b) and wet weights (Fig. 1c) are significantly above the control values ($P < 0.01$) by 36h after the beginning of the treatment, and increase rapidly between day 2 and day 5. By day 5, these parameters have returned to the normal values. After this time, the rate of increase of numbers of nuclei decreases to 25% of the former value, whereas weights continue to increase linearly until at least day 14.

The results presented in Fig. 1 indicate that the type of response elicited in the prostate of the castrated rat by androgens depends on the number of cells in the tissue relative to normal. If this number is normal, as in animals castrated less than 3 days previously, proliferative activity is not induced, but secretory activity, as indicated by increase in wet weight, is stimulated. If this number is below normal, as in animals castrated longer than 3 days previously, hormone does induce proliferation, but only until the number of cells has returned to normal. After this time, some homeostatic mechanism shuts off proliferation, but hormone continues to stimulate secretion. It is uncertain whether secretory activity is stimulated during the proliferative phase, or whether proliferation must be completed before secretion commences. A report by Schmid *et al.* (1973) indicates that in rat seminal vesicle the latter possibility is the case.

Hence it is evident that, when one is administering hormone to castrated animals, the duration of castration must be chosen carefully so as to produce the response desired. For studies of secretory function of the prostate, the animals of choice are short-term castrated animals (1 day or less) or long-term castrated animals whose prostates have completely

regenerated; in these cases, proliferation does not occur. For studies of proliferation, long-term castrated animals (7 days or more) should be used, and only during the period when the cellular complement is below normal or has just returned to normal. It is quite likely that the biochemical processes and the manner in which the tissue handles steroid are different in these different circumstances. If one uses animals whose prostates are in the process of atrophy (2–6 days after castration), then interpretation of results is difficult, because this is a very unstable period and then one cannot be as certain of the response elicited by hormone as during the periods mentioned above.

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