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CELL BIOPHYSICS

Aqueous and Salt Solutions of Quinine of Low Concentrations: Self-organization, Physicochemical Properties and Actions on the Electrical Characteristics of Neurons

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Abstract—Self-organization, the physicochemical properties of aqueous and salt solutions of quinine and the effects of salt quinine solutions in a wide range of concentrations ($1 \cdot 10^{-22}$–$1 \cdot 10^{-3}$ M) on the electrical characteristics of the edible snail's identified neurons were studied. Similar non-monotonic concentration dependencies of physicochemical properties of aqueous and salt quinine solutions at low concentrations are obtained. This allows of predicting the occurrence of biological effects at low concentrations of quinine solutions. Intrinsic (within 5% of the interval) changes in membrane potential, the amplitude and duration of the neuron action potential under the influence of quinine salt solutions at concentrations of quinine of $1 \cdot 10^{-20}$, $1 \cdot 10^{-18}$, $1 \cdot 10^{-10}$ M are found. For these concentrations the extreme values of specific conductivity and pH are shown.

Keywords: self-organization, physicochemical properties, solutions, membrane potential, threshold potential, identified neurons

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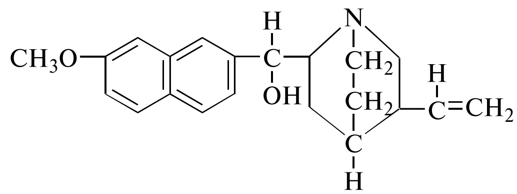
INTRODUCTION

In works [1–3] it is for the first time shown that in aqueous solutions of some substances at low concentration there form nanosized molecular ensembles called nanoassociates. Formation of nanoassociates is initiated by the dissolved substance under certain conditions, paramount of which come to be the availability of external physical fields (geomagnetic and low-frequency electromagnetic) [4] and certain structure of substance [5]. The disclosed phenomenon got the name of "effect of ultralow concentrations and electromagnetic fields" [4]. It is shown that formation of nanoassociates stipulates the emergence of unusual physicochemical properties of solutions of low concentrations and, what is especially important, correlates with bioeffects of these solutions [2, 3]. In work [3] upon studying the self-organization and physicochemical properties of physiological solution (0.16 M NaCl), containing a regulator of activity of microorganisms of low concentrations (10^{-13}–10^{-3} M), it is for the first time shown that regularities of physicochemical behavior of aqueous solutions of biologically active substances (BAS) of low concentrations are preserved also in physiological solution.

The present work has been undertaken with an aim of further confirmation of the possibility of manifestation of the "effect of ultralow concentrations and electromagnetic fields" in salt solutions of BAS, and also establishment of interconnection of the process of self-organization, physicochemical properties and bioeffects emerging under impact of salt solutions of BAS. For attainment of this foal in the quality of bioobject we chose an isolated preparation of the nervous system of a mollusk, capable of functioning only in physiological solution, while in the quality of BAS – a broadly known substance quinine (see structural formula), exerting many-sided action (antifebrile, disinfectant, anesthetic etc.) on a living organism as dependent on the applied dose. The main peculiarity of quinine as a pharmacological drug comes to be its antimalarial action. It is known that one of the mechanisms of action of antimalarial drugs comes to be blocking of Ca^{2+}-dependent K^+-channels [6, 7]. Prof. Herman et al., came to a conclusion that Ca^{2+}-dependent K^+-channels present as a key integrator in many biological systems and their activity may be modulated in broad limits by complicated and various mechanisms [8]. Sufficiently long ago it was established that isomers quinine and quinidine may serve as blockers of Ca^{2+}-dependent K^+-channels [9]. However quinine, apart from this, blocks, but less specifically than tetraethylammonium, the potential-dependent K^+-channels [10], and also is capable of decreasing the amplitude of potassium current [11]. Despite these accompanying effects, quinine at the present time in the main is used in the quality of blocker of Ca^{2+}-dependent K^+-channels [7, 9].



In the given work we have studied self-organization and physicochemical properties of water and salt solutions in a broad region of concentrations ($1 \cdot 10^{-22}$–$1 \cdot 10^{-3}$ M) of quinine, kept in natural and hypoelectromagnetic conditions, and also influence of salt solutions of quinine on electrical characteristics of identified neurons.

EXPERIMENTAL

In the work use was made of quinine produced by Sigma-Aldrich (USA). We have conducted two series of physicochemical experiments: in the first series we studied water solutions of quinine (solutions I), while in the second – salt ones (solutions II). Salt (physiological) solutions used in experiments with neurons of edible snail contained (mmol/L): NaCl – 78, KCl – 4.5, $NaHCO_3$ – 4.5, $MgCl_2$ – 6.7, $CaCl_2$ – 10. Solutions I and II of demanded concentration were prepared by a method of consecutive serial dilutions from initial solutions of quinine of concentration 110^{-3} M. Mixing was actualized with the use of a vortex Lab dancer (IKA, Germany).

Analysis of the sizes of particles was actualized by a method of dynamic light scattering (DLS) (analyzer Zetasizer Nano ZS, Malvern Instruments, Great Britain), physicochemical properties (electrical conductivity, pH) of solutions were studied by a method of conductometry (inoLab Cond Level 1, WTW, Germany) and pÍ-metry (inoLab pH, WTW, Germany), as described in works [1, 2]. The procedure of preparing samples for studying particle sizes provided necessary "dedusting" of solutions (use was made of disposable filters IsoDisc N-25-4 Nylon, 25 mm × 0.45 μm, Supelco, USA). All measurements were conducted after keeping the solutions in the course of 24 h in conditions of thermostating at 25 ± 0.1°C. Relative errors of measuring the electrical conductivity (χ, μS/cm) of solutions I and II did not exceed 15 and 1% respectively.

The influence of external physical fields (geomagnetic and low-frequency electromagnetic) on water solutions of quinine in a broad range of concentrations was studied analogously to work [4]. All investigated solutions were divided into two parts, one of which resided on the laboratory bench, while the other was placed into a shielding permalloy container, where action of fields is practically excluded (hypoelectromagnetic conditions). The mean value of magnetic induction (B) of external electromagnetic field in natural conditions equals 53000 nT, of residual field in the container – on a level of 10 nT.

Analysis of the influence of solutions I on electrical characteristics of command neurons was conducted on isolated preparations of the nervous system of a mollusk. We use an edible snail of Crimean population, the merit of which for studying the mechanisms of action of pharmacological drugs cones as availability of identifiable neurons (at the expense of their gigantism) not only by sizes and electrical characteristics but also by the role in certain forms of animal behavior. We registered the following parameters: membrane potential (V_m), amplitude of action potential (V_s), threshold of generation of evoked action potentials (V_t) and duration of action potentials (ts) of command neurons of defensive behavior. From the amplifier output the signal was recorded in digital form onto a computer with subsequent treatment. The quantization frequency during recording constituted 200 μs. Inasmuch as command neurons LPa3, PPa3, LPa2 and PPa2 in the norm come to be silent, then for evoking an action potential (AP) in an isolated preparation through the registering microelectrode to a cell we feed a pulse of current of rectangular shape of 1 s duration; the magnitude of stimulation current is selected minimal for AP generation. Therewith use is made of minimal current necessary for generation of two–three AP. Usually in experiments this magnitude of current varied from 1.7 to 3.5 nA.

RESULTS AND DISCUSSION

Upon studying the physicochemical properties (χ, pH) of water solutions of quinine it is shown that the concentration dependences of electrical conductivity and pH of solutions bear a nonlinear character (Fig. 1), typical for solutions of BAS capable of manifesting bioeffects in the region of low concentrations [1–3]. The whole range of concentrations conditionally may be divided into two intervals: ($1 \cdot 10^{-5}$)–($1 \cdot 10^{-3}$) M and ($1 \cdot 10^{-22}$)–($1 \cdot 10^{-6}$) M. In the first interval the values of χ decline from 93.5 to 8.4 μS/cm, while pH of solutions increases from 6.4 to 7.0. In the interval ($1 \cdot 10^{-22}$)–($1 \cdot 10^{-6}$) M the concentration dependences of χ and pH bear a nonmonotonic character. At concentrations $1 \cdot 10^{-10}$, $1 \cdot 10^{-20}$ M on the dependences of χ and pH we observe expressed maxima, indirectly testifying to formation and rearrangement of nanoassociates [1–5].

Study of self-organization of solutions I kept in natural and in hypoelectromagnetic conditions has shown that in both cases in the whole studied region of

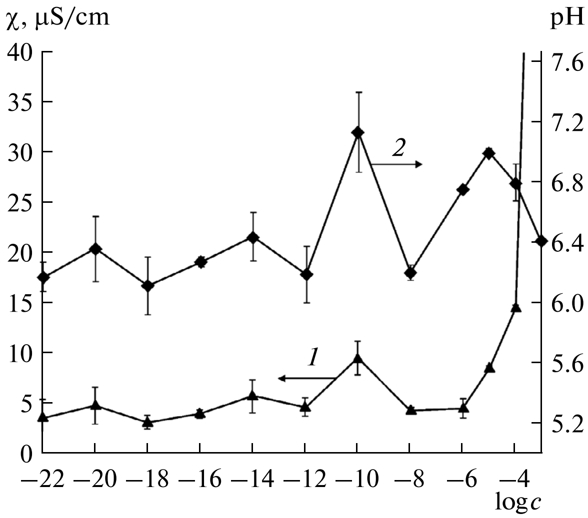


Fig. 1. Dependences of electrical conductivity (*1*) and pH (*2*) of water solutions of quinine on concentration.

concentrations ($1 \cdot 10^{-22}$–$1 \cdot 10^{-3}$ M) we have failed to reliably fix particles by the method of DLS.

It is known that about formation of nanoassociates and supramolecular domains forming in the region of concentrations below $1 \cdot 10^{-4}$ M one may indirectly judge by the results of studying the physicochemical properties of solutions kept in a permalloy container and on the laboratory bench [12].

In Fig. 2 we present the results of studying the physicochemical properties of solutions I kept on the laboratory bench (curve *1*) and in the permalloy container (curve *2*). Collation of concentration dependences of χ and pH of solutions I has shown that in the region of concentrations $1 \cdot 10^{-12}$–$1 \cdot 10^{-4}$ M the values of χ and pH of solutions I kept in the permalloy container are smaller than solutions kept on the laboratory bench. At concentrations below 10^{-12} M the physicochemical properties of solutions kept in the permalloy container practically correspond to properties of distilled water (2.5 μS/cm, pH 6.0).

In this way, disregarding that by the method of DLS we did not manage to reliably fix formation of nanostructures, on the basis of analysis of data obtained upon keeping solutions I in natural and hypoelectromagnetic conditions we may suppose that in the region of concentrations $1 \cdot 10^{-22}$–$1 \cdot 10^{-8}$ M there take place processes of self-organization, conditioning a nonmonotonic character of the concentration dependences of the properties of solutions [3, 4, 12].

In Fig. 3 (curve *1*) we present the results of studying the electrical conductivity (χ) of solutions II. The concentration dependence of electrical conductivity may be divided into two intervals: $1 \cdot 10^{-22}$–$1 \cdot 10^{-12}$ M, $1 \cdot 10^{-12}$–$1 \cdot 10^{-3}$ M. In the interval $1 \cdot 10^{-12}$–$1 \cdot 10^{-3}$ M the values of χ nonmonotonicaly change, with a maximum at quinine concentration equaling $1 \cdot 10^{-10}$ M. In spite of that quinine presents as a nonelectrolyte and is added into physiological solution at low concentration ($1 \cdot 10^{-10}$–$1 \cdot 10^{-6}$ M), the magnitude of χ of solution II in this interval of concentrations is significantly higher (by 6–9%) than in physiological solution, which confirms the results obtained in work [3]. In the second interval of concentrations the values electrical conductivity in the course of decreasing the concentration approach the value of χ of physiological solution (10130 μS/cm), while after $1 \cdot 10^{-16}$ M practically do not change.

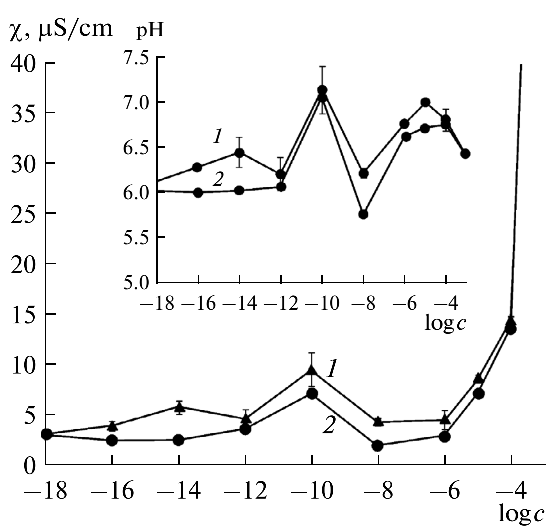


Fig. 2. Dependences of electrical conductivity and pH (inset) of water solutions of quinine on concentration in natural (*1*) and hypoelectromagnetic conditions (*2*).

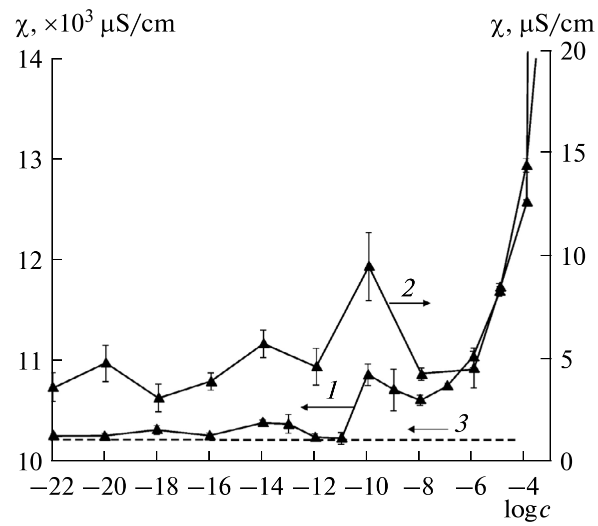


Fig. 3. Dependences of electrical conductivity of water solutions I (*1*) and salt solutions (*2*) on quinine concentration, electrical conductivity II (*3*) is presented for comparison.

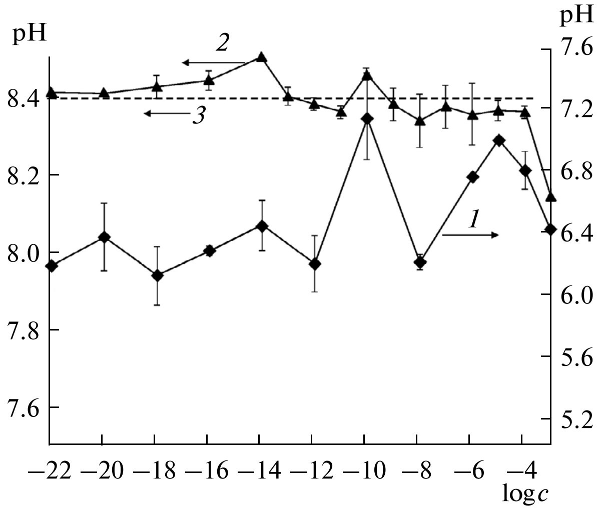


Fig. 4. Dependences of pH of water (*1*) and mixed I + II (*2*) solutions on quinine concentration. (3) pH of physiological solution shown for comparison.

As evident from data of Fig. 3, the concentration dependences of electrical conductivity of solutions I (curve *2*) and II (curve *1*) bear a symbate nonmonotonic character, most brightly expressed for water solution. In the case of solutions II the values of χ reliably differ from values of χ of physiological solution only in the interval of concentrations $1 \cdot 10^{-10}$–$1 \cdot 10^{-6}$ M.

An analogous nonmonotonic symbate course of concentration dependences is revealed upon studying the pH of solutions I (Fig. 4, curve *1*) and II (Fig. 4, curve *2*) with extrema at quinine concentrations equaling $1 \cdot 10^{-14}$ and $1 \cdot 10^{-10}$ M. In this way, the regularities of behavior of water solutions of quinine of low concentrations are preserved also in physiological solution.

Study of self-organization of physiological solution and solutions II by method of DLS has shown that the size distribution of particles in these solutions bears a polymodal character, formation of nanoassociates, just as in solutions I, could not be established.

However a nonmonotonic character of the concentration dependences of physicochemical properties of solutions I and II with maxima at concentrations $1 \cdot 10^{-10}$, $1 \cdot 10^{-14}$, $1 \cdot 10^{-20}$ M allows prognosticating a possibility of appearance of a bioeffect in the region of low concentrations of quinine solutions.

The given suggestion was checked upon studying the influence of salt solutions of quinine in a broad region of concentrations on the electrical characteristics of identified neurons (command neurons of defensive behavior) of edible snail.

In the course of biophysical experiments we have found reliable changes in the membrane potential (Fig. 5) of neurons under influence of solutions II

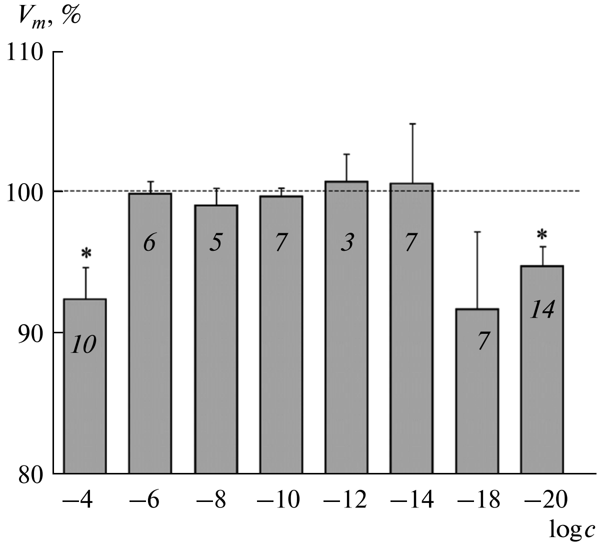


Fig. 5. Change in membrane potential of rest (V_m) of command neurons upon action of a water solution of quinine. Along the abscissa axis shown is the logarithm of quinine concentration, along the ordinate axis shown is the change of V_m relative to norm. * Reliable difference $p < 0.05$. Dotted line denotes initial level of V_m — 100%.

where quinine is present at concentrations of 10^{-4} and 10^{-20} M, and also changes in the amplitude of AP (Fig. 6) under influence of solutions II in which quinine resides at concentrations of $1 \cdot 10^{-18}$ and $1 \cdot 10^{-20}$ M. Reliable change in the duration of AP (Fig. 7) are found under influence of solutions II containing quinine at a concentration of $1 \cdot 10^{-10}$ M.

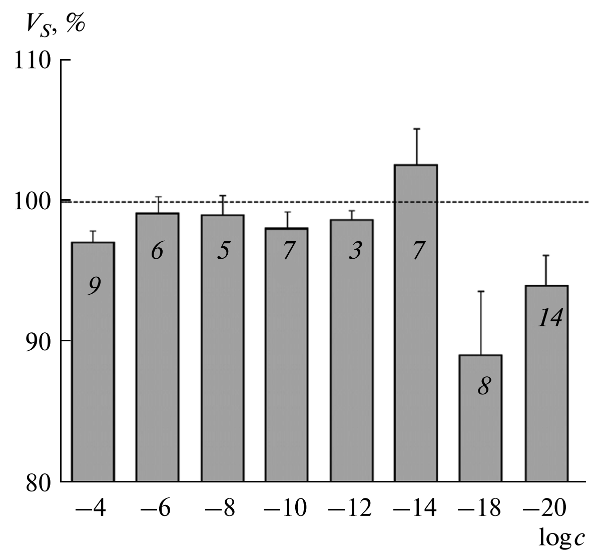


Fig. 6. Change in amplitude of AP (V_s) of command neurons upon action of a water solution of quinine. Along the abscissa axis shown is the logarithm of quinine concentration, along the ordinate axis shown is the change of V_s relative to norm. * Reliable difference $p < 0.05$. Dotted line denotes initial level of V_s — 100%.

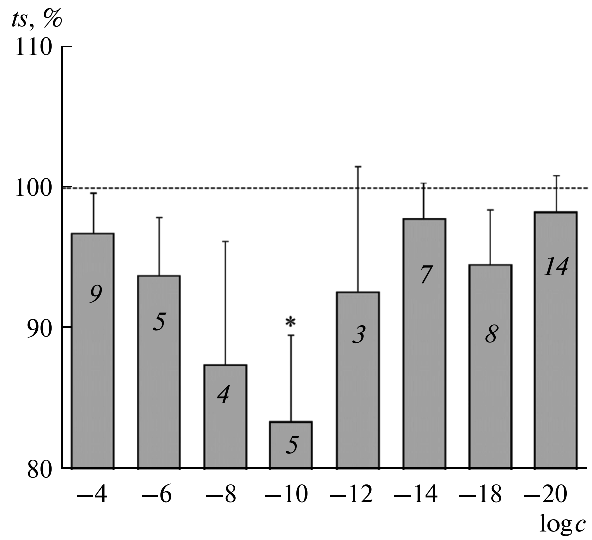


Fig. 7. Change in duration of A (*ts*) of command neurons upon action of quinine. Along the abscissa axis shown is the logarithm of quinine concentration, along the ordinate axis shown is the change of *ts* relative to norm. * Reliable difference $p < 0.05$. Dotted line denotes initial level of *ts* – 100%.

Analysis of the intervals of concentration in which there take place extremal changes of physicochemical properties and bioeffect of salt solutions of quinine testifies to their interconnection: extremal values of physicochemical properties and reliable changes of bioeffect are disclosed at the same concentrations (Figs. 3–7).

CONCLUSIONS

As a result of performed work we have obtained symbate nonmonotonic concentration dependences of physicochemical properties of water and salt solutions of quinine in the region of low concentrations. We have found reliable (inside a 5% interval) changes in membrane potential, amplitude of AP and duration of AP of snail neurons under influence of salt solutions containing quinine at concentrations $1 \cdot 10^{-20}$, $1 \cdot 10^{-18}$, $1 \cdot 10^{-10}$ M, at which shown are extremal values of electrical conductivity and pH of water and salt solutions of quinine. It is established that the results of studying the physicochemical properties of water and salt solutions of low concentrations may be used for prognosticating the bioeffects of quinine solutions in this region of concentrations.

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