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Effects of arginine-vasopressin and its functional analogues on contractile vacuole of *Amoeba proteus*: possible mechanisms of signal transduction

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Summary

Study of effects of arginine-vasopressin (AVP) and its functional analogues on the contractile vacuole of the amoeba *Amoeba proteus* has esished that AVP (10 μM) increases frequency of vacuole contractions. This effect is reproduced by antagonist of receptors of the V_2 type desmopressin (10 μM), but is suppressed by antagonist of receptors of the V_1 type. Apart from desmopressin, acceleration of the vacuole contraction is also caused by activator of adenylyl cyclase forskolin (25 μM). Cyclic adenosine monophosphate (cAMP, 1 μM) produces a dual effect on the vacuole contraction: it either increases its frequency like AVP, desmopressin, and forskolin, or suppresses the AVP effect without producing its own action. The non-selective blocker of adenosine receptors aminophylline inhibits the AVP-like effect of cAMP. This indicates either effect of cAMP on adenosine receptors or conversion of cAMP into adenosine through the action of 5-ectonucleotidase. In either case, we are dealing with extracellular cAMP receptors, which have been earlier established in the amoeba *Dictyostelium discoideum*. Results of our experiments indicate specificity of the effect of arginine-vasopressin as well as a mixed character of signal transduction from membrane areas sensitive to AVP and combining features of receptors both of the V_1 and of the V2 type.

Key words: water transport, arginine-vasopressin, cyclic adenosine monophosphate, adenosine, *Amoeba proteus*, contractile vacuole

Introduction

The first studies on effect of neuropeptides on the amoeba *Amoeba proteus* have shown arginine-vasopressin (AVP) and lysine-vasopressin to stimulate pinocytosis (Josefsson et al., 1975). Since a similar action was produced by substances with a quite different, not infrequently antagonistic effect, this action of the hormones was considered non-specific (Josefsson and Johansson, 1985). Couillard et al. (1989) have revealed a stimulating effect of AVP and of a number of its analogues on water permeability of membrane or, more

correctly, on activity of the amoeba contractile vacuole, that is an organ of the water-salt homeostasis in Protozoa (Patterson, 1980). Subsequently, the authors published the only work (Mayers and Couillard, 1990) to report that the capability of accelerating vacuole contraction in the amoeba *A. proteus* was peculiar not only of vasopressin and its analogues, but also of other substances of the amphiphilic nature, specifically, the synthetic antagonist of receptors of the V_2 type SKF101926. Based on this, the authors suggested that under effect of vasopressin and other amphiphilic substances, mycellar structures with high water permeability appeared in the amoeba outer membrane, and, hence, this effect was non-specific. The lack of effect of cyclic adenosine monophosphate (cAMP) and euphylline argue in favor of this suggestion. Thus, it looked as if this effect was produced by the hormone itself, without intracellular messengers.

We have decided to check this suggestion, quite interesting from the evolutionary point of view, by using agonists and antagonists of vasopressin receptors as well as activator of adenylyl cyclase and cyclic adenosine monophosphate. The goal of the work was to study signal transduction from receptors or structures of another type, which perceive action of AVP on the amoeba outer membrane. Finally, it was planned to compare the established pathways of the signal transduction in amoeba and in vertebrates.

Material and methods

The experiments were performed on the culture of free-living, freshwater amoebae *A. proteus*. The amoebae were kept in the Prescott medium (Prescott and Carrier, 1964) at 20–22°C and were fed with *Tetrahymena pyriformis* every 48–72 hr; the amoebae were used in the experiment in 24 hr after feeding. The animals were placed in a hollow of the object glass, 0.5 mm^3 in volume, filled with the Prescott medium with studied substances, and were covered with the cover glass. The frequency of vacuole contractions was observed under the microscope at a magnification ×25. Recording of the contractions was done visually and was controlled by a videosystem mounted on the microscope eye-piece and using software of a Matro Inspector image analyzer (Canada). The following preparations were used in the work: arginine-vasopressin (10 μM, Sigma), an agonist of receptors of the V_2 type desmopressin (10 μM, RBI), an activator of adenylyl cyclase forskolin (25 μM, Sigma), cyclic adenosine monophosphate (cAMP) (1 μm, Sigma), a non-selective inhibitor of adenosine receptors of the A_2 type aminophylline (10 μM, Sigma). To characterize receptors, added to the incubation medium also were antagonists of V_1- and V_2-receptors, respectively, [β-mercapto-β, β-cyclopentamethylenepropionyl[1], O-Eth-Thyr[2], Val[4], Arg[8]]-vasopressin (10 μm, Sigma) and [Adamantanacethyl[1], O-Eth-d-Thyr[2], Val[4], Aminobutyryl[6], Arg[8,9]]-vasopressin (10 μM, Sigma). The vacuole contraction frequency was measured in 3 animals, 5 contractions in each animal. The results of the measurements were processed statistically, using non-parametric test, and differences were considered significant at $p < 0.05$.

Results

Frequency of the vacuole contractions of *Amoeba proteus* increases statistically significantly on administration of arginine-vasopressin into the medium (Fig. 1). The AVP effect is inhibited only by antagonist of receptors of the V_1 type, but not of the V_2 type (Fig. 3). At the same time, the agonist of receptors of the V_2 type desmopressin also leads to a rise of frequency of the vacuole contractions (Fig. 1).

Data on the character of signal propagation from the AVP receptors in amoebae are lacking. According to our data, acceleration of contractions of the amoeba vacuole is produced by activator of adenylyl cyclase forskolin and by cAMP (Fig. 1). The stimulating effect of cAMP on the vacuole contraction frequency is inhibited by aminophylline, a non-selective blocker of A_2 receptors (Fig. 2) (Schaefeet al., 2001).

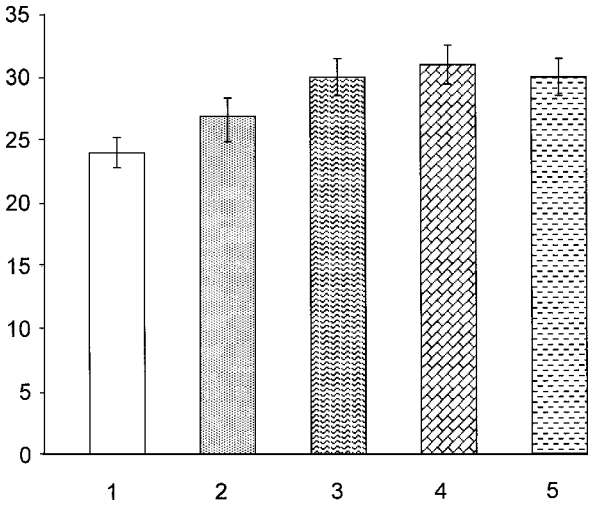

Fig. 1. Effects of arginine-vasopressin, desmopressin, cyclic adenosine monophosphate, and forskolin on frequency of vacuole contractions in *Amoeba proteus*. Abscissa: 1 – control, 2 – AVP 10 μM, 3 – desmopressin 10 μM, 4 – cAMP 1 μM, 5 – forskolin 25 μM. Ordinate: frequency of contractions in arbitrary units (one contraction/min × 100). Vertical bars on the top of columns: standard deviation of the mean.

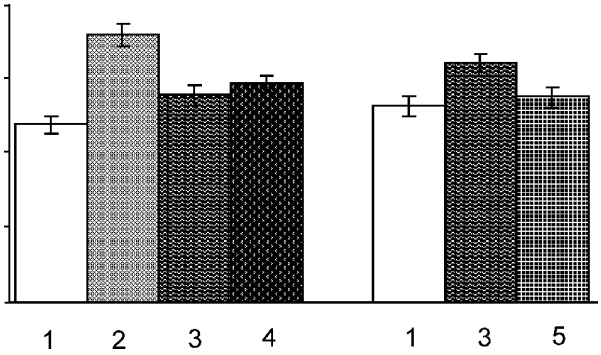

Fig. 2. Frequently of vacuole contractions in *Amoeba proteus* in the presence of arginine-vasopressin, cyclic adenosine monophosphate, and aminophylline. Abscissa: 1 – control, 2 – AVP (10 μM), 3 – cAMP (1 μM), 4 – cAMP (1 μM) + AVP (10 μM), 5 – cAMP (1 μM) + aminophylline (10 μM). Ordinate: frequency of contractions in arbitrary units (one contraction/min × 100). Vertical bars on the top of columns: standard deviation of the mean.

In studying the cAMP effect, we have revealed its significant variability. Sometimes, during several weeks, the amoebae showed no ability to respond to a sufficiently wide range of cAMP concentrations by an acceleration of the vacuole contractions. However, this refracterness was only apparent, as cAMP at this period suppressed the AVP effect (Fig. 2).

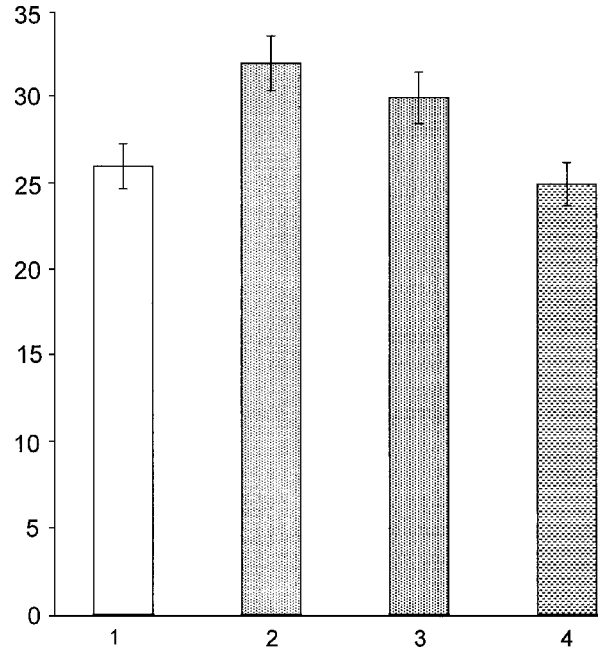

Fig. 3. Effect of AVP on frequency of vacuole contractions in *Amoeba proteus* in the presence of antagonists of arginine-vasopressin. Abscissa: 1 – control, 2 – AVP 10 μM, 3 – AVP (10 μM) + antagonist of receptors of AVP of the V_1 type (10 μM), 4 – AVP (10 μM) + antagonist of receptors of the V_2 type (10 μM). Ordinate: frequency of contractions in arbitrary units (one contraction/min × 100). Vertical bars on the top of columns: standard deviation of the mean.

Discussion

Thus, we were able to confirm the data of Couillard and co-authors (1989) and Mayers and Couillard (1990) about the ability of AVP to increase frequency of vacuole contraction of *Amoeba proteus*. However, our data contradict the conception of these authors about the amphiphilic nature of the AVP action. Such mechanism might have taken place, but it undoubtedly is not the main one. First of all, the AVP effect is eliminated by an antagonist of receptors of the V_1 type and is reproduced by the activator of receptors of the V_2 type desmopressin. This combination of effects seems paradoxical, but we have also obtained some other data confirming specificity of the AVP effect. Acceleration of the vacuole contraction is produced by activator of adenylyl cyclase forskolin and cAMP. Hence, we are dealing with the system of signal transduction. This system combines features of activation of vasopressin receptors of the V_1 and V_2 types. A similar character of transduction was observed in our earlier studies on action of AVP and its analogues on the apical membrane of the urinary bladder of the brown frog *Rana temporaria* (Bagrov and Manusova, 1999). The osmoregulation in the organism of vertebral animals is known to operate by action of AVP on the basolateral membrane of the cells sensitive to this hormone (urinary bladder and skin of anuran amphibians, collective ducts of mammalian kidney). The functional significance of the signal apparatus located on the cell apical membrane has so far remained obscure (Yoshitomi et al., 1996; Bagrov and Manusova, 1999). It is possible that this system is of purely atavistic character. In this case, similarity of pathways of signal transduction from the AVP receptors located on the apical membrane of the urinary bladder cells of the frog and on the external membrane of the amoeba is of particular interest. Similarity of peculiarities of signal transduction in these objects also includes action of cAMP. In both cases, cAMP acts as an activator of extracellular receptors, which was repeatedly shown in *Dictyostelium discoideum* (Soede and Inshall, 1994). The signal transduction from cAMP receptors in *Dictyostelium discoideum* is rather unique, as it is accompanied by activation of adenylyl cyclase and phospholipase C (Hereld and Devreotes, 1992).

Adenylyl cyclase is known to be a component of signal propagation from receptors of the V_2 type, whereas phospholipase C is activated at stimulation of

receptors of the V_1 type (Yoshitomi et al., 1996). Thus, in this case, a mixed character of the signal propagation is also observed. The ability of the non-specific blocker of adenosine receptors, aminophylline, to inhibit the cAMP effects might indicate action of cAMP on these receptors located on the cell surface. However, the extracellular cAMP itself can be converted into adenosine by the enzyme 5-ectonucleotidase (Orlov and Maksimova, 1999). Most likely, cAMP affects amoeba via its own receptors that, probably, are close to the adenosine ones. Such receptors were studied in detail in *Dictyostelium* (Hereld and Devreotes, 1992). It is known that cAMP is the main factor of regulation and development in *Dictyostelium* at its amoeboid stage (Soede et al., 1994). Naturally, we cannot rule out penetration of cAMP into the cell and its action as the second messenger. The mechanism of the cAMP action has remained much more obscure. Its analysis is handicapped by the fact that the cAMP formed in the cell can, on the one hand, leave the cell and affect external receptors (Dinauer et al., 1980), while, on the other hand, regulate sensitivity of the external receptors to intracellular cAMP (Van Haastert, 1994). The studies referred to above have shown that in *Dictyostelium*, intracellular mechanisms of various cAMP actions include the same elements as during activation of AVP receptors in vertebrates. Our preliminary data indicate that in *A. proteus*, action of cAMP is not sensitive to effects of modulators of the protein kinase C activity, blockers of calmodulin and calcium channels, that clearly affect the AVP action. As to AVP, it has remained unclear, with which endogenous substance the receptor apparatus responding to AVP interacts. Parasitic amoebae have been shown to synthesize and release peptides forming pores in the host's organism (Leippe et al., 1991). However, the amoebae themselves can hardly be the objects of such action. Amoebae release the so-called chemotactile peptides that stimulate pinocytosis (Josefsson et al., 1972).

In conclusion, it is to be stated that there are all grounds to consider the AVP action on *A. proteus* in terms of hormonal regulation.

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