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EFFECT OF CLORPROMAZINE ON ULTRASTRUCTURE OF EPITHELIAL CELLS AND CELL CONTACTS IN THE FROG BLADDER

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Chlorpromazine causes swelling and vacuolation of cells of the mucous membrane of the frog urinary bladder and prevents their response to antidiuretic hormone (ADH) by increased permeability for water. The structure of the zone of intercellular contacts was undisturbed, and this could be the reason for the impermeability of the chlorpromazine-treated epithelium for water.

KEY WORDS: chlorpromazine; permeability for water; antidiuretic hormone; swelling of the cell; intercellular contacts.

For the last two decades the problem of the mechanism of the increased water transport under the influence of antidiuretic hormone (ADH) has been a subject of intense discussion. According to one hypothesis, ADH increases the permeability of the apical plasma membrane of the cell for water [6, 7], whereas according to another hypothesis it is the permeability of the ground substance that is increased [1, 4]. Chlorpromazine prevents ADH from increasing the absorption of water [3]. The object of the present investigation was to compare the effect of chlorpromazine on the ultrastructure of the cell and intercellular contacts during its inhibition of the ADH effect.

EXPERIMENTAL METHOD

The bladder of a frog was filled with Ringer's solution diluted with water 1:10, with the mucous membrane on the inner side, and immersed in aerated Ringer's solution. The degree of permeability for water was estimated from the volume of water absorbed from the bladder along the osmotic gradient [5]. Chlorpromazine and and ADH were added to the Ringer's solution on the side of the mucous membrane. For the electron microscopic investigation, bladders in different functional states were transferred for 3-5 min into a 2.5% solution of glutaraldehyde made up in Ringer's solution with cacodylate buffer (pH 7.4). The bladder wall was then incised,

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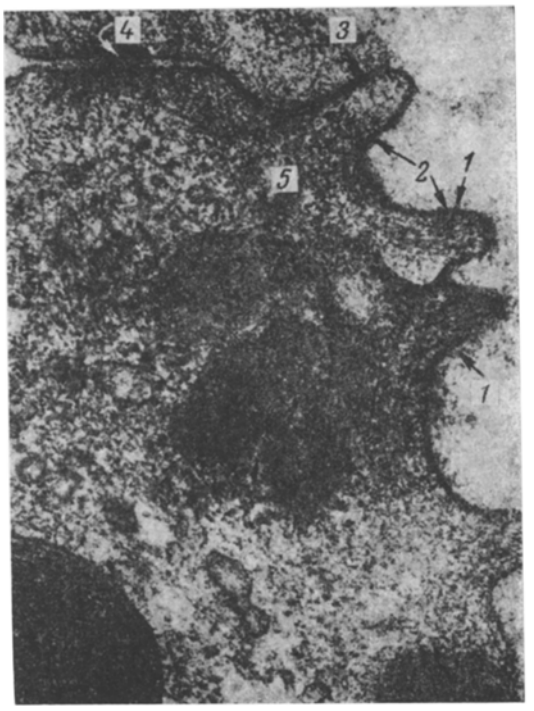

Fig. 1

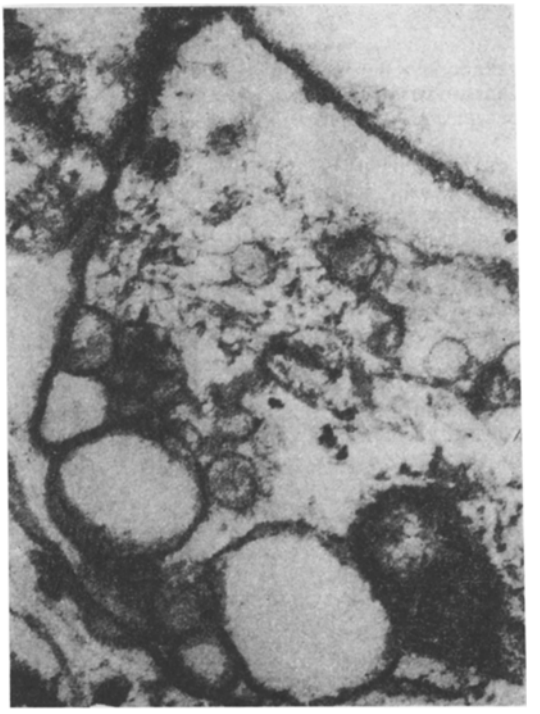

Fig. 2

Fig. 1. Cell of mucous membrane of frog bladder. Control: 1) glycocalyx; 2) apical plasma membrane; 3) dense contact; 4) desmosome; 5) microtubules; 75,000×.

Fig. 2. Cell of mucous membrane of frog bladder treated with chlorpromazine, 1 μmole/ml. Transparency of apical region of cell, vacuolation of cytoplasm, unusual arrangement of microtubules and microfilaments; dense contact and desmosome have normal structure; 65,000×.

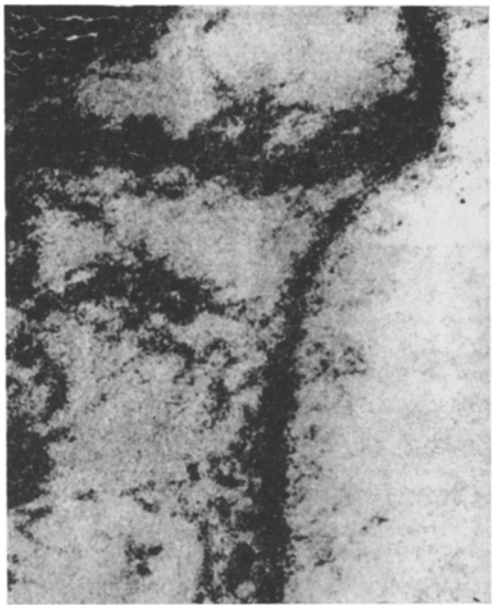

Fig. 3. Part of epithelial cell of mucous membrane treated with 1 μmole/ml chlorpromazine (high power). Structure of dense contact and plasma membrane preserved, destruction of microtubules; 160,000×.

the contents evacuated, and fixation continued in the same solution for 60 min at 5°C. Pieces of bladder wall were rinsed with buffered isotonic sucrose solution and transferred for 50 min to 1% OsO_4 solution. Dehydration was carried out in alcohols of increasing concentration and in absolute acetone. The material for examination was embedded in Araldite and sections were stained with stained with lead citrate and uranyl acetate and examined in the electron microscope.

EXPERIMENTAL RESULTS

On the addition of pituitrin P (an ADH preparation) in a dose of 30 milliunits/ml to the Ringer's solution on the side of the mucous membrane, permeability for water was increased after 30 min from 0.041 ± 0.008 to 2.44 ± 0.19 mg/(cm^2 · min) ($P < 0.001$; $n = 9$). Under these conditions a sharp increase was observed in the size of the intercellular spaces together with swelling of the cells [2]. The increase in the water content in the cells could be the result of increased permeability of the apical plasma membrane for water and subsequent movement of water through the cell. Another possibility is that under the influence of ADH the permeability of the intercellular space was increased and water moved along it from the hypotonic solution within the bladder into the Ringer's solution on the side of the serous membrane and penetrated through the lateral membrane into the cells, causing them to swell. The apical membrane of the cells in the epithelium of the bladder has low permeability for water, but the lateral and basal plasma membranes, on the other hand, are always readily permeable for water [4].

The addition of chlorpromazine (1 μmole/ml) to the Ringer's solution on the side of the serous membrane 30 min before the hormone completely prevented ADH from increasing permeability for water: 0.043 ± 0.011 mg/(cm^2 · min) in the control period and 0.044 ± 0.022 mg/(cm^2 · min) 30 min after addition of ADH to bladders in incubated with chlorpromazine ($n = 10$). The results of the study of the ultrastructure of the epithelium of the intact urinary bladder (Fig. 1) and after the action of ADH agreed completely with previously published data [2] and, consequently, only those structural changes induced by chlorpromazine will therefore be described. Chlorpromazine led to well-marked vacuolation and swelling of the epithelial cells. The microtubules and filaments were irregularly distributed over the cytoplasm. The structure of the microtubules showed considerable changes and their internal structure also was disturbed. In the apical regions of the cell, for a distance of up to 0.2 μ the cytoplasmic structures had disappeared, especially the microtubules and microfilaments characteristic of this zone. The mitochondria, endoplasmic reticulum, and nuclear structures were swollen (Figs. 2 and 3).

Despite these gross changes in the intracellular structures, no visible disturbances of the structure of the plasma membranes of the epithelial cells could be found: The three-layered character of the lipoprotein membrane and the structural elements of the glycocalyx were clearly visible. The zone of intercellular contacts likewise was preserved and showed no visible changes as a result of the action of chlorpromazine (Fig. 3). After preliminary treatment of the tissue of the bladder with chlorpromazine, the addition of ADH caused no changes in its structure compared with the action of chlorpromazine. These results agree with those of functional investigations showing that under the influence of ADH permeability for water after treatment with chlorpromazine was the same as before.

Chlorpromazine thus causes marked swelling and vacuolation of the epithelial cells accompanied by the complete absence of transepithelial flow of water. Consequently the swelling of the cells observed under the influence of ADH cannot be used as an argument in support of the hypothesis of the transcellular movement of water. On the other hand, the widening of the intercellular spaces which is always observed when the flow of water is increased, the complete interlocking of the cells under the influence of chlorpromazine, and the inability of ADH to increase permeability for water after treatment of the bladder tissue with chlorpromazine can be regarded as proof of the role of the ground substance and intercellular contact in the epithelium in the regulation of permeability for water. The results suggest that chlorpromazine stabilizes the organic component (protein, lipid, or carbohydrate) of the membranes and so prevents any possible increase in the degree of intercellular permeability through the action of mediators formed in the cell under the influence of ADH.

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ULTRASTRUCTURAL CHANGES IN EXPERIMENTAL TETANUS

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In experiments on rats an electron-microscopic study was undertaken of the caudal part of the hypothalamic-hypophyseal neurosecretory system in experimental tetanus. Activation of the system was found in the early stages after injection of tetanus toxin, evidently on account of the injection of heterologous protein, for a similar effect was given by injection of the inactivated toxin. Parallel changes were observed in vascular permeability, lipid metabolism, and the clotting system of the blood.

KEY WORDS: hypothalamic-hypophyseal neurosecretory system; tetanus toxin.

Light-optical studies of the hypothalamic-hypophyseal neurosecretory system (HHNS) in experimental tetanus have demonstrated the successive stages of the secretory disturbances: activation, preceding the appearance of the clinical features, and followed by inhibition of synthesis and liberation of neurohormones after injection of tetanus toxin [3]. However, many of the finer details of the response of the components of the posterior lobe of the pituitary still await discovery, for which electron-microscopy is the essential method.

This paper describes a study of the ultrastructural changes in the caudal part of the HHNS at different periods after injection of tetanus toxin.

EXPERIMENTAL METHOD

Experiments were carried out on 16 male albino rats weighing about 250 g. A lethal dose of tetanus toxin in 0.24 ml 0.85% sodium chloride solution was injected intramuscularly into the left calf. After 24 h the rats developed signs of local tetanus, and after 3 days general ascending tetanus with spontaneous convulsions, followed by death on the fourth day. Another group of animals received the same dose of inactivated toxin (heated to 56°C for 2 h). The control animals received physiological saline but the general conditions of the basic experiments remained the same.

Material was taken for electron microscopy 5 h and on the third day after injection of the toxin. Pieces of the posterior lobe of the pituitary were fixed in glutaraldehyde in phosphate buffer and postfixed in osmium tetroxide solution, dehydrated in acetone, and embedded in a mixture of Epon and Araldite. Ultrathin sections, stained with lead citrate and uranyl acetate, were examined in the UÉMV-100 and JEM-100B electron microscopes.

EXPERIMENTAL RESULTS

The ultrastructure of the posterior lobe of the pituitary of the rats receiving physiological saline was virtually indistinguishable from that in intact animals. Nerve fibers and terminals contained many neurosecretory elementary granules, a few empty and synaptic vesicles, and also small mitochondria. The endings of the axons were in contact with capillaries, a special feature of which was their fenestrated endothelium. Glial cells, represented by pituicytes, corresponded to astrocytes and oligodendrocytes of the neuroglia. Mast cells, characteristic of the neurohypothesis of dogs and opossums [1, 4], are not found in rats.

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