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Effect of Amiridine and Tacrine on Potassium Currents in the Nerve Fiber

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The mechanism of the positive therapeutic effect of derivatives of aminoacridine and aminoquinoline, tacrine (T) [10] and amiridine (A) [3], in dementia of different genesis and in Alzheimer's disease is still to be clarified. Analysis of the spectrum of pharmacological activity of A and T and their derivatives, as well as of a known blocker of K^+ channels, 4-AP, has cast doubt on both the traditional explanation of the action of these preparations (by their anti-cholinesterase activity) and the possibility of the action potential (AP) being markedly affected by them [1]. Despite the fact that a blocking effect of T on Na^+ and different types of K^+ channels has been discovered for various objects in a number of studies [5,7,9], the efficacy of blocking has proved to be quite low: IC_{50} for potassium currents reportedly varies from 50 to 500 μM, whereas a clinical effect has been observed for a concentration in the blood serum of just 0.02 μM.

The aim of the present study was to analyze in detail the previously discovered [2] effects of A and T on steady-state K^+ currents over the range of membrane potentials (MP) near the resting potential (RP) in connection with the possible effect of these agents on RP.

MATERIALS AND METHODS

The experiments were carried out on isolated nerve fibers of *Rana ridibunda* frogs by recording the ionic currents under MP clamp conditions after Dodge and Frankenhaueser [4] using Sigworth's modification of this method [8], which makes it

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possible to compensate for series impedance. After the fiber was placed in the bath and connected with the aid of salt bridges to the electrodes, the ends of the fiber in isotonic KF solution in the side baths were transsected at the midpoint of the internodal distance. Solutions of the following composition (mM) were used as the external ones: 1) Ringer solution (NaCl 110, KCl 2.5, $CaCl_2$ 2, Tris 5; pH 7.2) and 2) isotonic solution (KCl 112, $CaCl_2$ 2, Tris 5; pH 7.2)). The experiments were carried out at a solution temperature of 12-14°C. The currents responding to stepwise shifts of MP from the initial holding potential (which in all experiments was equally adjusted to a level of 100 mV according to the absolute scale) were recorded on film by taking photos from the oscillograph monitor. The effects of the preparations on RP and AP were studied in separate experiments performed in a current clamp mode.

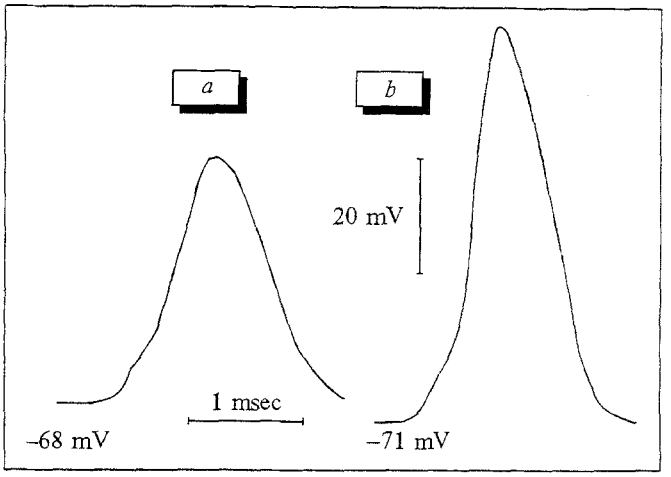

Fig. 1. AP recordings for current clamp mode before (*a*) and after (*b*) addition of T (1 μM) to external Ringer's solution. The value of RP according to the absolute scale of potentials is shown for each recording. AP was established at a level of −68 mV prior to replacement of the solution with that containing T by the passage of a direct depolarizing current.

RESULTS

Regular changes of RP exceeding the possible experimental error (some 1-2 mV) for the effect of A and T were revealed just for specific conditions, namely: the fiber had a rather high intrinsic RP close to the standard value (-75 mV), and before replacing the solutions the membrane was depolarized several mV by passing a current from the outer source. Under these conditions the preparations in a concentration of 1 μM caused a 3-4-mV shift of RP toward hyperpolarization and an increase of the amplitude and slope of AP, as is shown in Fig. 1.

When A and T were added to the Ringer solution, a slight blocking of Na^+ and outward K^+ currents was observed under voltage clamp conditions. IC_{50} for Na^+ currents was 0.5 mM.

The blockade of the K^+ current was also weak, though somewhat more marked (IC_{50} varied from fiber to fiber in the range of 50-100 μM), this being consistent with above-mentioned data in the literature. The effect of A and T on the K^+ current was investigated near the RP by using isotonic KCl solution as the external one. In this case the reversion potential of the K^+ current attained an approximate value of 0 mV due to the K^+ concentrations being equal inside and outside the fiber; within the MP range in question, K^+ currents were inward currents and had a rather high amplitude (up to 3-5 nA), providing for high-resolution measurements. The K^+-current (I_K) records obtained in isotonic solution in the control and in the presence of A in a concentration of 1 μM are presented in Fig. 2. At test potential values of -70 and -60 mV a pronounced A-induced increase of the K^+ current as compared with the control may be observed, and at a potential of -50 mV the currents are approximately the same. A similar effect was observed for the action of T in the same (1 μM) concentration. In the volt-ampere characteristic (Fig. 3, *a*), this effect manifests itself as a shift of the descending branch of the curve (of the zone of activation, i.e., of the increased proportion of opened K^+ channels) to negative MP values (toward hyperpolarization). The

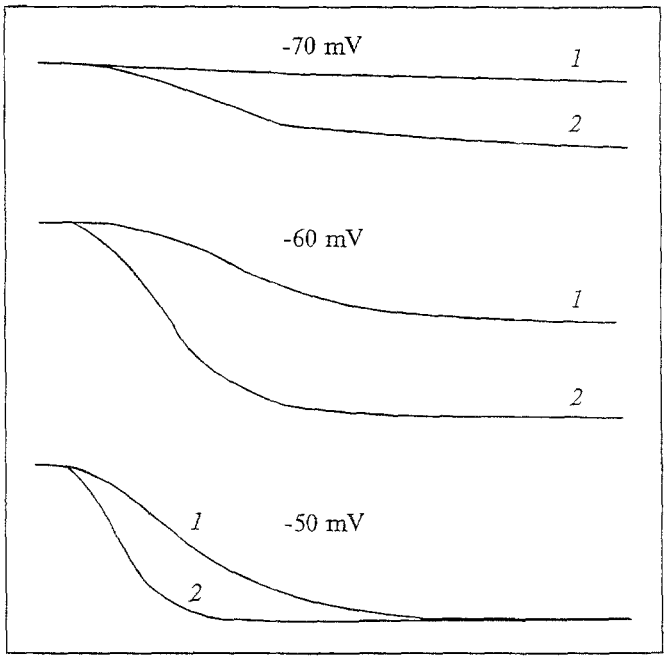

Fig. 2. Recordings of K^+ currents in response to test shift of potential in the control (*1*) and in the presence of A (1 μM) (*2*) in isotonic KCl solution. Figures near each pair of recordings show the test potential.

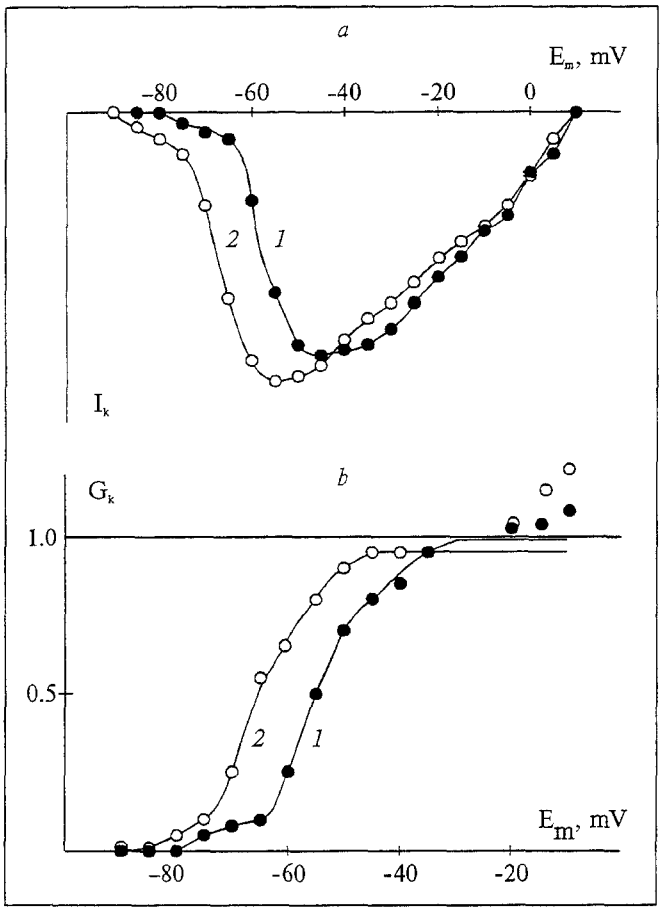

Fig. 3. Steady-state values of K^+ current (*a*) and conductance (*b*) as a function of MP in the control (*1*) and in the presence of A (*2*). *a*) continuous lines are drawn through the experimental points "by rule of thumb;" *b*) points are calculated according to experimental values of I_k taken from *a*.

shift was dose-dependent; when the concentrations of the preparations were 0.01-0.1 μM, this shift constituted 1-2 mV, attaining its 10-mV maximum at a concentration of 1 μM.

Thus, along with the previously described blocking of ionic currents by T in high concentrations, we discovered a shift of the zone of K^+-current activation, which was caused T and A in concentrations comparable to therapeutic ones (0.01-1 μM).

Under physiological conditions, when the K^+ current is an outward current, the increased number of opened channels (due to the shift of the zone of potential sensitivity) will result in membrane hyperpolarization. Hence, A and T will raise the RP. This rise will cause an increase of the proportion of excitable Na_+ channels (due to inactivation being prevented), this providing for the generation of AP with a higher amplitude and slope of increase, as was observed in the experiment presented in Fig. 1. Qualitatively, the shift phenomenon provides an explanation for the therapeutic effect of the preparations, since this shift results in an increased reliability of generation and transmittance of the nervous impulse, important elements for information processing in the CNS. Let us assess the quantitative characteristics of the expected shift of RP, caused by the preparations, in relation to their concentrations and to the initial value of RP in the Ringer solution. After addition of the preparations at an RP value of E_1, the new equilibrium will be established at a potential of E_2, for which an increment of outward current I_k, depending on the shift of the conductance curve, will be equal to the increment of the inward leakage current, i.e.:

$$G_k^2[E_2]\times(E_2-E_k) - G_k[E_1]\times(E_1-E_k) = \\ =-G_L\times(E_2-E_1), \qquad (1)$$

where $G_k[E_i]$ is the K^+ conductance at E_1 in the control Ringer solution; $G_k^2[E_2]$ is the K^+ conductance at E_2 in the presence of preparation; G_L is the leakage conductance, independent of E_m; E_k is the equilibrium K^+ potential (equal to -90 mV in the Ringer solution).

In numerical calculations with formula (1) the control dependence G_k vs. E_m presented in Fig. 3, *b* was used, the points being calculated for E_k=10 mV by using the experimental values with the formula: $G_k = I_k/(E_m-E_k)$, and normalized to the G_k^{max} value at the inflection point of the G_k-E_m dependence for E_m values close to -30 mV. The experimental points for E_m values to the left of -30 mV were described by the theoretical curve of the form:

$$G_k/G_k^{max} = 1/[1+\exp((E_{1/2}-E_m)/K)].$$

In the control curve $E_{1/2}$ (the midpoint of the curve) was -55 mV, the slope of the curve K=4.5 mV. The theoretical curve of conductance for the effect of A was obtained by shifting the control curve by value S along the E_m axis, i.e., $G^2_k = G_k[E_m-S]$, where S=10 mV for a concentration of agent [A]=1 μM and S=2 mV for [A]=0.1 μM. The maximal value G_k^{max} of G_k in the Ringer solution was assessed as follows. In special experiments with the use of the averaging method for raising the signal-to-noise ratio, I_k was measured in the Ringer solution for MP depolarized to -60 mV. This value was 0.2 nA, resulting in:

$$G_k[-60\ mV] = 0.2\ nA/(-60+90)\ mV = \\ =6.6\times10^{-9}\ Ohm^{-1}.$$

According to the control conductance curve in Fig. 3, *b*, $G_k[-60]=0.25\ G_k^{max}$; hence, $G_k^{max}=26\times10^{-9}$ Ohm^{-1}. The leakage conductance G_l was taken as 25×10^{-9} Ohm^{-1}, based on resting impedance of the

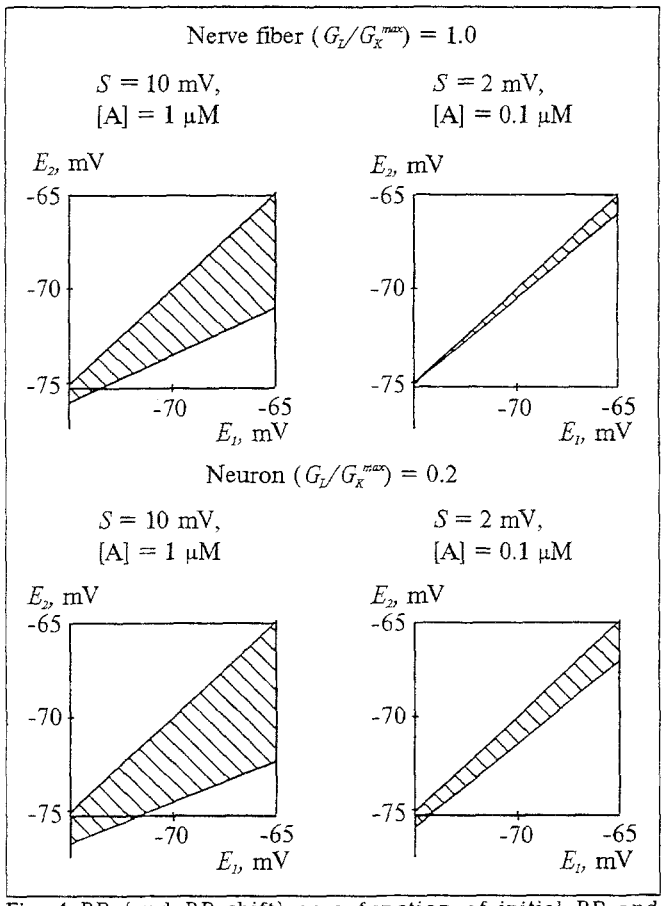

Fig. 4. RP (and RP shift) as a function of initial RP and concentration of agents in solutions containing A and T (the values of preparation–induced shift S of $G_k - E_m$ curve). In each diagram the top line corresponds to $E_2 - E_1$, and the bottom line shows the dependence of E_2 on E_1 calculated with formula (1).

membrane of the intact Ranvier's node, which constitutes 40×10^6 Ohm [4]. The results of calculations for the nerve fiber are presented at the top of Fig. 4. Here, the vertical section of the hatched zone presents the hyperpolarizing RP shift vs. the initial value of E_1. One may see that the shift is minimal for an E_1 equal to the RP of the intact fiber (-75 mV) and rises as the absolute value of the initial RP drops. Note the good agreement between the calculated value of the RP shift for an initial RP of -68 mV and the results of the experiment presented in Fig. 1. It should be mentioned that little effect is produced by low concentrations of the preparations (< 0.1 μM) on the RP of the nerve fiber. The lower part of Fig. 4 illustrates an attempt to predict the effects of A and T on the nerve cell. The neurocyte was simulated by reducing the G_l/G_k^{max} ratio 5-fold on the basis of the fact that the specific leakage conductance of the neurocytes is more than one order of magnitude lower than the specific leakage conductance of the membranes of Ranvier's node. It is seen that the pronounced increase in the value of the RP shift allows for a physiological effect at very low concentrations of the preparations (about 0.1 μM).

In summary of the above, the assumption may be made that since the RP shift will be minimal in intact nerve cells and in cells with an increased leakage conductance (membrane lesions), the therapeutic effect of A and T will manifest itself in cells characterized by a low excitability due to the failure of cell metabolism, this resulting in a reduction of the RP or in a decreased density of the channels providing for AP generation.

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