SUBCELLULAR DISTRIBUTION OF SOME INORGANIC CATIONS IN THE VAGINAL EPITHELIUM OF THE RAT

ELSA B. SCHUCHNER, ALICIA PACZY AND R. H. ASCH
Centro de Investigaciones sobre Reproducción, Facultad de Medicina, Buenos Aires, Argentina
(Received 2nd November 1971, accepted 23rd February 1972)

Recent studies have demonstrated that a saturated aqueous solution of potassium pyroantimonate can be used as a precipitant of inorganic cations as well as a fixative (Tandler, Libanati & Sanchis, 1970; Schuchner & Tandler, 1972). Tissue fixed by this saturated solution alone (Tandler et al., 1970; Kierszenbaum, Libanati & Tandler, 1971; Schuchner & Tandler, 1972; Schuchner, Foix & Borenstein, 1972; Stockert & Schuchner, 1972) or by a potassium pyroantimonate/osmium tetroxide solution (Komnick, 1962; Spicer, Hardin & Greene, 1968; Hardin & Spicer, 1970) showed the presence of electron-opaque antimonate precipitates that could be analysed by microprobe. Analysis provided evidence that the precipitates represented such inorganic cations as Ca, Mg or Na (Tandler et al., 1970; Kierszenbaum et al., 1971). In the present study, the subcellular distribution of inorganic cations was studied in the vaginal epithelium of control and experimental rats.

Ten 60-day-old rats, used as controls, were killed during pro-oestrus. Twenty-four experimental rats were allocated equally to two groups: (1) ovariectomized, and (2) ovariectomized and primed with a single subcutaneous dose of 1 µg oestradiol in 0.1 ml peanut oil. Six rats from each group were perfused (Palay, McGee-Russell, Gordon & Grillo, 1962) with a saturated solution of potassium pyroantimonate, pH 9.2 (Riedel-De Haen Ag., Seelze, Hannover, Germany, analytical reagent). The vagina was dissected out and immersed in a large amount of fixative for 3 hr at room temperature (22°C). The tissue was hardened in a formaldehyde–potassium pyroantimonate solution, washed with distilled water and post-osmicated as previously described (Kierszenbaum et al., 1971). After dehydration, the tissue was embedded in Maraglas epoxi-resin.

Four control rats and six rats from each of the experimental groups were killed without previous perfusion and the vaginae were directly immersed in the fixative and processed as described. Thin sections (about 0.1 µm) were cut in a Porter Blum microtome mounted in Formvar-coated grids and were examined unstained with a Siemens Elmskop I electron microscope.

Removal of the antimonate deposits was accomplished by floating the grids on a drop of 1/400 oxalic acid solution for 1 min and washing in distilled water. The sections were stained with uranyl acetate and lead citrate.
The nuclei in the vaginal epithelium of the control rats showed coarse electron-opaque deposits in the nucleoli and fine precipitates in the chromatin area. Similar nuclei in the ovariectomized rat contained almost no cation precipitates. The few deposits visible were mainly distributed in the nucleoli. In the vaginal epithelium of the rats treated with oestradiol, heavy antimonate deposits were evident in the nuclei. The patchy distribution was not coincident with the nucleoli but was largely confined to the interchromatin areas, which probably correspond to areas containing ribonucleoprotein (Pollard, 1970). These nuclear precipitates were ring-shaped with external hexagonal edges (Pl. 1, Figs 1 to 3) and resembled in-vitro Mg antimonate deposits (E. B. Schuchner, unpublished work). Microprobe analysis provided evidence of a high nuclear concentration of magnesium. Electron microscopy of the cytoplasm of the control and experimental vaginal epithelial cells showed abundant electron-opaque deposits in the desmosomes, which in some places were closely packed together in rows (Pl. 1, Figs 6 to 8). The abundant cation antimonate precipitates in the dense plaque of the desmosomes as seen in the transitional cells (Pl. 1, Figs 1, 7 and 8) were probably due to the concentration of calcium. The microprobe analysis provided evidence of high levels of calcium in the cytoplasm. Removal of antimonate deposits from the sections with a 1/400 oxalic acid solution and post-staining of the grids with uranyl acetate and lead citrate showed that the precipitate was located mainly in the dense plaque anchoring cytoplasmic tonofilaments. After perfusion of six rats with 0.02 M-EDTA (Vargas Linares & Burgos, 1965) followed by potassium pyroantimonate, few antimonate precipitates could be detected in the vaginal epithelium and there was a significant expansion of the intercellular space and loss of density of the desmosomes (Pl. 1, Figs 4 and 5).

It has been suggested that one of the primary actions of oestrogen is to induce synthesis in the nucleus of a small group of proteins by RNA polymerase (Noteboom & Gorski, 1963; Uí & Müller, 1963). Pollard, Martin & Shorey

EXPLANATION OF PLATE 1

Fig. 1. Microphotograph of the intermediate area of the vaginal epithelium of an ovariectomized rat primed with oestrogen. The tissue was fixed by a saturated solution of potassium pyroantimonate. N: nucleus; C: cytoplasm. × 3000.

Fig. 2. High magnification of an intermediate cell fixed by potassium pyroantimonate showing electron-opaque deposits in the interchromatin areas. No antimonate cations could be seen in the nucleolus. N: nucleus; n: nucleolus; C: cytoplasm; ic: interchromatin. × 12,500.

Fig. 3. High magnification of an intermediate cell fixed by glutaraldehyde Caulfield solution. N: nucleus; n: nucleolus; C: cytoplasm; cr: chromatin. × 12,000.

Fig. 4. Low-power view of the vaginal epithelium of a rat perfused by EDTA and potassium pyroantimonate. A significant increase in the intercellular space can be seen. N: nucleus; n: nucleolus; C: cytoplasm; is: intercellular space. × 4000.

Fig. 5. High magnification of an area of Fig. 4 showing the intercellular space, is: intercellular space. × 10,000.

Fig. 6. Cell junctions of two intermediate cells fixed by glutaraldehyde Caulfield solution. C: cytoplasm; d: desmosome; is: intercellular space; ab: adhering band. × 10,000.

Fig. 7. Similar to Fig. 6. Fixed by post-pyroantimonate solution C: cytoplasm; d: desmosome; is: intercellular space. × 10,000.

Fig. 8. Similar to Fig. 6. Cell junctions are completely covered by electron-opaque deposits. d: desmosome. × 10,000.
(1966) noted that the first change in the nucleus of the vaginal epithelial cells of ovariectomized mice primed with oestrogen occurred in the volume of the nucleolus, probably due to an increase in the granular component following oestrogen stimulation. Recently, Pollard (1970), using the staining technique of Bernhard (1969), reported dense nuclear aggregations of heavily stained material. The relation to oestradiol treatment probably indicates that the material, which is easily distinguishable from chromatin before the nucleolar changes take place, is ribonucleic in origin. Similar areas showed antimonate deposits, probably indicating the need for high levels of magnesium and RNA polymerase to initiate the RNA and protein synthesis induced by oestrogen.

Electron microscope studies of vaginal epithelial cell junctions were carried out in a number of species: guinea-pig (Burgos & Wislocki, 1958), hamster (Vargas Linares & Burgos, 1965) and human (Burgos & Vargas Linares, 1970). Moscona (1961) pointed out that maintenance of the junctions depends on high calcium concentrations. Vargas Linares & Burgos (1965) also studied the vaginal epithelium after perfusion with EDTA. Following such treatment, the desmosomes showed loss of density and definition, expansion of the intercellular space and splitting with evident separation of the cells. Our observations confirm that the Ca cation is an important element in the maintenance of cell junctions.

This work was supported by a grant from The Population Council Inc., New York. The authors want to thank Dr J. C. Stockert for valuable criticism of the manuscript.

REFERENCES


Burgos, M. H. & Wislocki, G. B. (1958) The cyclical changes in the mucosa of the guinea pig’s uterus, cervix and vagina and in the sexual skin, investigated by the electron microscope. Endocrinology, 63, 106.


