PHYSIOLOGY

Possible Absorption of Antidiuretic Hormone in Isolated Rat Intestine

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Arginine-vasopressin or arginine-vasotocin hormones introduced into the small intestine are absorbed and exert a hydrosmotic effect. The amount of absorbed antidiuretic hormone increased when it is introduced with intestinal peptidase inhibitor aprotinin. These data attest to the possibility of absorption of pituitary hormones (vasotocin and vasopressin) in rat small intestine without losing their physiological activity.

Key Words: *rat intestine; vasopressin; permeability; frog urinary bladder*

Peptide hormones are secreted by endocrine glands into the blood, while in case of hypofunction leading to hormone deficiency they are administered via intranasal or parenteral routes [2,12]. Introduction of peptide hormones into the gastrointestinal tract (GIT) in clinical practice is low efficient because of their degradation to amino acids, di-, and tripeptides under the effect of digestive peptidases [4,7,8]. In our previous experiments we observed antidiuretic effect of vasopressin not only after injection of the hormone to rats, but also after its administration into the stomach through a gastric tube [6]. However, the site of hormone absorption and the possibility of hormone absorption in intact small intestine remained unclear. Here studied the possibility of absorption of antidiuretic hormone (ADH) in the small intestine.

MATERIALS AND METHODS

Special method was developed for this study. The experiments were carried out on Wistar rats and *Rana temporaria L.* frogs. Under ether narcosis, a 12-cm segment of the small intestine (20 cm from the place of its junction with the duodenum) was isolated. The intestinal content was removed by washout with Ringer solution (20 ml) for mammals through a syringe. Then two 2-3-cm fragments were cut out. The distal end of the isolated intestine was ligated, 0.1 ml Ringer saline with or without ADH was introduced through the proximal part, and then this part was also ligated. The fragment was placed into a chamber thermostabilized using a UH 16 thermostat.

The chamber contained 12.5 ml aerated Ringer solution containing (in mM): 154.0 NaCl, 5.63 KCl, 1.15 $CaCl_2$, 2.38 $NaHCO_3$, 5.5 glucose. Osmolality of this saline was 305 mOsm/kg H_2O. The isolated fragments of the small intestine were incubated at 37°C. The saline was agitated with air bubbles. Twenty minutes after the start of incubation 10 ml of the incubation medium was transferred to a vessel with 3.85 ml cold solution containing (in mM): 3.35 KCl, 0.8 $CaCl_2$, 2.38 $NaHCO_3$, 5.5 glucose, osmolality 21 mOsm/kg H_2O was added. This maneuver enabled rapid change in osmolality, ionic composition, and temperature of Ringer saline for mammals and turned it into physiological saline for the cold-blooded animals. The resultant solution contained (in mM): 111 NaCl, 3.35 KCl, 0.8 $CaCl_2$, 2.38 $NaHCO_3$, 5.5 glucose, osmolality 225 mOsm/kg H_2O, temperature 23°C. Six to seven

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preliminary prepared frog urinary bladders were transferred to this saline. The bladders were filed with hypotonic solution (osmolality 25 mOsm/kg H_2O). To this end, Ringer solution (225 mOsm/kg H_2O) was 10-fold diluted with distilled water. The bladders were bathed in standard cold-blooded Ringer saline. Single change of bathing Ringer saline before measurements of hormone concentration considerably improved sensitivity of the method due to elimination of autocoids. The concentration of the hormone transported through the intestinal wall into Ringer solution was measured by the increase in osmotic permeability of the urinary bladder. The hydroosmotic method for measuring ADH activity using frog urinary bladder was described elsewhere [13]. During incubation of urinary bladders the temperature was stabilized at 23°C using a Ministat 650 (MTA Kutesz). Agitation and aeration of the saline were performed with an AP-1 Aquael compressor. The urinary bladders filled with hypotonic saline were weighted in a VLE-200 electronic balance (Gosmetr) coupled to a computer. The changes in osmotic permeability were calculated in μl/min×cm^2. The data were compared with the relations between osmotic permeability and concentration of examined ADH (arginine-vasopressin, AVP or arginine-vasotocin, AVT) determined in the parallel experiments. This approach made it possible to calculate the amount of hormone absorbed across the intestinal wall.

RESULTS

Osmotic permeability of frog urinary bladders remained unchanged during their incubation with small intestine fragments filled with 0.1 ml Ringer solution (Fig. 1). Therefore, the intestine secreted no agents affecting water permeability of urinary bladder epithelium and thereby imitating the action of ADH. When Ringer solution with AVP was introduced into the intestine, water permeability of frog urinary bladders increased, which attested to absorption of AVP in the intestine (Fig. 1). This effect was comparable with the action of the same hormone applied to the serosa (Fig. 1). Similar effect was produced by AVT, but in considerably lower concentrations (Fig. 2). This peculiarity can be explained by the fact that in excaudate amphibian vasotocin acts as natural ADH [10], and its affinity to V_2 receptors in epithelial cells of urinary bladder is higher. In mammals, AVT is produced by the pineal gland [11].

Introduction of AVT into intestinal preparations increased osmotic permeability of frog urinary bladder (Fig. 2). The hormone was partially absorbed and stimulated ADH receptors in basolateral membranes of urinary bladder epithelial cells. According to calibration curve, the amount of AVT absorbed over 20 min

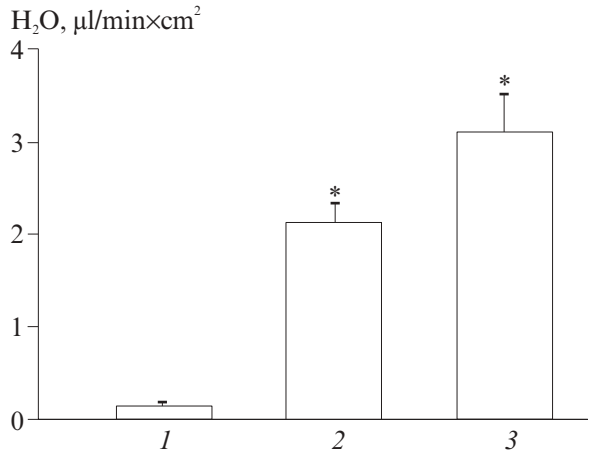

Fig. 1. Potentiating effect of arginine-vasopressin (AVP) absorbed from rat small intestine preparation on osmotic permeability of frog urinary bladder. *1*) control Ringer solution, *n*=10; *2*) AVP, 0.1 μmol/100 mg intestine, *n*=8; *3*) application of AVP to serosa of the urinary bladder, 0.05 nmol/ml, *n*=10. $p^*<0.001$ compared to the control. Here and in Figs. 2 and 3: ordinate, water flow with osmotic gradient.

from the intestine was 0.046±0.004 pmol, when it was used in concentration of 0.1 nmol per 100 mg intestine weight.

A great part of ADH is cleaved by intestinal peptidases, so introduction of ADH with peptidase inhibitor should prevent ADH hydrolysis and potentiate absorption of the hormone. For inhibition of peptide hydrolysis in the small intestine we used aprotinin, a natural inhibitor of serine proteases. As expected, combined introduction of AVT and aprotinin potentiated the hydrosmotic effect of AVT (Fig. 3).

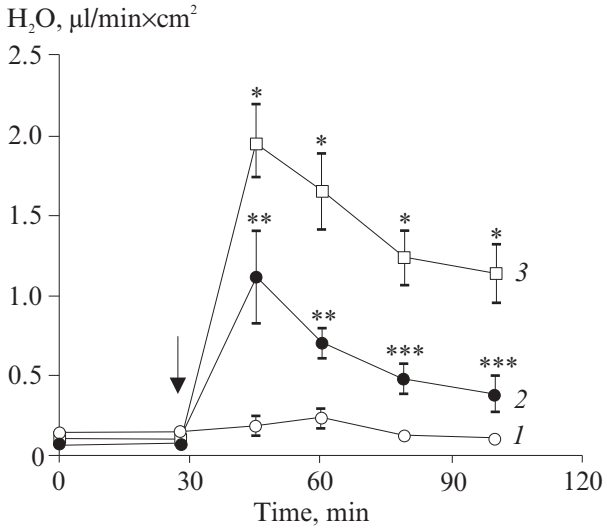

Fig. 2. Effect of arginine-vasotocin (AVT) introduced into the rat small intestine (*2*) or applied to urinary bladder serosa (*3*) on osmotic permeability of frog urinary bladder. *1*) control Ringer solution, *n*=10; *2*) AVT, 0.1 nmol/100 mg intestine, *n*=33; *3*) AVT, 0.01 nmol/ml, *n*=10. The arrow marks addition of AVT to Ringer's solution or transfer of frog urinary bladders to Ringer's solution after incubation of AVT-containing small intestine preparation in this solution. $^*p<0.001$, ** $p<0.01$, *** $p<0.05$ compared to the control.

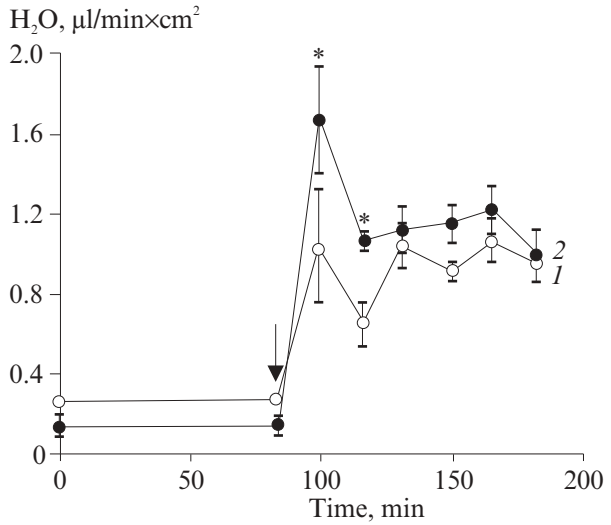

Fig. 3. Effect of arginine-vasotocin (AVT) and aprotinin introduced into rat small intestine on osmotic permeability of frog urinary bladder. *1*) AVT, 0.1 nmol/100 mg intestine, *n*=10; *2*) AVT+aprotinin, 0.047 and 0.045 nmol/100 mg intestine, respectively, *n*=10. The arrow marks transfer of frog urinary bladders to Ringer solution after incubation of small intestine preparation with AVT or AVT+aprotinin in this solution. **p*<0.01 compared to AVT.

Thus, ADH is absorbed in the small intestine and retains its ability to interact with ADH receptor and increase osmotic permeability of cell membranes for water. The small intestine *per se* does not exert such effect, so it is caused by ADH absorbed through the intestinal epithelium. ADH is the only known agent increasing water permeability in collecting tubules [1,5]. Of primary importance is the finding that the small intestine can absorb cyclic nonapeptide hormone without changes in its specific activity. The measurements of ADH activity were performed by physiological method, but not by radioimmunoassay. This method was chosen in order to examine the possibility of ADH absorption in the isolated small intestine with preservation of physiological potency to increase water permeability of the epithelium and to control the osmotic parameters. The cells of frog urinary bladder specifically respond to vasotocin in very low concentrations (femtomolar range). No other agent can increase the osmotic permeability of epithelium at such low concentration.

The concentration of AVP in human plasma is about 10^{-12} M [9]. According to our data, urinary bladder of *Rana temporaria L.* responds to AVT in a concentration of 2×10^{-12} M. Physiological tests showed that rat small intestine can absorb cyclic nonapeptides, *e.g.* vasopressin and vasotocin, without changing their physiological activity related to stimulation of V_2 receptors.

Thus, not only specific endocrine gland can supply hormones into blood plasma. Under specific conditions ADH can be absorbed in the small intestine. For example, in some pathological states such as inadequate ADH secretion, other organs produce it [2]. We demonstrated hyperproduction of ADH during acute pneumonia in children, which underlies hyposmosis and hypersthenuria [3]. Evidently, normalization of the endocrine status requires effective functioning of the ADH inactivation system. This role is probably performed by the kidneys, where ADH is filtered out by glomerules, reabsorbed by cells in the proximal part of the nephron, and finally hydrolyzed in these cells under the effect of lysosomal enzymes.

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