VASOPRESSIN-STIMULATED HYDROLYSIS OF PHOSPHATIDYLINOSITOL-4,5-DIPHOSPHATE AND ITS CONNECTION WITH PROSTAGLANDIN BIOSYNTHESIS IN FROG URINARY BLADDER EPITHELIUM

R. G. Parnova and D. L. Firsov

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A leading role in the regulation of water metabolism in land vertebrates is played by vasopressin. Ideas regarding the cellular mechanisms of the antidiuretic action of vasopressin have recently been considerably widened. Evidently not only receptors of V_2 type, coupled with adenylate cyclase, and playing a leading role in the hydroosmotic response [1, 5], but also receptors of the V_1 type, linked with stimulation of phosphoinositide metabolism [1, 14], are involved in the regulation of water permeability by vasopressin. It is considered that the stimulation of prostaglandin (PG) secretion observed under the influence of vasopressin [8, 13] also is connected with the V_1 type of receptor, but the molecular mechanisms of activation of their biosynthesis are not clear. The aim of the present investigation was to study the receptor-mediated phosphoinositide response and its functional role and to study the possible molecular mechanisms of its link with stimulation of PG secretion.

EXPERIMENTAL METHOD

Experiments were carried out on the isolated urinary bladder of the frog *Rana temporaria* L, placed in Ringer's solution and filled with hypoosmotic (22 milliosmoles) Ringer's solution. Permeability for water was measured gravimetrically [2]. Vasopressin ("Calbiochem"), neomycin sulfate ("Serva"), indomethacin ("Zeszczuw," Poland), and radioactive precursors were added to the physiological saline from the side of the serous membrane of the bladder. The bladders were incubated for 1 h at room temperature with aeration in physiological solutions containing 200 μCi/ml of [^{3}H]-myoinositol ("Amersham") or 2.5 μCi/ml of [^{3}H]-arachidonic acid (Isotope Institute, Hungary), in the presence of 0.05% bovine serum albumin ("Calbiochem"). Total lipids were extracted by the method in [7]. Labeled neutral lipids were analyzed by thin-layer chromatography on "DC-Alurolle" plates (Kieselgel 60, "Merck"), in a system of hexane—diethyl ether—acetic acid (55:19:1.5 by volume). 1,2-Diacylglycerol (1,2-DAG) was identified with the aid of a reference substances ("Sigma"). Labeled phospholipids were analyzed by two-dimensional microthin-layer chromatography on silica-gel KSK [3]. Phosphatidylinositol-4,5-diphosphate (PIP_2) was extracted with a mixture of solvents chloroform—methanol—HCl (200:100:1 by volume) and analyzed by thin-layer chromatography as described previously [4]. Zones corresponding to lipids were transferred into flasks for scintillation counting. The radioactivity of the lipids was calculated as a percentage of the total count from the plate. The PGE concentration was measured by radioimmunoassay in physiological saline in which the bladders were incubated, and also in the wall of the bladders with the aid of a "Dade" kit (Baxter Travenol Diagnostics Inc., USA). Protein was determined by the method in [10]. The concentration of inositol-1,4,5-triphosphate in the urinary bladder wall was determined with the aid of commercial kits "Amersham"). The radioactivity of all samples was measured on an SL 4000 liquid scintillation counter ("Roche Bioélectronique," France).

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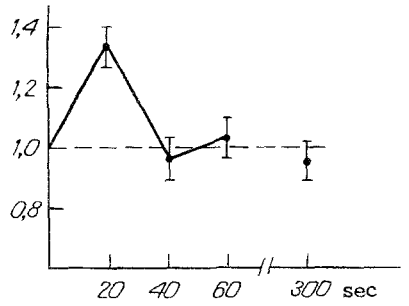

Fig. 1

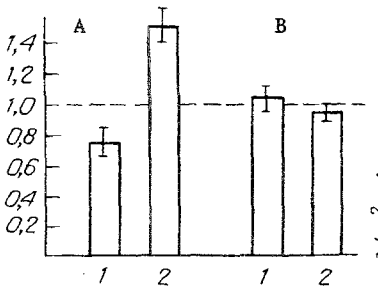

Fig. 2

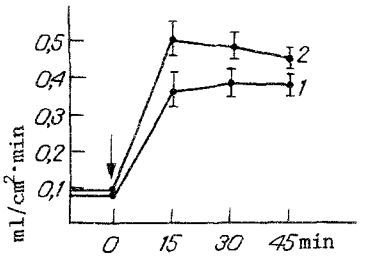

Fig. 3

Fig. 1. Effect of vasopressin ($4 \cdot 10^{-9}$ M) on metabolism of 1,2-diacylglycerol, labeled with [^{3}H]-arachidonic acid. Abscissa, time after addition of hormone; ordinate, level of change in radioactivity compared with control, taken as 1, and indicated by broken line.

Fig. 2. Effect of vasopressin acting for 20 sec on hydrolysis of PIP_2 and release of inositol-1,4,5-triphosphate in absence (A) and presence (B) of 10^{-4} M neomycin. 1) PIP_2, 2) inositol-1,4,5-triphosphate. Ordinate, level relative to control, taken as 1 and indicated by broken line.

Fig. 3. Potentiation of hydroosmotic effect of vasopressin by neomycin: 1) Vasopressin, 2) vasopressin + 10^{-4} M neomycin. Abscissa, time, in min; Ordinate, flow of water along osmotic gradient in μl/min·cm^2. Arrow indicates addition of $4 \cdot 10^{-9}$ M vasopressin.

EXPERIMENTAL RESULTS

A 1.4-fold increase in the concentration of 1,2-DAG, labeled with [^{3}H]-arachidonic acid (Fig. 1), was observed in the wall of the urinary bladder 20 sec after addition of $4 \cdot 10^{-9}$ M vasopressin to the Ringer's solution on the side of the serous membrane. The maximal degree of PIP_2, assessed as the loss of [^{3}H]-PIP_2 (Fig. 2), was noted at the same time. This is in agreement with our previous data indicating release of inositol triphosphate after the action of vasopressin for 20 sec in this tissue [1]. Thus under the influence of vasopressin, receptor-mediated activation of phosphoinositide-specific phospholipase C took place in cells of the frog urinary bladder, leading to increased formation of 1,2-DAG and inositol triphosphate.

For a functional evaluation of the observed phosphoinositide response we used neomycin, an aminoglycoside binding PIP_2 and preventing its hydrolysis [9]. Preincubation of the urinary bladders for 30 min in Ringer's solution with 10^{-4} M neomycin completely blocked hydrolysis of PIP_2 by phospholipase C (Fig. 2), and which was observed in its absence 20 sec after addition of the hormone. Neomycin alone, in a concentration of 10^{-5}-10^{-4} M, did not change the basal level of osmotic permeability of the bladder wall, but strengthened the response to vasopressin, however, probably as a result of blocking of the phosphoinositide response (Fig. 3).

PG, which inhibits adenylate cyclase activity in urinary bladder cells, are among the known modifiers of the antidiuretic action of vasopressin, and they depress the cAMP level and osmotic permeability under the influence of vasopressin [11, 15]. As was expected, addition of indomethacin (10^{-6} M) to the incubation medium led to potentiation of the hydroosmotic effect of vasopressin. The mechanism of activation of PG secretion under the influence of vasopressin is not clear, but we know that this regulation is effected at the level of lipases which release arachidonic acid from lipids, and not at the stage of conversion of arachidonic acid into PG [15]. It has been shown on other objects, for example, on a culture of mesangial cells, that an essential condition for activation of phospholipase A is previous activation of phospholipase C [12]. To discover whether correlation exists in the frog urinary bladder between the increase in PG secretion under the influence of vasopressin and the phosphoinositide response observed, we measured the PGE concentration in the bladder wall and incubation medium 5 min after addition of the hormone, in the absence and in the presence of neomycin. The PGE concentration in the bladder wall without neomycin was unchanged, whereas that in the incubation medium increased by 3.6 times. Preincubation of the bladders for 30 min in a solution with 10^{-5} M neomycin depressed PGE production by 62.0% (Table 1). Thus the fall in PGE secretion when the phosphoinosi-

TABLE 1. PGE Concentration in Wall of Frog Urinary Bladder and in Physiological Saline in Contact with Tissue of Serous Membrane

Experimental conditions	PGE (μg/mg protein)	
	in bladder wall	in physiological saline
Control	7,24±0,73 (4)	5,67±1,23 (8)
Neomycin, 10^{-5} M	—	7,18±1,39 (6)
Vasopressin, $4 \cdot 10^{-9}$ M	7,17±9,62 (13)	20,58±3,75 (10)
Vasopressin + neomycin	5,82±0,94 (8)	12,89±1,39 (11)

Legend. PGE concentration in physiological saline calculated per milligram protein of bladders incubated in the given volume; number of determinations shown in parentheses.

tide cycle was blocked suggests that activation of lipases, releasing arachidonic acid from lipids, correlates at least partially with activation of phospholipase C.

Investigation of the effect of vasopressin on metabolism of bladder wall lipids, labeled with [^{3}H]-arachidonic acid showed that the level of radioactivity in phosphatidylcholine, the plasmalogen form of phosphatidylethanol amine, phosphatidylserine, and phosphatidylinositol, under the influence of the hormone, was virtually identical with the control. A significant fall of radioactivity of the lipids was found 5 min after addition of vasopressin in two lipids: 1,2-DAG (by 14.8 ± 5.1%, n = 9) and in the diacyl form of phosphatidylethanolamine (by 7.0 ± 3.1%, n = 8). Probably these two lipids are the main sources of free arachidonate in the urinary bladder, and 1,2-DAG-lipase and phospholipase A_2 are enzymes whose activity is largely determined by activation of phospholipase C. The observed increase in the inositide triphosphate concentration in the first few seconds of action of the hormone, through an increase in Ca release from the intracellular depots, may directly activate phospholipase A_2. On the other hand the 1,2-DAG formed during hydrolysis of PIP_2 by phospholipase C may itself act as the source of free arachidonate and of PG. This sequence of biochemical reactions (phosphatidylinositol—1,2-DAG—arachidonic acid) has been found in platelets [6].

Thus in the process of regulation of the osmotic permeability of urinary bladder cells by vasopressin, secondary intermediaries are involved: hydrolysis products of PIP_2 by phospholipase C. Their role is probably to participate in the feedback system involved in the regulation of osmotic permeability by vasopressin. One result of activation of the phosphoinositide response and one way of effecting feedback regulation is intensification of PG biosynthesis, which may be associated both with an increase in the concentration of 1,2-DAG — the possible substrate for PG synthesis — and with increased activity of phospholipase A_2.

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