J. steroid Biochem. Vol. 23, No. 2, pp. 231–233, 1985
Printed in Great Britain.

0022-4731/85 $3.00 + 0.00

SHORT COMMUNICATION

BINDING OF [^{3}H]ALDOSTERONE TO A SINGLE POPULATION OF CELLS WITHIN THE RAT EPIDIDYMIS

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(*Received* 19 *November* 1984)

Summary—Using the dry-mount autoradiographic technique, a single population of cells within the rat epididymis, the clear cells, have been shown to bind [^{3}H]aldosterone at a nuclear site. Competitive binding experiments demonstrated that aldosterone was more potent than desoxycorticosterone than testosterone in reducing the nuclear uptake of radioactive aldosterone. Furthermore, the other epididymal cells (principal and basal cells) in all regions of the epididymis were not significantly labelled; occasional labelling was noted in some endothelial and stromal cells. It is suggested that aldosterone may play a role in controlling the intracellular and transcellular movement of ions and water necessary for concentrating absorbed macromolecules in the clear cell.

INTRODUCTION

There are several similarities between the mechanisms of transepithelial ion and water transport in the kidney and the epididymis [1–3]. This may not be surprising in view of their similar embryological origins. Recently, there have been several reports in the literature describing the effects of diuretics and aldosterone-antagonists on the water-concentrating ability of the mammalian epididymis [4, 5]. However, the mechanisms by which aldosterone is able to control water movement across the epididymal epithelium is now known. Therefore, the present study was initiated to determine (i) whether there are receptors for aldosterone located within the epididymis as has been shown for the kidney [6–8] and, if so, (ii) which cells contain these receptors. To answer these questions, the dry-mount autoradiographic method [9–11] was used for the localization of [^{3}H]aldosterone in the rat epididymis.

EXPERIMENTAL

Animals

Adult male Sprague–Dawley rats (200–250 g; Hilltop, Philadelphia, U.S.A.) were housed in the University Vivarium under a 12 h light/12 h dark (lights on at 06.00 h) cycle with free access to food and water.

Chemicals and isotopes

[^{3}H]Aldosterone (sp. act. 77.0 Ci/mmol) was purchased from Amersham (Arlington Heights, IL, U.S.A.). Aldosterone, testosterone, desoxycorticosterone were purchased from the Sigma Chemical Co. (St Louis, U.S.A.).

Preparation of tissues

Rats were anesthetized with sodium pentobarbital (Nembutal, 50 mg/kg) and bilaterally adrenalectomized. The animals were fed *ad libitum* and their drinking water replaced with 0.9% sodium chloride. Control animals underwent a sham operation. Seventy-two hours later, the following 4 groups of 2–3 animals were administered intravenously as follows: group 1 received vehicle (20% ethanol in saline), group 2 received 50 μg aldosterone, group 3 received 50 μg testosterone and group 4 received 50 μg desoxycorticosterone. Each animal received either vehicle or the above steroid approx 20–25 min before the administration of 0.6 μg [^{3}H]aldosterone. One hour later, each animal was sacrificed, epididymides excised, cut into 3 regions (caput, corpus and cauda) and frozen quickly in liquid Freon (−150°C). The epididymal regions were then sectioned at 4 μm in a cryostat at −30°C and freeze-dried overnight.

Individual sections were mounted onto dessicated, emulsion-coated slides and exposed for 400 days at −20°C [9–11]. The sections were then photographically processed and stained. Silver grains were counted over nuclei of 40 cells for each cell type (see below) for each animal using a Zeiss microscope at 1250×. The data were analyzed by the Poisson distribution [12], then a one way analysis of variance followed by Duncan's multiple range test ($P < 0.05$). The criteria used for identification of each epididymal cell type was that originally published by Reid and Cleland (1957) [13] and Hamilton (1975) [14]. Three cell types were identified: clear, basal and principal cells. The clear cell was recognized as having considerable vacuolization within the apical region of the cell and were only seen within the cauda region of the rat epididymis. The basal cells had their nuclei orientated along periphery of the duct, whereas the nuclei of the principal cells were rounder and positioned towards the base of the cell.

In an initial series of experiments following the above protocol, localization of silver grains was confined to the clear cell. Furthermore the concentration of steroids used (equimolar concentrations of non-radioactive steroid and radioactive aldosterone) in the competition studies was insufficient to cause a reduction of silver grains within the clear cell of all animals; hence, another series of experiments were designed as described above. It was not possible to combine the data for both sets of experiments because the time of development of the silver grains in the initial set of experiments was 753 days as compared to 400 days for the second set.

RESULTS

Radioactivity was localized primarily within the clear cell (Figs 1 and 2) although occasional labelling was noted in some endothelial and stromal cells. The number of silver

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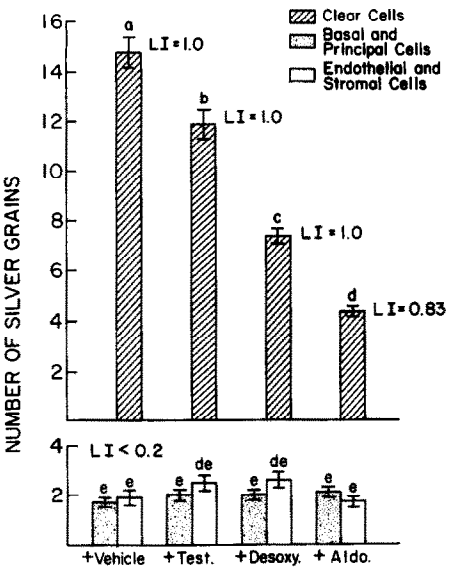

Fig. 1. Mean ± SEM number of silver grains overlying the nuclei of clear cells, basal and principal cells, and endothelial and stromal cells within the cauda region of the rat epididymis. Animals were adrenalectomized for 72 h and then received either vehicle, 50 μg testosterone, 50 μg desoxycorticosterone or 50 μg aldosterone approx 20 min prior to the administration of [^{3}H]aldosterone. One hour later each epididymis was prepared for dry-mount autoradiography. A labelling index (LI) of 1.0 indicated that 100% of the cells observed had a significant number of silver grains overlying the nuclei above that of background [12]. Mean values having the same lower case letter are not significantly different ($P < 0.05$).

grains overlying the nucleus of each epididymal cell-type from the cauda region were as follows (mean ± SEM); principal cells, 1.95 ± 0.2 grains; basal cells 1.60 ± 0.16 grains; clear cell, 14.8 ± 1.9 grains (n = 80–100 cells from 2–3 animals per group). At the concentrations used, aldosterone, desoxycorticosterone and testosterone reduced the nuclear labelling of [^{3}H]aldosterone within the clear cell by approx 70, 50 and 20% respectively (Fig. 1). Each treatment did not significantly affect the labelling of the other epididymal and interstitial cell types; therefore, the data obtained from the localization of silver grains within the principal and basal cells has been combined for each treatment group (Fig. 1).

DISCUSSION

The results from this study suggest that [^{3}H]aldosterone bound to specific sites within the nucleus of the clear cell. Furthermore, the competition experiments demonstrated that aldosterone was a more potent competitor than desoxycorticosterone than testosterone. In view of the known mineralocorticoid activity of desoxycorticosterone, it was not surprising to find that this steroid had some effect in limiting the nuclear uptake of [^{3}H]aldosterone.

It was surprising however, to find specific labelling associated with only one epididymal cell type since the recent findings of Turner and Cesarini (1983) [5] and Wong *et al.* (1978) [2] demonstrated that aldosterone-antagonists affected the water-concentrating ability along the entire length of the epididymis. Since the initial segment of the rat epididymis is very efficient at absorbing water from the lumen [1], we would have predicted labelling of either the principal or the "narrow" cells within this region. The latter cell type has been suggested to be the precursor cell to the clear cell during development [15]. Since it was difficult to identify the "narrow cell" in light microscopic preparations, then there still remains the possibility that this cell contains nuclear aldosterone receptors.

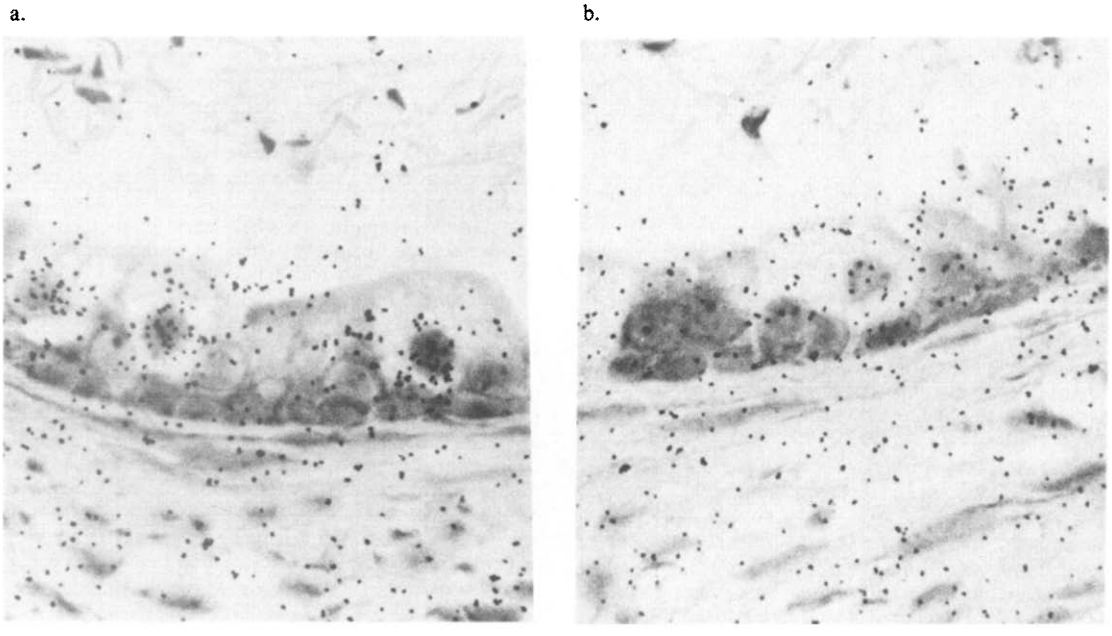

Fig. 2. Photomicrographs of the cauda epididymal epithelium from rats which had been adrenalectomized for 72 h and then received either (a) vehicle alone or (b) 50 μg aldosterone 1 h prior to administration of [^{3}H]aldosterone. Note the higher number of silver grains overlying the nucleus in each clear cell in (a) compared to (b). Magnification × 1500.

In view of the known absorptive functions of the clear cell within the rat epididymis [16, 17], it is tempting to speculate that the role of aldosterone in this cell is one of controlling the local intracelluar and transcellular movement of water and ions. This would be necessary for concentrating macromolecules within vesicles once the molecules have entered the cell. Hence, aldosterone may control ion and water movements within individual cells as well as across complete epithelia. Whether aldosterone induces citrate synthase or other proteins in the clear cell, as it does in the kidney and bladder [18–23], remains to be elucidated.

Acknowledgements—We would like to thank S. K. Lau for excellent technical assistance. Supported by grants; RCDA (DAK) HD00243 from the National Institutes of Health, Grant AM 22125 from the Diabetes Research Training Center and Grant 5S07RR05431-22 from the Biomedical Research Support Award (BTH).

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