EFFECT OF ANTIDIURETIC HORMONE ON THE ULTRASTRUCTURE OF THE KIDNEY

V. S. Orlov

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During the antidiuretic reaction induced in rats by injection of antidiuretic hormone after moderate water loading, by contrast with injection of the hormone without water loading, widening of the intercellular spaces and basal labyrinth in the epithelium of the collecting tubules and of the thin descending segment of the loop of the nephron, evidently connected with the presence of an intratubular pressure gradient under these conditions, was found. After injection of the hormone in both variants, vacuolation of the cytoplasm and widening of the perinuclear space of the interstitial cells of the renal medulla took place. The results confirm the hypothesis that intercellular contact plays a role in the transepithelial reabsorption of water in the kidney during the urine concentration process, and they also point to participation of the interstitial cells in this process.

KEY WORDS: *kidney; antidiuretic reaction; antidiuretic hormone; transepithelial reabsorption of water; interstitial cells of the renal medulla.*

The ability of the kidneys to produce concentrated urine and so to preserve water for the body when it is deficient is under the control of antidiuretic hormone (ADH). In the presence of ADH the epithelial wall of the collecting tubules becomes permeable to water. The increased concentration gradient around the collecting tubules, created by the "pumping" of sodium from the outer segment of the nephron into the renal medulla and by the work of the hairpin countercurrent system, facilitates the passage of water from them into the hypertonic interstices [4, 11, 12]. However, the actual path by which water passes through the cell wall is still debated [2]. There are data in the literature (electron micrographs) on the formation of the intercellular space during the action of ADH on the wall of the frog urinary bladder [3], and also in experiments on isolated collecting tubules from the rat [7] and rabbit [8] kidney during isotonic perfusion and in the collecting tubules of the kidney of rats with diabetes insipidus [10, 15]. The role of other structures of kidney tissue in the concentrating and diluting function of the kidney still awaits elucidation.

In the investigation described below an electron-microscopic study was made of the path followed by water through the epithelial wall in the kidney of normal rats during the antidiuretic reaction to injection of ADH.

EXPERIMENTAL METHOD

Male Wistar rats were "standarized" as regards deprivation of food and water for 2.5-3 h before the beginning of the experiments. Antidiuresis was induced in 6 rats by subcutaneous injection of ADH (vasopressin) in a dose of 25 milliunits/100 g body weight. Water in a volume equivalent to 3% of the animal's body weight, warmed to 37°C, was injected into the stomach of 8 rats through a catheter 20 min before the injection of vasopressin. A group of five "standardized" rats served as the control. The animals were killed for electron-microscopic investigation 1.5 h after the beginning of the experiments. The dynamics of development of antidiuresis was first studied in a group of nine rats in which the osmolarity of the urine was measured.

EXPERIMENTAL RESULTS

Injection of vasopressin was followed by a rise in the osmolarity of the urine until sacrifice of the animals up to 2.17 osmoles/kg (Fig. 1) The study of the ultrastructure of

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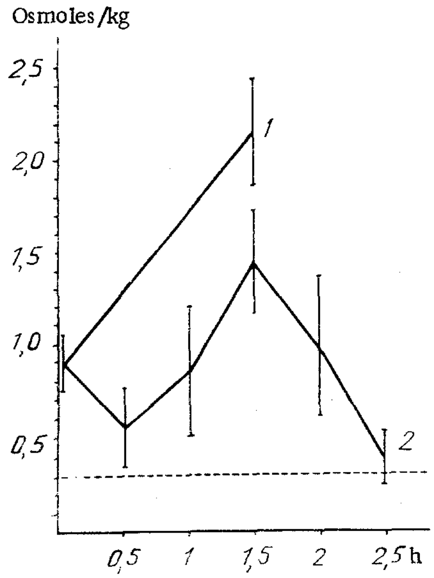

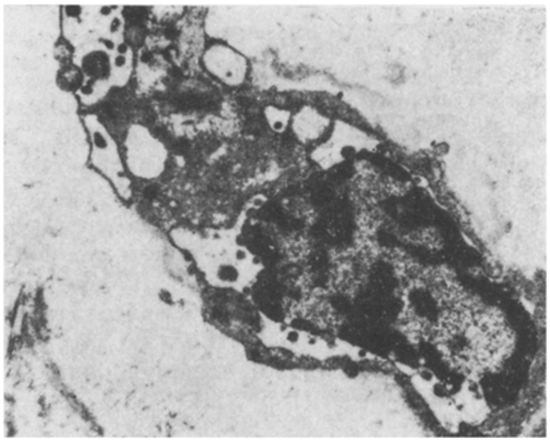

Fig. 1 Fig. 2

Fig. 1. Concentrating work of kidneys based on data of osmometry of urine. 1) Injections of ADH; 2) injection of ADH after water loading. Broken line corresponds to osmolarity of blood plasma (0.3 osmole/kg).

Fig. 2. Vacuolation of cytoplasm and widening of perinuclear space of interstitial cell following adminstration of ADH (6100×).

the renal tubules at that moment in fact showed no difference from the control. However, the interstitial cells of the inner layer of the medulla were vacuolated, with a considerably widened perinuclear space, which was connected with the vacuoles and with the granular cytoplasmic reticulum (Fig. 2).

The dynamics of the change in osmolarity of the urine during induction of the antidiuretic reaction after water loading is shown in Fig. 1, point 2, from which it is clear that the concentrating power of the kidneys was appreciably reduced in this experiment. Electron-microscopic study of the kidney tissue showed widening of the spaces of the basal labyrinth and lateral intercellular spaces in the thin descending segment of the loop of the nephron, as a result of which a single system of extracellular tubules and cavities, separated from the interstices only by the basement membrane, was formed. The terminal bridges between the cells retained their normal structure (Fig.3a). Characteristic widening of the lateral intercellular spaces in the form of a channel 0.1-0.15 μ wide was found in the collecting tubules in this experiment, often with the formation of cavities or chambers along its course, and communication between this channel and the widened system of the basal labyrinth (in the control the width of the intercellular space varied from 0.025 to 0.04 μ). In the terminal bridge of the neighboring epithelial cells and in the region of the desmosomes the channel was narrowed. From the side of the tubular lumen the terminal bridges created a form of "plug" in the widened lateral intercellular space (Fig. 3b). The structure of the basement membrane of the narrow segment and of the collecting tubules was indistinguishable from the control. In the interstitial cells of the inner layer of the renal medulla the same characteristic vacuolation of the cytoplasm was observed, together with a sharp increase in width of the perinuclear space, just as in the experiment in which vasopressin was given without water loading.

It can tentatively by suggested that widening of the intercellular spaces and basal labyrinth appeared as the result of the formation of a hydrostatic pressure gradient in the tubular lumen, as in experiments on isolated collecting tubules [7, 8]. In other words, widening of the extracellular spaces in the tubular epithelium under these conditions depended on the flow of water through the wall of the tubule. Clearly the flow of liquid along the tubule itself was not the cause of widening of the extracellular spaces, for even during very intensive diuresis this phenomenon is known to be absent. After injection of ADH, the sodium concentration in the renal medulla rises sharply [11]. For intercellular spaces

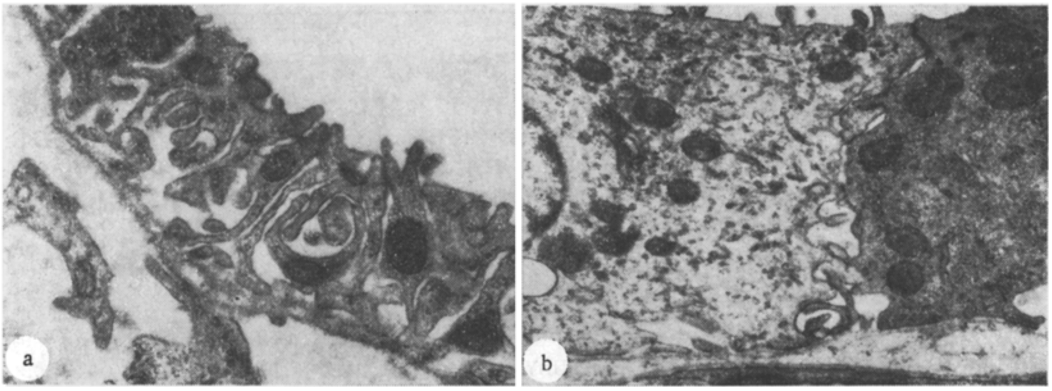

Fig. 3. Widening of intercellular spaces and spaces of basal labyrinth following administration of ADH after water loading: a) in epithelium of thin descending segment of nephron loop (15,000×); b) in epithelium of collecting tubule (13,000×).

to be revealed in the tubular epithelium, hyperosmosis must evidently be created in the interstices, with activation of the mechanism of an antidiuretic reaction by ADH. In the collecting tubules this mechanism consists of an increase in the active transport of sodium and potassium ions [6], aimed at increasing the osmotic pressure in the renal medulla and opening up the intercellular space. In the latter case, hyaluronidase plays a definite role [1, 3].

The state of hyperosmosis of the interstices of the medulla, created during development of the antidiuretic reaction, probably has an important role in the function of the thin segment of the nephron loop. Ultrastructural features of cells of the descending and ascending segments of the nephron loop are known to reflect differences in their function [5, 13]. The formation of intercellular spaces and widening of the basal labyrinth only in the descending segment of the loop in the present experiments were evidence of the high permeability of this part of the nephron and they confirm the results of physiological investigation [12]. The descending segment of the nephron loop, together with the collecting tubules, can be assumed to participate in the process of urine concentration, and ADH exhibits its action on this part of the nephron indirectly through hyperosmosis of the tissue of the renal medulla.

The interstitial cells may perhaps play an important role in the creation of hyperosmosis of the medulla, as is shown by changes taking place in them under the influence of ADH. These changes are most likely connected with osmotic mechanisms. The cell nucleus is known to be distinguished by a high salt concentration. For instance, according to data for rat liver cells, there are substantial concentration gradients for several ions between the nucleus, cytoplasm, and extracellular space, as well as "direct" communication between the nucleus and the extracellular space through the cytoplasmic reticulum [14]. These concentration gradients are fully comparable with changes in the osmolarity of the medulla in extreme states of the osmoregulatory function of the kidney.

It can be concluded from these results that the ability of the extracellular spaces (the intercellular spaces and basal labyrinth) in the epithelium of the collecting tubules to widen under the influence of ADH is a morphophysiological manifestation of the process of facultative reabsorption of water in the kidney, controlled by ADH, during the antidiuretic reaction; two other components of the inner medulla of the kidney — the thin segment of the nephron loop and the interstitial cells — also participate in this process.

LITERATURE CITED

1. A. G. Ginetzinsky (A. G. Ginetsinskii), Nature, 182, 1218 (1958).
2. Yu. V. Natochin and K. Chapek, Methods of Investigation of Ion and Water Transport [in Russian], Leningrad (1976).
3. Yu. V. Natochin (Y. V. Natochin) and E. I. Shakhmatova, Endocr. Exp., 2, 151 (1968).
4. R. W. Berliner, Kidney Int., 9, 214 (1976).
5. R. E. Bulger, L. D. Griffith, and B. F. Trump, Science, 151, 83 (1966).
6. J. Diezi, P. Micoud, J. Aceves, et al., Am. J. Physiol., 224, 623 (1973).
7. C. E. Ganote, J. J. Grantham, H . L. Moses, et al., J. Cell Biol., 36, 355 (1968).

8. J. J. Grantham, C. E. Ganote, M. B. Burg, et al., J. Cell Biol., 41, 562 (1969).
9. M. Imai and J. P. Kokko, J. Clin. Invest., 53, 393 (1974).
10. J. P. Kokko and C. C. Tisher, Kidney Int., 10, 64 (1976).
11. H. Levintin, A. Goodman, G. Pigeon, et al., J. Clin. Invest., 41, 1145 (1962).
12. T. Morgan and R. W. Berliner, Am. J. Physiol., 215, 108 (1968).
13. L. Osvaldo and H. Latta, J. Ultrastruct. Res., 15, 144 (1966).
14. G. Siebert, in: The Cell Nucleus (Proceedings of an International Symposium on the Structure and Function of the Cell Nucleus) [in Russian], Moscow (1972, p. 219.
15. C. C. Tisher, R. E. Bulger, and H. Valtin, Am. J. Physiol., 220, 87 (1971).

COMPARATIVE INDIRECT IMMUNOFLUORESCENCE STUDY OF MYOID CELLS OF THE EMBRYONIC AND ADULT HUMAN THYMUS

É. V. Gnezditskaya and L. V. Beletskaya

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It was shown by the indirect immunofluorescence method that the lower content of muscle antigens in the myoid cells of the embryonic than in the adult human thymus is due to the higher secretory activity of these cells in the early stages of embryogenesis. Because of the increased secretory activity of the myoid cells the internal medium of the embryonic thymus contains more common antigens with muscle tissue than the adult human thymus. The fact that the functional activity of the myoid cells correlates during individual development with the rate of formation of lymphoid tissue confirms the view that heteroorganic antigens provide the lymphocytes of the thymus with information of the structure of autoantigens necessary for the formation of natural immunologic tolerance to them.

KEY WORDS: *embryo; thymus; indirect immunofluorescence.*

The presence of cells morphologically similar to muscle cells in the epithelial tissue of the human and animal thymus has frequently been described by histologists [6, 7]. Modern methods of investigation have shown that this similarity is not limited to morphological likeness. For instance, myofibrils have been found in the cytoplasm of the myoid cells by electron microscopy [15]. The antigenic similarity of the components of the cytoplasm of myoid cells and the components of muscle fibers of skeletal muscle and myocardium have been demonstrated by the indirect immunofluorescence method [4, 12]. The same method also has revealed other heteroorganic antigens in the epithelial tissue of the thymus, namely antigens common to various epithelial tissues of man and animals [1, 3, 8, 9, 14]. As regards the functional role of these heteroorganic structures of the thymus epithelium it has been suggested that they serve as the source of information on the structure of autoantigens that is required for the formation of natural immunologic tolerance to the body's own antigens in the process of differentiation of the lymphoid cells of the thymus [1, 5].

The object of the present investigation was to study the immunomorphological features of the myoid cells in the embryo, i.e., during the period of active differentiation of thymocytes and the formation of the T system of the body, and to prepare them with the corresponding properties of myoid cells of the adult human thymus, during the period of decline of the function of the organ immediately before its involution.

EXPERIMENTAL METHOD

Experiments were carried out by the indirect immunofluorescence method using pure antibodies against human immunoglobulins labeled with fluorescein isothiocyanate, [10]. Sera of patients with myasthenia, reacting with skeletal muscle and myocardial antigens in dilutions

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