A NEW MECHANISM OF ACTION OF VASOPRESSIN ON NEURON MEMBRANES

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The neurotropic mechanisms of action of arginine-vasopressin (VP) have hitherto been studied mainly on models in which synaptic processes were studied [7, 8, 11]. The possible mechanisms of the effect of VP on electrically sensitive ionic channels of neuron membranes and the probable consequences of such reactions as a rule have not been considered, and have virtually not been studied. In recent years, however, data indicating the high sensitivity of these structures to endogenous peptides and neurotransmitters have been published [1, 6].

The aim of the present investigation was to study the action of VP on electrically sensitive ionic channels conducting sodium (I_{Na}), calcium (I_{Ca}), and slow (I_K^s) and fast (I_K^f) potassium membrane currents in 85 neurons of the mollusk *Lymnaea stagnalis*.

EXPERIMENTAL METHOD

In the investigation described below a method of intracellular dialysis and perfusion of neurons under membrane voltage clamping conditions [2] was used. I_{Na} and I_{Ca} were separated from each other by means of a calcium-free or sodium-free perfusion solution [4]; K^+ ions in the dialyzing solution were replaced by Cs^+ ions, which do not pass through the membrane. $I_K^{s,f}$ were recorded with the aid of sodium-free and calcium-free perfusion solutions, on account of which, first, inward currents were blocked and, second, ionic channels of the calcium-dependent potassium current were inactivated [10]. The action of VP was estimated as a percentage increase or decrease of the amplitude of the currents under the influence of VP compared with the normal state, taken as 100%. Current–voltage characteristic curves of single neuron membranes were plotted for a particular current under normal conditions and under the influence of VP. On the basis of the effects of VP "dose–effect" graphs were plotted. The results were subjected to statistical analysis by Student's t-test with a level of significance of $p \leq 0.05$.

EXPERIMENTAL RESULTS

VP significantly reduced the amplitude of in concentrations of between $1 \cdot 10^{-15}$ and $1 \cdot 10^{-6}$ M. The maximal effect of VP on I_{Na} was observed in a concentration of $1 \cdot 10^{-6}$ M. VP also blocked I_{Ca} In this case the action was complex in character. In a concentration of $1 \cdot 10^{-15}$ M VP blocked I_{Ca} by 41.7 ± 20.1%, but with an increase in the concentration of VP there was some decrease in the blocking effect of the peptide (concentration of $1 \cdot 10^{-13}$ M); finally, within the concentration range from $1 \cdot 10^{-12}$ to $1 \cdot 10^{-6}$ M it was relatively ineffective (Fig. 2). VP had no significant effect on I_K^s (Fig. 1). The action of the peptide on I_K was twofold. Application of VP to the membrane in concentrations of $1 \cdot 10^{-16}$ to $1 \cdot 10^{-15}$ M led to a significant increase in the amplitude of this current (Fig. 1). In concentrations of over $1 \cdot 10^{-9}$ M VP blocked I_K, whereas by its action on the membrane in concentrations of between $1 \cdot 10^{-16}$ and $1 \cdot 10^{-14}$ M it reduced the nonspecific leakage current (NLC) on

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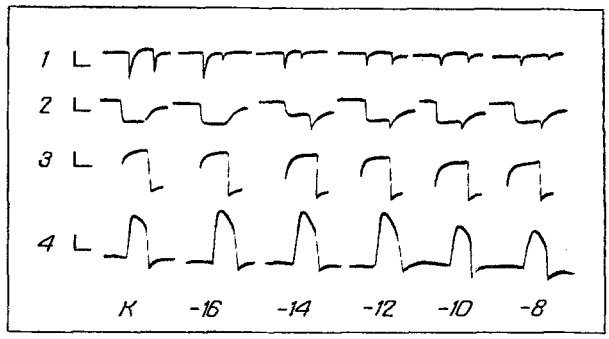

Fig. 1. Traces of ionic currents under normal conditions and under the influence of vasopressin (VP). Horizontal axis — logarithm of VP concentration. C) Control. 1) I_{Na} (sodium current), 2) I_{Ca} (calcium current), 3) I_K^s (slow potassium current), 4) I_K^f (fast potassium current). Calibration: 1) 20 nA, 50 msec; 2) 5 nA, 140 msec; 3) 20 nA, 400 msec; 4) 20 nA, 50 msec.

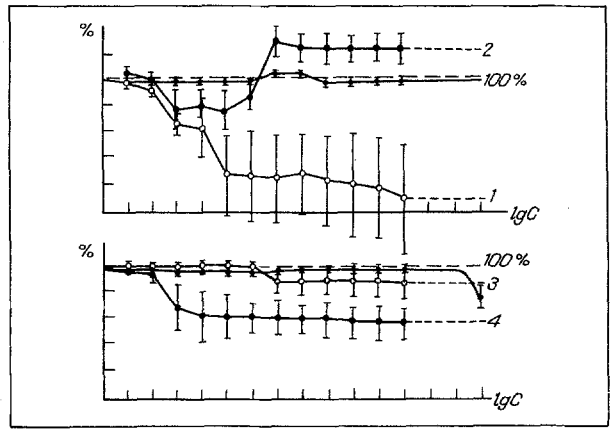

Fig. 2. "Dose—effect" curves showing action of vasopressin on ionic channels. Abscissa, logarithm of VP concentration; ordinate, amplitude of ionic current (in per cent of normal level, taken as 100%). Short vertical lines indicate confidence intervals. 1) I_{Na}, 2) I_{Ca}, 3) I_K^s, 4) I_K^f.

average by 23.7 ± 10.5 nA. In concentrations of between $1 \cdot 10^{-13}$ and $1 \cdot 10^{-6}$ M it increased NLC by 38.2 ± 14.8 nA. Higher concentrations of the peptide led to an abrupt and irreversible increase in NLC. The kinetics of activation and inactivation of all currents was not significantly changed by the action of VP. Plotting the corresponding current—voltage characteristic curves of the membrane revealed no change in voltage-dependence of the ionic channels after application of VP to the membrane in adequate doses.

This investigation showed for the first time that VP, in adequate concentrations, can significantly change the amplitude of ionic currents through electrically excitable channels of neuron membranes. It is clear that blocking I_{Na} may lead to impairment of action potential (AP) generation in neuron nets, and that blocking I_{Ca} may lead to shortening of the AP plateau [2, 5]. Moreover, since Ca^{2+} performs the role of secondary messenger in the cell [9], reduction of its entry into the neuronal cytoplasm may have a significant effect on intracellular metabolism and, in particular, on activity of several phospholipases and protein kinases. If ionic channels probably are involved in the regulation of excitability of the neuronal somatic membrane in the interspike interval [2], and for that reason VP, depending on its concentration, may either lower or raise the threshold of membrane excitability between AP. This dual effect of VP on I_K^f is possibly due to complex interaction of this peptide with subunits of the ionic channel. It may be that under the influence of various concentrations of VP a "frame shift" of interaction between hormone and chemosensitive subunits of the electrically sensitive ionic channel takes place [6]. The possibility likewise cannot be ruled out that the I_K^f channel has more than one binding site for VP. In our view, similar mechanisms may lie at the

basis of the dual dynamics of the change in NLC under the influence of VP. Probably one of the basic mechanisms of the change in amplitude of NLC under the influence of the preparations is opening, blocking, or inactivation of chemosensitive ionic channels [3].

Thus VP has a significant effect on the properties of electrically sensitive ionic channels of the neuron membrane. The important point is that VP, in these same concentrations, effectively activates chemosensitive extrasynaptic neuron membrane receptors. It is possible that under the influence of VP, cooperative relations begin to form between these two systems regulating excitability of the neuron membrane, aimed at securing the most effective possible regulation of cellular excitability.

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EFFECT OF HEPARIN AND THROMBOPLASTIN ON THE HALF-LIFE OF ^{125}I-PROTEIN C IN CIRCULATING RAT BLOOD

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Protein C is a vitamin K-dependent protein of the blood clotting system which circulates in the blood in the form of the proenzyme — a serine proteinase precursor [12]. Conversion of protein C into its active form (activated protein C) takes place in the body under the influence of thrombin [6]. Activation of protein C is accelerated by several orders of magnitude by the endothelial membrane protein thrombomodulin, which forms an equimolar complex with thrombin, which increases the affinity of thrombin for protein C [3]. Unlike other vitamin K-dependent proteinases of the blood clotting system, namely factors II, VII, IX, and X, which are procoagulants, activated protein C exhibits anticoagulant and profibrinolytic properties. The anticoagulant effect is due to the fact that activated protein C inactivates factors V (Va) and VIII (VIIIa) and, consequently, it inhibits thrombin generation [7]. The profibrinolytic action of activated protein C is connected with the fact that it interacts with the inhibitor of tissue plasminogen activator, with which this activator exists in the form of a complex. This leads to elevation of the

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