Arginine-Vasopressin Effect on the Content of Cyclic Nucleotides, Cellular Protein Phosphorylation, and Adhesion to Plastic of Peritoneal Mouse Macrophages

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Intracellular concentration of cAMP and phosphorylation of 15 kD protein in cultured mouse peritoneal macrophages are increased in the presence of arginine-vasopressin. Vasopressin decreases adhesion of macrophages to plastic.

Key Words: *macrophages; arginine-vasopressin; cyclic nucleotides; protein phosphorylation; cell adhesion*

Vasopressin (VP) is a neuropeptide which is probably involved in functional interactions between the central nervous and immune systems [5]. Mononuclear macrophagal cells are an object of its immunomodulating effect. Some researchers claim that these cells are the main target of specific binding of VP among circulating blood cells of man [8]. Few studies of functional effects and mechanisms of VP effect on macrophages have been carried out on cells of different species using different structural analogs of VP, which may account for their contradictory results. Studies on mouse and rat cells showed activation of phagocytosis in macrophages under the effect of VP [4,10]. Study of intracellular signalization systems activated during specific binding of VP to human circulating blood mononuclear phagocytes showed an increase in the content of intracellular cAMP in the presence of the hormone [8,12], which is typical of phagocytosis inhibition at all its stages [2]. Studies of specific VP binding by mononuclear macrophagal cells of circulating blood of humans and rats carried out by different analogs and selective antagonists of VP, brought the authors to contradictory conclusions on the type of receptors (V_1 or V_2) for this neuropeptide [7-9].

Further studies of the mechanisms underlying the effect of VP on macrophages were devoted to its effects on the intracellular content of cyclic nucleotides, cellular protein phosphorylation, and macrophage adhesion to plastic on the same cell model: cultured mouse peritoneal macrophages.

MATERIALS AND METHODS

Macrophages of CBA mice were isolated from peritoneal lavage by adhesion on the glass. Nonadherent cells were removed by washing in warm incubation medium, and adhesive cells (mainly macrophages) were washed from the glass by cold Ca^{2+} and Mg^{2+}-free phosphate saline buffer.

For assessing the effect of VP on the intracellular content of cAMP and cGMP, macrophages were preincubated for several hours at 37°C and then incubated for 10 min with 10^{-9}, 10^{-8}, 10^{-7}, and 10^{-6} M [Arg^8]-vasopressin (Sigma), after which cold trichloroacetic acid was added. The specimens were immerced in boiling water for 5 min, homogenized,

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and centrifuged. The supernatant was extracted with ether and lyophilized, dry residue was dissolved in buffer for measuring cyclic nucleotides with kits for cAMP and cGMP radioimmunoassay (Chemapol). The concentration of cellular protein in the specimens was measured routinely [13].

Phosphorylation of macrophagal cellular proteins under the effect of VP was evaluated by incorporation of ^{32}P after a 2-h incubation of cells in medium containing ^{32}P-orthophosphoric acid (12 MBq/ml, Izotop) for the production of endogenic pool of ^{32}P-ATP, after which the specimens were incubated with or without 10^{-6} M arginine-VP, 10^{-4} M methylisobutyl xanthine, and 10^{-3} M dibutyril-cAMP for 60 min at 37°C. The reaction was stopped by adding cold buffer containing 100 mM NaF, 10 mM EDTA, 80 mM sucrose, and 10 mM Tris, pH 7.4. The specimens were centrifuged for 5 min at 1000*g*, the supernatant was discarded, and buffer of the following composition was added to the precipitate: 1% sodium dodecyl sulfate, 5% 2-mercaptoethanol, 5% glycerol, 1 mM EDTA, 5 mM bromophenol blue, and 10 mM Tris (pH 8.0), and boiled for 15 min. For eletrophoretic separation of protein, the specimens were layered onto 5% stack and 14% separating polyacrylamide gel. Electrophoresis was performed in the Laemmli's system [11] at 150 V for 5 h. After electrophoresis the gels were stained, vacuum-dried, and autoradiographed.

Adhesion of cells to plastic was assessed by total cellular protein content after incubation of cell suspension in the wells of 96-well plates in Medium 199 with fetal calf serum at 37°C for 1 h in the presence of 10^{-9}, 10^{-8}, and 10^{-7} M arginine-VP and removal of nonadherent cells by shaking and washing in a warm incubation medium.

Results were statistically processed using Student's *t* test.

RESULTS

Radioimmunoassay of cAMP and cGMP in macrophages after 10-min incubation with arginine-VP in concentrations 10^{-9}-10^{-6} M revealed a dose-dependent increase in the intracellular content of cAMP which was statistically significant at VP concentrations of 10^{-7} and 10^{-6} M ($p<0.01$), while the content of cGMP virtually did not change (Fig. 1). The cGMP/cAMP ratio in the cells decreased. These results agree with the data obtained on human circulating blood mononuclear phagocytes [8]. An increase in cAMP level in cells is typical of activation of type V_2 receptors of VP associated with antidiuretic activity of this hormone at the level of renal tubular epithelium. An increase in the intracellular cAMP concentration can lead to activation of cAMP-dependent protein kinases and phosphorylation of cellular proteins; therefore, we studied macrophagal protein phosphorylation in the presence of VP. A 15-kD protein was detected, phosphorylated upon addition to cells of VP and dibutyril-cAMP (Fig. 2), presumably a substrate of

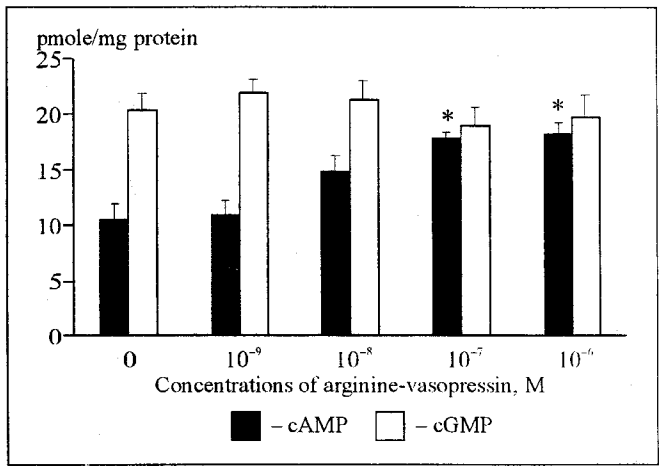

Fig. 1. Changed content of cyclic nucleotides in peritoneal macrophages in the presence of vasopressin. Here and in Fig. 3: *$p<0.01$ *vs.* the control.

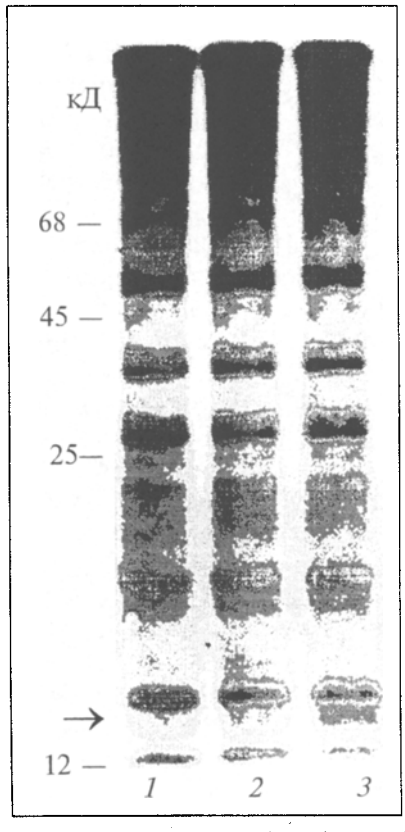

Fig. 2. Phosphorylation of CBA mouse peritoneal macrophage proteins under the effect of vasopressin (autoradiogram). 1) control; 2) vasopressin, 10^{-6} M, and methylisobutyl xanthine, 0.1 mM; 3) dibutyril-cAMP, 1 mM. Arrow shows 15 kD protein.

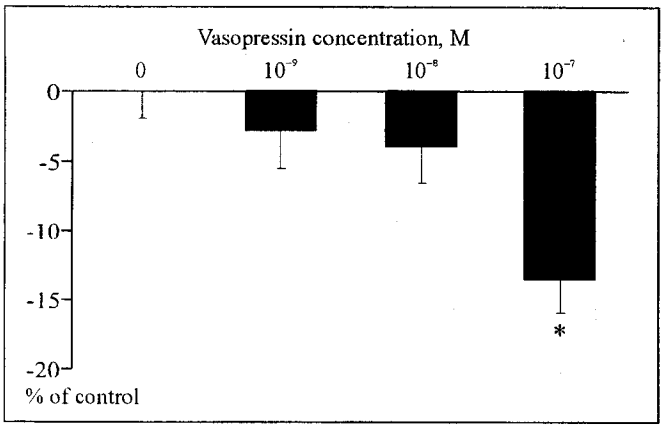

Fig. 3. Changed macrophage adhesion to plastic in the presence of vasopressin.

cAMP-dependent protein kinases activated during VP binding to macrophagal receptors. The 15-kD protein is a protein of the membranous fraction of the renal tubular epithelium phosphorylated in the presence of VP [3]. Which characteristics of macrophagal membranes (if it is a membrane-associated protein) can change in the presence of VP? A characteristic feature of macrophages is the capacity of adhesion. Study of VP effect on macrophage adhesion to plastic revealed a decrease in this ability in the presence of 10^{-7} M VP ($p<0.01$, Fig. 3). Although such an inhibition of cell adhesion combined with increase of cAMP concentrations in them and a decrease in intracellular cAMP/cGMP ratio, which was observed at the same concentration of the hormone, is in line with current views on the direction of these intracellular messengers' action in the regulation of macrophagal function, these results can hardly be interpreted as a decrease in the functional activity of macrophages in the presence of VP. Some authors confirmed the stimulation of phagocytosis with VP — the results of experiments on peritoneal mouse macrophages *in vitro* at VP concentrations compatible to those we used [4]; this neuropeptide activates human macrophage chemotaxis [14]. At certain concentrations cAMP increases the migration capacity of macrophages, which can be explained by decreased adhesion of these cells [6]. *In vivo* response of macrophages to inflammation, their chemotaxis and inhibition of leukocyte adhesion induced by chemoattractants, are in good correlation; reversible cell adhesion is required for leukocyte locomotion [15]. Thus, the notion of discreteness of signs of macrophage activation and independent mechanisms of their activation characteristic of these polyfunctional cells is justified [1].

We cannot say for sure that the detected changes in intracellular content of cAMP, phosphorylation of 15 kD protein, and decreased adhesion of macrophages to plastic are components of the same chain of cell events triggered by the hormone, but detection of these shifts in the same cell model in the same range of hormone concentrations permits such a hypothesis.

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