EXTRASYNAPTIC ACTION OF VASOPRESSIN, OXYTOCIN, AND VASOTOCIN NEUROPEPTIDES ON ELECTRICALLY OPERATED IONIC CHANNELS AT THE MOLLUSK NEURONAL MEMBRANE

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Techniques of intracellular dialysis and neuronal perfusion in the visceral ganglion of Lymnaea stagnalis used during voltage-clamping at the neuronal membrane helped to ascertain that a concentration of 1×10^{-16}-1×10^{-6} M neuroactive peptides (vasopressin, oxytocin, and vasotocin) alter the amplitude of electrically-operated transmembrane ionic currents considerably without affecting the kinetics of current activation and inactivation and surface potential at the membrane. The experimental conditions applying made it possible to record incoming sodium and calcium currents separated from each other as well as outward delayed and transient potassium currents. It was found that electrically-operated cerebral currents could either increase or decline in amplitude under the effects of peptides applied at different concentrations to the membrane of the same unit. Receptors of the peptides investigated in this study are thought to be located within the structure of electrically-operated channels at the neuronal membrane.

INTRODUCTION

Neurophysiological research uses experimental modeling of isolated mollusk neurons extensively [3, 8, 9-11]. The fact that mechanisms of electrogenesis present at the neuronal membrane of mollusks and vertebrates do not differ from each other is a major premise [8], enabling mollusk neurons to be used in experimental modeling for interpreting the action of different biologically active substances on neurons of vertebrate animals. Most known neurotransmitters functioning in the central nervous system (CNS) of vertebrates (acetylcholine, catecholamines, serotonin, γ-aminobutyric acid, etc.) have been found in the gastropod nervous system [2, 9, 14]. A peptidergic system has been found in the nervous system of gastropod mollusks involving both mollusk neuropeptides themselves and some peptides found in vertebrates, such as opiates and substance P [2, 15, 23, 25, 26]. Arginine vasopressin (VP), oxytocin (OT), and vasotocin (VT) and fragments of these are thought to be present in the gastropod nervous system [5, 23]. Chemoreceptors for biologically active substances in vertebrate and invertebrate CNS, including neuropeptide receptors, are located not just at synapses but also at extrasynaptic sites on the neuronal soma and processes [2, 4, 14]. A certain proportion of extrasynaptic chemoreceptors at the neuronal membrane could apparently be associated with electrically-operated ionic channels at the membrane [1, 3, 10, 21]. The extrasynaptic action of neuropeptides and other physiologically active substances could well be composed of two principal components; influences on chemically-operated receptor–ionophoric complexes at the membrane and electrically-operated ionic channels. The alteration in the functional state of electrically-operated ionic channels under the action of physiologically active substances on the membrane could either be associated with the presence of specialized chemoreceptors within the structure of these channels [1, 14-16, 21] or with altered intracellular metabolism resulting from change in the activity of second messenger systems [2, 4, 8, 12, 14, 22]. The study of the neurotropic mechanisms underlying the action of chemically and physiologically similar peptides (VP, OT, and VT) has mainly been conducted previously on models investigating synaptic processes produced in the organism by applying these substances [6, 18, 19]. Mechanisms potentially underlying the effects of VP, OT, and VT on extrasynaptic electrically-operated ionic channels at the neuronal membrane have not yet been examined.

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Bearing the above factors in mind, we conducted research into the effects of VP, OT, and VT on functionally active ionic channels of sodium (I_{Na}), potassium (I_{Ca}) delayed (I^S_K) and transient (I^F_K) potassium current at the membrane in the gastropod mollusk Lymnaea stagnalis.

METHODS

Experiments were conducted using techniques of intracellular dialysis and neuronal perfusion with voltage-clamping at the membrane [8]. Transmembrane ionic currents were recorded by electrophysiological equipment consisting of a spike generator serving as stimulator, and electronic clamping arrangements for maintaining a given voltage at the neuronal membrane also amplifying ionic current, an oscillograph, photographic recorder, and a spike discriminator. A dialyzing substance free of potassium ions and perfusing solution free of sodium and calcium ions were used alternately to separate ionic currents, according to the aims of the experiment [7, 8, 11]. The standard intracellular dialyzing solution was composed as follows (mM): KCl: 120; Tris OH-1: 3; max. pH 7.4. Calcium-free solutions: CsCl: 120, Tris OH-1: 3; max. pH 7.4. The standard perfusing (extracellular) solution contained (mM): NaCl: 100, KCl: 2, $CaCl_2$: 2, $MgCl_2$: 5, Tris OH-1: 3; max. pH 7.4; for the sodium-free solution: Tris Cl: 105, $CaCl_2$: 10, $MgCl_2$: 5, and Tris OH-1: 3; max. pH 7.4; for the calcium-free solution: NaCl: 105, $MgCl_2$: 5, Tris OH-1: 3; max. pH 7.4. The calcium- and sodium-free perfusing solutions were used to divide off I_{Na} and I_{Ca}, with Cs^+ (which blocks potassium ionic channels) replacing K^+ in the dialyzing solution. Recording of I^S_K was achieved by using the calcium-free external (perfusing) solution, resulting in inactivation of ionic channels for the passage of calcium-dependent I_K [24]; I_{Na}, I_{Ca}, and I^SK were only recorded at a holding potential of −50 mV − circumstances inactivating I^F_K [8]. A holding potential of −110 mV was used to separate off isolated I^F_K. Test potentials ranging between −40 and +30 mV were selected for different units in the course of recording I_{Na}, I_{Ca}, and I^S_K. Test potentials varied between −100 and −30 mV when investigating I^F_K. It should be mentioned that selected holding and test potentials did not vary throughout the course of a given experiment. The three substances VP, OT, and VT were applied to the outer side of the neuronal membrane. Pepide action was assessed by the increase or reduction in the amplitude of the appropriate electrically operated ionic currents expressed as a percentage of control level. Once each of the peptide concentrations used had been applied over a 5-30 min period, washout of the preparation continued until the current investigated completely returned to control amplitude. Peptide solutions were introduced into the experimental chamber holding the neuron by means of a special syringe. Standard extracellular solution was applied to the neuronal membrane in control experiments. Dose–response curves were produced, based on the effects of the preparations used. Current–voltage relationships (CVR) were obtained for certain currents affecting individual units under the action of experimentally-applied substances and in controls. Decline in the amplitude of I_{Ca} during neuronal dialysis was monitored throughout all experiments involving this current. Dialysis was curtailed in order to limit this effect. Leakage current at the membrane was compensated for by using a converter with negative feedback as and when required. Findings underwent statistical processing, applying Student's t test with $\alpha \leq 0.05$. Research investigating the effects of VP, OT, and VT on ionic channels was conducted on 85, 111, and 88 units respectively. Action of these peptides on I_{Na}, I_{Ca}, I^S_K, and I^F_K respectively was investigated in 21, 18, 29, and 17 units in the case of VP, 30, 15, 51, and 15 for OT, and 21, 10, 38, and 19 for VT. Peptide influence on the functional activity of electrically-operated ionic channels was analyzed over a concentration range of 1×10^{-16}-1×10^{-6} M. Lower peptide concentrations proved ineffective in this study, while higher concentration levels amplified nonspecific leakage current ast the membrane in most cases.

RESULTS

In 14 out of 21 units, VP blocked I_{Na} at all the above concentrations (see Fig. 1). Blockade reached its peak when using VP at a concentration of 1×10^{-6} M, with amplitude measuring 19.3 ± 14.8% of control level; accordingly, blockade equaled 80.7 ± 14.8% (mean ± SEM). A decline in the amplitude of I_{Ca} was also observed under VP action, although the increase in intensity of blockade followed a more complex pattern (see Fig. 1). A significant reduction in this current under VP influence was only noted in 11 out of 18 test units. VP only produced a slight effect on I^S_K. Statistically significant results evidencing slight blockade of this current were only obtained from 21 out of 29 neurons. Amplitude of I^F_K only declined by an average of 19.0 ± 4.4% even under the effects of VP at a concentra-

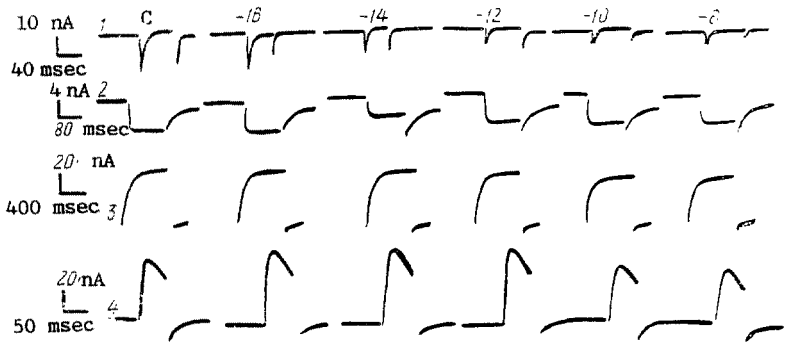

Fig. 1. Oscillograms of transmembrane ionic currents in controls (C) and under the effects of vasopressin. Peptide concentrations (logarithmic scale) shows above oscillograms; C: controls; series 1) sodium, 2) potassium, 3) potassium (holding), and 4) transient potassium current (test potentials of −10, +10, +10, and −40 mV respectively).

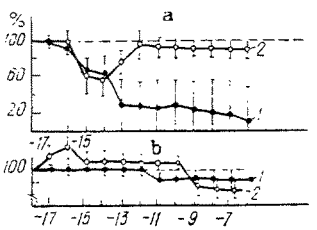

Fig. 2. Dose–response curve (a and b) for vasopressin action on electrically-operated ionic currents at the membrane. Abscissa: peptide concentrations (logarithmic scale); ordinate: current level as of controls. Vertical columns: confidence intervals (a) ≤ 0.05; a, plot 1) sodium and 2) potassium current; b, plot 1) potassium (holding) and 2) transient potassium current.

tion of 1×10^{-6} M. The influence of VP on I^F_K at the membrane on 13 out of 17 test neurons was effective and of a more complex nature. Application of lower VP concentrations (of 1×10^{-16} and 1×10^{-15} M) to the neuronal membrane led respectively to a 20.8 ± 3.2% and 29.1 ± 4.2% increase in I^F_K amplitude. Further increasing VP concentration from 1×10^{-14} to 1×10^{-9} M led to the onset of response very similar in amplitude at the membrane, corresponding to the plateau on the dose–response curve seen in Fig. 2 above the abscissa. Applying VP at concentrations in excess of 1×10^{-9} M to the neuronal membrane led to the onset of I^F_K blocking action in the ionic channel system: this peaked when using the same peptide at a concentration of 1×10^{-6} M.

Significant blockade of I_{Na} was produced by OT in 17 out of 30 tests units. The action of this peptide largely resembled that of VP (see Fig. 3). It also blocked I_{Ca} in 10 out of 15 neurons. Effective OT concentration levels were higher than with blockade of I_{Na} in such cases, however. In addition, OT blocked I^S_K and I^F_K at the membrane of 38 out of 51 and 11 out of 15 test units respectively (see Fig. 4). Finally, VT, in common with VP and OT, successfully blocked I_{Na} at the membrane of 13 out of 21 test units (see Fig. 5). It produced a varying action on I_{Ca}. Significant results were obtained for 7 units, with 1×10^{-13} and 1×10^{-12} M VT increasing I_{Ca} amplitude by 34.2 ± 8.8 and 20.4 ± 6.8% respectively (see Fig. 6). This peptide blocked I_{Ca} at a starting concentration of 1×10^{-13} M. It also produced diverging effects on I^S_K and I^F_K ionic channels. Lastly, VT proved effective in relation to I^F_K at the membrane of 29 out of 38 test neurons (17 out of 19 units in the case of I^F_K).

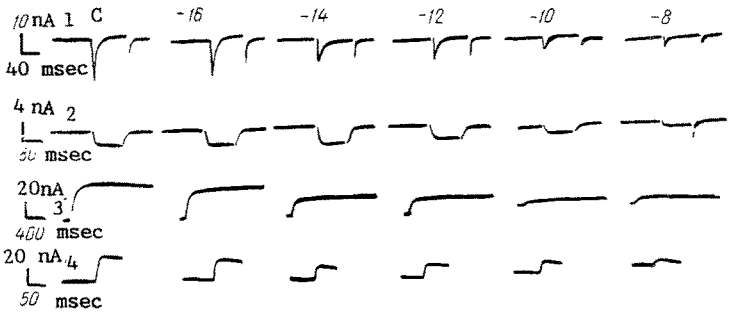

Fig. 3. Oscillograms of transmembrane ionic current in controls (C) and under oxytocin action. Remaining notations as for Fig. 1.

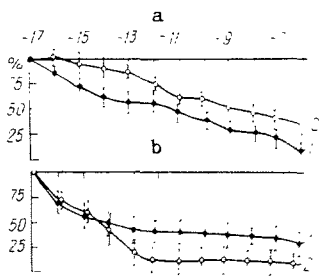

Fig. 4. Dose–response curve for oxytocin action on electrically-operated ionic currents at the membrane.

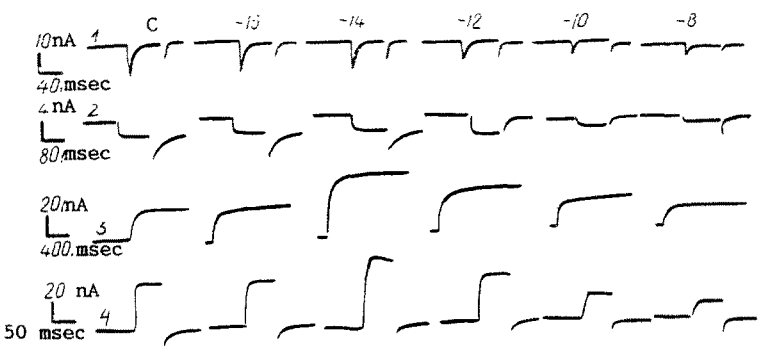

Fig. 5. Oscillograms of transmembrane currents in controls (C) and under vasotocin action. Remaining notations as for Fig. 1.

Fast onset of peptide effects (lasting a few seconds) was a distinctive feature of the action on all our test peptides on electrically operated ionic channels at the membrane. In addition, the amplitude of such fast-developing effects remained constant for some hours. We should also mention the fact that activation and inactivation kinetics of all the electrically operated channels investigated during these observations, involving VP, OT, and VT application to the membrane, remained unchanged. Plotting of selected CVR for individual units based on different currents for action of experimentally-applied substances and in controls revealed no shift in these curves in relation to the abscissa.

DISCUSSION

This study has shown how sufficient concentrations of VP, OT, and VT [2, 5, 6] can significantly modulate the amplitude of electrically operated ionic channels at the neuronal membrane and that, considering their CVR did not shift in relation to the voltage axis, this occurrence could not be put down to the three preparations acting on surface potential of the membrane. Findings from the literature [8, 9, 13] indicate that the blockade of I_{Na}

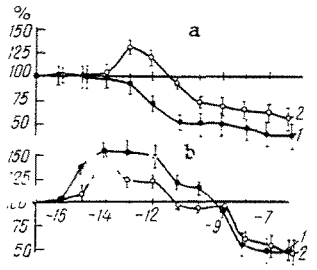

Fig. 6. Dose–response curve (a and b) for vasotocin action on electrically-operated ionic currents at the membrane.

produced by these substances could render generation of action potentials (AP) more difficult in neuronal modules; blockade of I^S_K and increased I_{Ca} could lead respectively to foreshortening and prolongation of the AP plateau. Since Ca^{2+} acts as second messenger in nerve cells, moreover, Na^{2+} increase or decrease in neuronal cytoplasm manifests in the intracellular metabolism and in the activity of cAMP and cGMP systems in particular [8, 22]. Ionic channels carrying I^S_K are responsible for repolarizing the membrane at the point of AP generation, while those carrying I^F_K probably regulate excitation at the neuronal soma during the interspike interval [8, 13, 17]. It should be mentioned that the complex pattern of how different doses of VP, OT, and VT influence the functional activity of electrically-operated ionic channels means that no well-grounded conclusion on raised or reduced neuronal excitation thresholds under the action of these peptides may be drawn without special verification. The above-mentioned findings in the literature would justify such an analysis in each individual case of VP, OT, and VT action using a given concentration. The double-sided effect of VP and VT on the determined population of the ionic channels agrees well with the multidirectional effects of these peptides observed at the systemic level [6, 18, 19]. The double-sided effect of VP and VT on ionic channels could well indicate that the relevant chemoreceptors at the membrane interacting with these peptides do not have a single binding site [2, 12]. The high specificity of VP, OT, and VT action revealed in this study would suggest the existence of receptors specific to these substances at the membrane. Our finding of lack of response to the peptides at the membrane of numerous nerve cells could well point to the existence of such receptors in these units. The extensive duration of response to peptides observed in electrically operated ionic channels during our experiments apparently indicates that intracellular biochemical systems help to produce such effects. Were this not the case, peptide action would obviously be short-lived, firstly because inactivation of chemoreceptors would take place and secondly because of destruction of the peptide by specialized proteolytic mechanisms located at the neuronal plasma membrane [2, 9, 12, 14]. The systems most likely to potentiate long-term changes in the operation of electrically-driven ionic channels is the metabolic system of neuronal second messengers; cGAMP, cAMP, cGMP, and inositol triphosphate, which can influence phosphorylation of ionic channels by the appropriate protein kinase [2, 8, 12, 22]. Chemoreceptors for VP, OT, and VT activating second messengers might be located both within and outside the structure of electrically operated ionic channels [2, 20, 21]. Further research will be required in order to establish a given receptor location. All three substances — VP, OT, and VT modulated functional activity of sodium, calcium, and potassium electrically operated channels at the neuronal membrane at concentrations similar to those at which they had proved active on chemically operated receptor-ionophore complexes at the membrane [2, 20, 21]. The possibility should be considered that cooperative relationships for the purpose of the best means of regulating neuronal excitability set in between the two systems for controlling this excitability under the action of these peptides on the somatic neuronal membrane.

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