Ornithine Decarboxylase Activity and [125 I]Iododeoxyuridine Incorporation in Rat Prostate

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The relationship between ornithine decarboxylase activity and [125 I]iododeoxyuridine incorporation was studied in prostates from castrated rats (aged 5, 26 and 80 weeks) injected daily with testosterone for up to 10 days. The results suggest that ornithine decarboxylase activity is a parameter of secretory activity, rather than growth, in the ventral prostate. In the dorsolateral prostate, ornithine decarboxylase activity tends to parallel [125 I]iododeoxyuridine incorporation.

Ornithine decarboxylase (EC 4.1.1.17) is thought to be the rate-limiting step in the synthetic pathway of the polyamines putrescine, spermidine and spermine (Williams-Ashman *et al.*, 1972). Ornithine decarboxylase activity has been shown to increase during growth or regeneration, and in response to the administration of growth-inducing factors both *in vivo* and in cell culture (Roger *et al.*, 1974; Hogan *et al.*, 1974; Panko & Kenney, 1971; Russell & Snyder, 1969). Activity is high in tissues involved in rapid metabolism, such as neoplastic and embryonic tissues.

Ornithine decarboxylase activity is also very high in the rat ventral prostate, since the organ is engaged in the active secretion of polyamines. Castration results in a rapid decline in ornithine decarboxylase activity, which can be restored to normal values by the injection of testosterone (Pegg & Williams-Ashman, 1968) but not cyclic AMP (Mangan *et al.*, 1973). These findings, coupled with the fact that the enzyme is thought to have an extremely short half-life, would suggest that ornithine decarboxylase may be a sensitive and specific parameter of androgen action in the prostate.

In the present communication, we have examined the relationship between ornithine decarboxylase activity and DNA synthesis in ventral prostates from immature and adult castrated rats injected with testosterone. DNA-synthetic activity was monitored by using [125 I]iododeoxyuridine.

Materials and methods

Wistar rats aged 5, 26 and 80 weeks were used. Castration was performed via the scrotal route under light ether anaesthesia. After 7 days, testosterone (400 μ g/100 g body wt.) in propylene glycol (0.1 ml) was injected subcutaneously each day for up to 10 days. Animals were killed by cervical dislocation. The ventral prostate was quickly dissected free of fat and capsule *in situ*, excised and maintained on ice for a short time. A portion of the material was teased into

2 mm³ explants for incubation with [125 I]iododeoxyuridine. Some material was retained for routine histology, and the remainder homogenized. In the case of the 5-week-old animals, the testes were also examined histologically. Cross-sections of the tubules showed an immature pattern with predominantly early spermatids, although later stages were represented in a few regions.

5-[125 I]Iodo-2-deoxyuridine and L-[1- 14 C]ornithine monohydrochloride were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Pyridoxal 5'-phosphate, dithiothreitol and testosterone were obtained from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K.

[125 I]Iododeoxyuridine incorporation. Explants (approx. 20 mg) were incubated for 6 h with 2 μ Ci of [125 I]iododeoxyuridine (100 mCi/mg) in 3 ml of Eagle's basal medium. Explants were removed, blotted and weighed. Tissue was fixed in ethanolic Bouin's fluid for 24 h and then washed four times in 70% (v/v) ethanol to remove unbound 125 I (Shipman *et al.*, 1975). Samples were counted for radioactivity in a Gamma Set 500 isotope counter.

Ornithine decarboxylase activity. Tissue samples were homogenized in ice-cold water containing 1 mM-dithiothreitol, in a Teflon-glass pestle and mortar. Portions of the homogenate were taken for assay. The assay medium (1 ml total volume) contained L-[1- 14 C]ornithine monohydrochloride (220 000 d.p.m./assay tube), 1 mM-dithiothreitol, 0.1 mM-pyridoxal phosphate and 50 mM-sodium phosphate buffer, pH 7.2. The reaction vessel was a test tube (75 mm $\times$ 15 mm) with a side arm, containing 300 μ l of 5 M-H₂SO₄ as described by Fisher & Davies (1974). The rubber stopper of the tube was pierced by a glass tube, sealed at the upper end. A wick of rolled glass-fibre paper was inserted into the lower end and the exposed paper was impregnated with 10 μ l of 20% (w/v) KOH. Incubations were carried out in a shaking water bath at 37°C for up to 2 h. The reaction was halted and 14 CO₂ driven off by tipping acid from the side arm into the assay medium. A further incubation

for 30 min ensured complete absorption of the $^{14}\text{CO}_2$ by the wick.

The wick was then transferred to 10 ml of scintillation fluid [4 g of 2,5-diphenyloxazole, 0.129 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene/litre of toluene; toluene-Triton X-100, 3:2, v/v]. Acetic acid (30 μl) was added to each vial to avoid chemiluminescence caused by the KOH, and the glass-fibre discs were broken up by shaking.

Vials were counted for radioactivity in a Philips scintillation counter, with an efficiency of 75–80%. Boiled enzyme blanks were run to correct for non-enzymic decarboxylation, as well as water blanks.

Protein determination. Protein was determined by the method of Geiger & Bessman (1972), which corrects for the presence of dithiothreitol. Bovine serum albumin (Sigma) was used as standard.

Results and discussion

[^{125}I]Iododeoxyuridine is specifically incorporated into DNA and has provided a useful approach to measurements of cellular proliferation and cell loss, as the problems of reutilization inherent with [^3H]-

thymidine are lessened (Dethlefsen, 1971, 1974; Clifton & Cooper, 1973). It has an additional advantage that quantitative methods for morphological examination can be carried out after determination of the radioactive content of the tissue samples (Shipman *et al.*, 1975). In the present communication the incorporation of [^{125}I]iododeoxyuridine into prostates of immature and adult testosterone-treated castrated rats is reported.

Ventral prostates from three age groups of animals were used (5, 26 and 80 weeks). In the 80-week age group, the dorsolateral prostates were also examined. The results are shown in Fig. 1.

The pattern of incorporation of [^{125}I]iododeoxyuridine in ventral prostates from the testosterone-treated adult castrated rats (Figs. 1b and 1c) is similar to that described by Lesser & Bruchovsky (1973), using other parameters of DNA-synthetic activity. In the ventral prostate of immature rats (Fig. 1a) there is a sharp peak in the incorporation of [^{125}I]iododeoxyuridine on day 3 and the magnitude of the response is much greater than that seen in tissue from the older animals. This difference between tissue from immature and adult animals has also been

Fig. 1. Incorporation of [^{125}I]iododeoxyuridine (●) and ornithine decarboxylase activity (▲) in prostates from castrated rats injected with testosterone (400 $\mu\text{g}/100\text{g}$ body wt.)

(a) and (b) Ventral prostates from 5- and 26-week-old animals respectively; (c) and (d) ventral and dorsolateral prostates respectively from 80-week-old rats. Four to eight animals were used/point. Pooled material was used for ornithine decarboxylase activity. [^{125}I]iododeoxyuridine incorporation is given as means $\pm$ S.E.M. ($n = 6$). The vertical axis is the same for [^{125}I]iododeoxyuridine incorporation and ornithine decarboxylase activity in all graphs except (a).

noted in experiments with cultured prostate (L. J. Donaldson & G. H. Thomas, unpublished work).

In contrast with the dorsolateral prostate (Fig. 1d), ornithine decarboxylase activity in the ventral prostate does not correlate with [125 I]iododeoxyuridine incorporation. Thus tissues from the immature and 80-week-old testosterone-treated castrated rats show a very similar and apparently linear increase in ornithine decarboxylase activity with time, despite a 5-fold difference between the two groups in terms of [125 I]iododeoxyuridine incorporation.

The lack of a clear correlation between proliferative activity and ornithine decarboxylase activity is also shown by comparison of the number of metaphases accumulated *in vivo* with the enzyme concentration in fresh ventral prostate. The number of arrested metaphases accumulated/h per 10^3 epithelial cells after vincristine sulphate administration were 12.3 ± 0.7 and 0.56 ± 0.06 for immature (6 weeks) and young adult (26 weeks) rats respectively (L. J. Donaldson & G. H. Thomas, unpublished work), compared with ornithine decarboxylase activities of 7517 and 8512 d.p.m. of $^{14}\text{CO}_2$ /h per mg of protein respectively.

In many systems there appears to be a correlation between polyamine concentration, or ornithine decarboxylase activity, and DNA synthesis. However, there can be marked polyamine accumulation in tissues undergoing RNA synthesis without concomitant DNA synthesis (Russell, 1973). We have shown that a proliferative surge can occur *in vitro* in human prostate in the presence of a decayed polyamine-synthetic pathway (Fuller & Thomas, 1975). The present work suggests that ornithine decarboxy-

lase activity is a parameter of secretory activity, rather than growth, in the rat ventral prostate. As such, it may have useful applications in the study of the role of testosterone metabolites in the regulation of growth and differentiation.

- Clifton, K. H. & Cooper, J. M. (1973) *Proc. Soc. Exp. Biol. Med.* **142**, 1145–1151
- Dethlefsen, L. A. (1971) *Cell Tissue Kinet.* **4**, 123–138
- Dethlefsen, L. A. (1974) *Cell Tissue Kinet.* **7**, 213–222
- Fisher, S. K. & Davies, W. E. (1974) *J. Neurochem.* **23**, 427–433
- Fuller, D. J. M. & Thomas, G. H. (1975) *J. Anat.* in the press
- Geiger, P. J. & Bessman, S. P. (1972) *Anal. Biochem.* **49**, 467–473
- Hogan, B., Shields, S. & Curtis, D. (1974) *Cell* **2**, 229–233
- Lesser, R. & Bruchovsky, N. (1973) *Biochim. Biophys. Acta* **308**, 426–437
- Mangan, F. R., Pegg, A. E. & Mainwaring, W. I. P. (1973) *Biochem. J.* **134**, 129–142
- Panko, W. B. & Kenney, F. T. (1971) *Biochem. Biophys. Res. Commun.* **43**, 346–350
- Pegg, A. E. & Williams-Ashman, H. G. (1968) *Biochem. J.* **109**, 32p–33p
- Roger, L. J., Schanberg, S. M. & Fellows, R. E. (1974) *Endocrinology* **95**, 904–911
- Russell, D. H. (1973) in *Polyamines in Normal and Neoplastic Growth* (Russell, D. H., ed.), pp. 1–13, Raven Press, New York
- Russell, D. H. & Snyder, S. H. (1969) *Endocrinology* **84**, 223–228
- Shipman, P. A. M., Littlewood, V., Riches, A. C. & Thomas, G. H. (1975) *Br. J. Cancer* **31**, 570–580
- Williams-Ashman, H. G., Coppoc, G. L. & Weber, G. (1972) *Cancer Res.* **32**, 1924–1932