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A bistable membrane potential at low extracellular potassium concentration

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Abstract

In order to understand the electrochemical behavior of a living cell at a low extracellular potassium concentration, a model is constructed. The model involves only the ATP driven sodium–potassium pump, and the sodium and potassium channels. Predictions of the model fit the N-shape of the current–voltage characteristic at low extracellular potassium. The model can, furthermore, quantitatively account for the experimentally observed bistability of the membrane potential at low extracellular potassium concentration. A crucial role in the control of the transmembrane potential appears to be played by how the permeability of the inward rectifying potassium channels depends on the transmembrane potential and on the extracellular potassium concentration.

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1. Introduction

A living cell is alive by virtue of an electrochemical gradient across the membrane. The inside of a skeletal muscle cell is approximately -75 mV relative to the outside. In mammals the intracellular potassium concentration ($[K^+]_i$) is approximately 30 times as high as the extracellular concentration ($[K^+]_o$) (150 vs. 5 mM), whereas the intracellular concentration of sodium ($[Na^+]_i$) is approximately one tenth of the extracellular

concentration ($[Na^+]_o$) (14 vs. 140 mM). The electrochemical gradient is maintained by a complex system of pumps and channels. Pumps use the energy of ATP hydrolysis to move ions against the electrochemical gradient. The most prominent pump is the Na,K-ATPase. This membrane protein transports 3 Na^+ ions to the outside and 2 K^+ ions to the inside of the cell for every hydrolyzed ATP. Both the concentration and the electric gradients ‘want to bring’ Na^+ to the inside of the cell. But Na^+ channels are mostly closed and flow is thus prevented. For K^+ the electric and the chemical potential have opposite directions and almost balance each other out. The net driving

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force for K^+ across the cell membrane is thus very small. A significant portion of the K^+ channels is open and this actually helps stabilize the electric potential around a value where it compensates for the concentration gradient of K^+ . In many types of cells the Inward Potassium Rectifier (IRK, Katz [1] called it the anomalous rectifier) is the most prominent K^+ channel. This channel rectifies inwardly, i.e., it passes inward current (K^+ flow into the cell) more easily than outward current. A beneficial consequence of the rectification is that it protects the cell against fast depletion of the intracellular potassium at more positive potentials. It has furthermore been discovered that the permeability of the IRK is proportional to $1/\sqrt{[K^+]_o}$. This appears to be connected with a binding site for K^+ on the extracellular part of the channel [2]. A bound potassium ion decreases the permeability of the IRK channel.

It is not difficult to understand what happens at low extracellular Na^+ . Because Na^+ channels are mostly closed, the transmembrane potential at rest is barely influenced. But because of the smaller electrochemical gradient for sodium at low extracellular Na^+ , the action potentials have smaller amplitudes and longer refractory times [3,4]. When extracellular K^+ gets low, the situation is more complex. The flow of K^+ across the membrane is relatively free and at low $[K^+]_o$ a hyperpolarization is necessary to compensate for an increased K^+ -chemical gradient. But such a hyperpolarization would lead to more leak of Na^+ into the cell. The capability, on the other hand, of the Na,K -ATPase to cycle faster to compensate for the leak, is compromised because of the lower extracellular potassium concentration that makes it harder to 'pick up' a K^+ ion on the outside. So it may turn out to be impossible to maintain the necessary hyperpolarization. In that case we get a breakdown and a switch to a depolarized steady state in which the potassium channels are closed. These intuitive arguments will be made more robust and more quantitative in a mathematical model which follows. We will see that there is a range of K^+ concentrations for which bistability occurs, i.e., both a hyperpolarized and a depolarized state are possible solutions. We will, furthermore, compare

the results of model calculations with actual experiments.

In our model and experiments we consider one cell in a bath. Single cell experiments in a bath with lower than physiological potassium concentration mimic to some extent what is happening in hypokalemia, i.e., a disorder in which the blood $[K^+]$ of the patient becomes too low. Because of an exchange mechanism between intracellular K^+ and extracellular H^+ , it is often associated with alkalosis (high blood pH). Hypokalemia can also be a result of renal malfunction, diuretic therapy, or increased aldosterone activity. The clinical manifestations relate to neuromuscular function and consist of decreased muscle potential, decreased muscle contractility, and even paralysis. In extreme cases the patient may die from paralysis of the respiratory muscles.

2. The model

The passive ion fluxes of K^+ and Na^+ through the membrane depend on the electrochemical gradients and on the permeabilities. Goldman derived the constant-field flux equation for the combined effect of a chemical and an electric potential [5]:

$$J_X(U) = - \frac{U([X]_i e^U - [X]_o)}{e^U - 1} P_X, \quad (1)$$

where, in our case, X stands for either Na^+ or K^+ and $[X]_i$ and $[X]_o$ represent the concentrations of the ions inside and outside the cell, respectively. J_X gives the current that flows from the outside to the inside. The variable U is a dimensionless measure of the electric transmembrane potential: $U = FV_m/RT = 38 V_m$ at 35 °C. F represents Faraday's constant ($9.65 \cdot 10^4$ C/mole, the number of Coulombs in a mole of ions) and V_m is the electric transmembrane potential in volts. P_X stands for the permeability of the membrane for a particular ion. We are interested in the steady states and we therefore do not take peculiarities of the kinetics as time delayed activation and inactivation into account. P_{Na} is a constant number, but P_K has a complicated dependence on V_m and $[K^+]_o$. The following empirical description has been estab-

lished for the potassium permeability [6–8]:

$$P_K(V_m, [K^+]_i, [K^+]_o) = P_0 + \frac{\bar{P}_K}{\sqrt{[K^+]_o}} \times (1 + e^{V_m - V_h/V_s})^{-1}. \quad (2)$$

P_0 constitutes the permeability due to potassium channels other than IRKs. The IRK channels are voltage gated and the term in parentheses describes how the open/closed partition follows a Boltzmann distribution when V_m varies. V_h is the voltage where the open/closed distribution is fifty–fifty. V_s fixes the sensitivity to V_m of the open/closed distribution [2]. It has been found [7] that V_h is very close to the actual equilibrium potential for potassium, i.e. $V_h = (RT/F) \ln([K^+]_o/[K^+]_i)$. However, sometimes small corrections have to be made to fit the experimental results [8]. The $\bar{P}_K/\sqrt{[K^+]_o}$ term describes how the permeability of an open channel depends on $[K^+]_o$.

At every catalytic cycle, the Na,K-ATPase transports 3 Na^+ 's from the inside to the outside and 2 K^+ 's from the outside to the inside. The transmembrane flux due to the pump thus depends on $[K^+]_o$ and $[Na^+]_i$ with second and third order Michaelis–Menten kinetics, respectively:

$$J_P([Na^+]_i) = \alpha J_P^{\text{Max}} \left(1 + \frac{K_m^K}{[K^+]_o}\right)^{-2} \left(1 + \frac{K_m^{Na}}{[Na^+]_i}\right)^{-3}. \quad (3)$$

Here J_P^{Max} is the maximal attainable flux, K_m^{Na} is the affinity for Na^+ , and K_m^K is the affinity for K^+ . The parameter α represents the stoichiometry value of the pump and equals -3 for the Na^+ now, and 2 for the equivalent K^+ now. The activity of the pump also depends on V_m and on the $[ATP]/([ADP][P])$ ratio [9]. However, for the range of V_m that we will be looking at and without contractions, the pump activity changes relatively little. We, furthermore, assume that the $[ATP]/([ADP][P])$ ratio is buffered at a sufficiently saturating level for the dependency on these concentrations to be ignored.

For both Na^+ and K^+ , the change per unit of time of the intracellular concentration, i.e. the time

derivative of $[X]_i$, can be expressed as the sum of the pump flux and the channel flux:

$$\frac{d[X]_i}{dt} = \frac{S}{B} (J_X + J_P) \quad (4)$$

B represents the cell volume and S represents the cell's surface area. For a long cylindrical cell with a $17.5 \mu\text{m}$ radius we have $B/S = 8.75 \cdot 10^{-6} \text{ m}$. We follow a physical convention that runs contrary to the use by most electrophysiologists and let a positive current of cations be one that goes from the outside to the inside. For the change in V_m , due to such currents we have:

$$\frac{dV_m}{dt} = \frac{F B}{C_m S} \sum_i \frac{d[X]_i}{dt}, \quad (5)$$

where F is Faraday's constant and C_m is the specific membrane capacitance. We take $q = F B / C_m S$, where q is now a constant that is dependent only on the geometry of the cell. Taking $C_m = 8.0 \cdot 10^{-6} \text{ F/cm}^2$ [10] leads to $q = 10555 \text{ V/M}$, where M represents the concentration in moles per liter.

3. Results and discussion

In 1977 Gadsby and Cranefield [11] did voltage clamp experiments in Purkinje fibers (which also contain IRK channels) at lower than physiological $[K^+]_o$ ($1\text{--}4 \text{ mM}$). For a range of values of V_m they measured the current necessary to maintain that voltage immediately after changing from the physiological to low $[K^+]_o$ extracellular solution. They found two steady states, i.e. voltages that required no current. In the resulting current–voltage graph this means that there are two points where the curve crosses the voltage axis with a positive slope resulting in a positive conductance ($dI/dV_m > 0$). To connect these positive-conductance-crossings it is necessary to have a negative conductance crossing ($dI/dV_m < 0$) in between. As a result one finds an N -shape curve (Fig. 1). In agreement with the qualitative arguments that we presented at the beginning of this report, they found that there is indeed a hyperpolarized steady state and a depolarized steady state. The two-

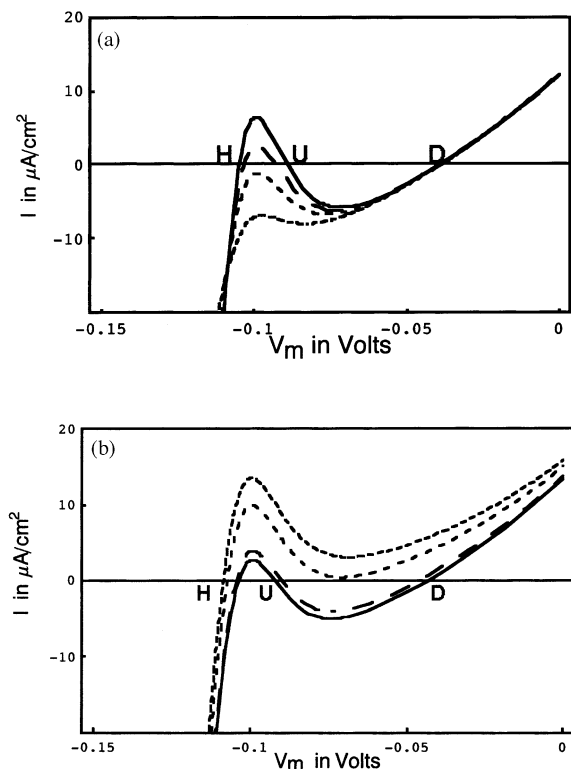


Fig. 1. The I/V relationships predicted by the model at the lower than physiological $[\text{K}^+]_o = 2$ mM. All the other intra- and extracellular concentrations are fixed at physiological values: ($[\text{K}^+]_i = 125$ mM, $[\text{Na}^+]_i = 16.7$ mM, and $[\text{Na}^+]_o = 147$ mM) while the imposed V_m is varied. Other parameters were given the following values: $K_m^{\text{K}} = 1$ mM, $K_m^{\text{Na}} = 7$ mM, and $J_P^{\text{Max}} = 3.1 \cdot 10^{-11}$ mol/cm²s, and $V_s = 6.5$ mV. (a) The blocking of IRK channels by increasing concentrations of barium is modeled by taking $P_{\text{Na}} = 2.5 \cdot 10^{-10}$ cm/s, $P_o = 1.25 \cdot 10^{-9}$ cm/s, $\bar{P}_K = 14.1 \cdot 10^{-9}$ M^{3/2} cm/s, and multiplying the value of \bar{P}_K with 1.0 (solid curve, no blocking), 0.8, 0.6 and 0.3 (dashed curves) respectively. The experimentally observed disappearance of the hyperpolarized state is present in the model, (b) The blocking of Na⁺ channels by increasing concentrations of TTX is modeled by taking $\bar{P}_K = 10.12 \cdot 10^{-9}$ M^{1/2} cm/s, $P_o = 1.32 \cdot 10^{-9}$ cm/s, $P_{\text{Na}} = 2.2 \cdot 10^{-10}$ cm/s and multiplying the value of P_{Na} by 1.0 (solid curve, no blocking), 0.9, 0.45 and 0.1 (dashed curves) respectively. In agreement with the experiments on Purkinje fibers, we see that for low P_{Na} only a hyperpolarized steady state is left.

valuedness is lost in a medium with $[\text{K}^+]_o$ greater than 4 mM or lower than 1 mM, or when either the Na⁺ or the K⁺ channels are blocked. In

voltage clamp experiments V_m is imposed as a fixed and time-independent parameter. This value of V_m can be entered into Eqs. (1) and (2). The voltage changes are sufficiently fast that $[\text{Na}^+]_i$ and $[\text{K}^+]_i$ can be assumed to not change, and we thus view these intracellular concentrations as constant parameters in time. Eqs. (1)–(5) contain a number of parameters whose values have been independently determined and they are listed in the legend of Fig. 1. The expressions for J_{Na} , J_{K} and J_{pump} can be evaluated and substituted into Eq. (4). The sum $F B \frac{d}{dt}([\text{Na}^+]_i + [\text{K}^+]_i)$ now

represents the total current across the membrane. Plotting the current against V_m at $[\text{K}^+]_o = 2$ mM indeed results in the experimentally observed N -shaped curve (Fig. 1). In Fig. 1a we see three values of V_m , that lead to a zero current. Point H indicates the stable hyperpolarized positive conductance, point U the unstable negative conductance and point D the stable depolarized positive conductance. In the hyperpolarized state the IRK channels are mostly open, whereas in the depolarized state they are mostly closed. A large variety of K⁺ channels is known (see e.g. [12]), and P_o in Eq. (2) represents an approximation of the cumulative effect of all the K⁺ channels that are not IRKs. An important dimensionless parameter in the analysis is $P_{\text{K}}/P_{\text{Na}}$, i.e. the ratio of the K⁺ and Na⁺ permeabilities. For excitable cells like skeletal and cardiac muscle cells, $P_{\text{K}}/P_{\text{Na}}$ has been found to be approximately 100, with the IRKs being responsible for approximately 90% of the P_{K} [13]. We therefore take P_o/P_{Na} to be approximately 10 and set \bar{P}_K such that the variable part of $P_{\text{K}}/P_{\text{Na}}$ (cf. Eq. (2)) comes out to be approximately 85 at $V_m = -75$ mV and $[\text{K}^+]_o = 5.7$ mM. Gadsby and Cranefield found that adding barium, a known blocker of K⁺ channels, leads to the disappearance of the hyperpolarized state and the N -shape. When we describe the influence of barium in the medium by a smaller value of $P_{\text{K}}/P_{\text{Na}}$, the model predicts the lower three curves in Fig. 1a. Fig 1b shows how the model also predicts the experimentally observed disappearance of the depolarized steady state when TTX, a potent blocker of some Na⁺ channels, is added to the

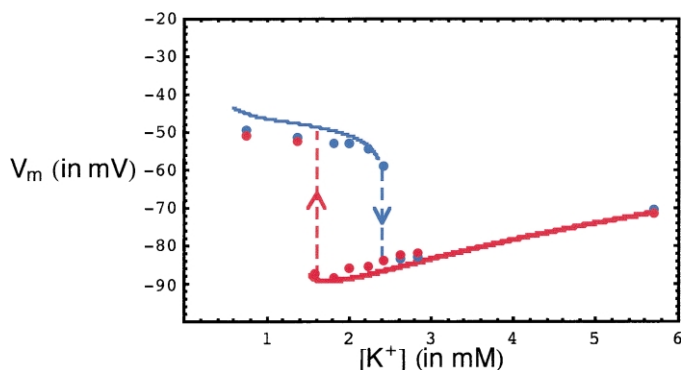


Fig. 2. The points represent the experimental results of Fig. 3. The curves are the prediction of the model. The dashed lines indicate the jumps occurring in the hysteresis loop. The parameter values entered into the model were $P_{Na}=6.6 \cdot 10^{-10}$ cm/s, $P_o=3.435 \cdot 10^{-9}$ cm/s, $\bar{P}_K=8.22 \cdot 10^{-9}$ M^{1/2} cm/s, $K_m^K=0.32$ mM, $K_m^{Na}=7$ mM, $J_p^{Max}=2.41 \cdot 10^{-10}$ mol/cm²s, and $V_s=9.68$ mV. For the value of V_h we took $(1/38) \ln([K^+]_o/[K^+]_i)+0.0024$. The value of V_o was derived from a steady state physiological situation at $[K^+]_o=5.7$ mM and $V_m=71.5$ mV and found to be -1279.2 V. At different values of $[K^+]_o$ the V_m of the steady state was calculated. When $[K^+]_o$ was varied, $[Na^+]_o$ was kept at a value such that $[K^+]_o+[Na^+]_o$ was constant at 0.148 M. All these parameters values are chosen in a range consistent with what is reported in the literature.

medium. Blocking a fraction of the Na⁺ channels means that P_{Na} decreases and thus that P_K/P_{Na} increases. The three upper curves in Fig. 1b represent the model predictions for increasing P_K/P_{Na} and they do indeed describe the results of the experiments of Gadsby and Cranefield [11]. All in all, our model is in full agreement with the observations of Gadsby and Cranefield.

In a living cell the transmembrane voltage is not a parameter that is imposed from the outside, but a dynamical variable that influences the intracellular concentrations and permeabilities and is, in turn, itself also influenced by these concentrations and permeabilities. Integrating Eq. (5), we can express V_m in terms of $[Na^+]_i$ and $[K^+]_i$:

$$V_m = q[Na^+]_i + q[K^+]_i - V_o. \quad (6)$$

At steady state, i.e. $\frac{d}{dt} \times [Na^+]_i = 0$ and $\frac{d}{dt} [K^+]_i = 0$, and with the physiological values of $[K^+]_o=5.7$ mM and $V_m=-71.5$ mV, we derive from Eq. (4) that $[Na^+]_i=18.4$ mM and $[K^+]_i=102.8$ mM. This gives $V_o=-1279.2$ V for the integration constant in Eq. (6) (so $|V_o/q|=121$ mM). Physically V_o represents the part of the

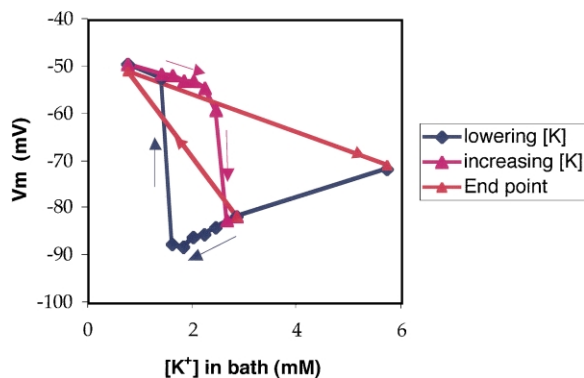


Fig. 3. The result of an experiment with an individual skeletal muscle cell. At first the concentration $[K^+]_o$ was decreased from 5.7 mM in a stepwise fashion. At every point indicated (blue diamonds) in the figure we waited for at least 4 min to let V_m relax to the steady state value. When depolarization occurred more time was needed and the cell was given approximately 45 min. After reaching 0.76 mM the process was reversed and $[K^+]_o$ was increased in a stepwise fashion until 2.85 mM (pink triangles). Again we waited for at least 4 min at every datapoint and for 45 min when repolarization occurred. Finally, we cycled $[K^+]_o$ in two large steps, 2.85 mM \rightarrow 0.76 mM \rightarrow 5.7 mM (orange triangles), giving the cell sufficient time after each step to relax to a new value of V_m . The experiments were performed at 35 °C. In eight more identical experiments hysteresis loops were observed with only slightly different ‘switch points.’

transmembrane potential due to charged particles other than Na^+ and K^+ . These include ions like Cl^- , Ca^{2+} , Mg^{2+} , etc. and the mostly negatively charged macromolecules (proteins, sugars, DNA, etc). For the situation at low $[\text{K}^+]_o$ we work as follows. Eq. (6) can be substituted in Eqs. (1) and (2), and when the net currents of Na^+ and K^+ in Eq. (4) are set equal to zero, we get algebraic equations from which we can solve numerically for $[\text{Na}^+]_i$ and $[\text{K}^+]_i$ with a simple Newton scheme. From these values V_m can eventually be calculated via Eq. (6). The theoretical curve in Fig. 2 was obtained by calculating V_m for many values of $[\text{K}^+]_o$.

In Eq. (6) numerical values of $(q[\text{Na}^+]_i + q[\text{K}^+]_i)$ and V_o eventually differ by less than 0.1%. The membrane potential of approximately 100 mV is indeed the result of only a μM magnitude difference in concentration between negative and positive ions. At first sight it may thus seem as though V_m would be greatly affected by small relative changes in $[\text{Na}^+]_i$, $[\text{K}^+]_i$ and V_o . However, the steady state values and the bistability of V_m , appear remarkably robust when Eqs. (1)–(6) are solved for $[\text{Na}^+]_i$ and $[\text{K}^+]_i$ at different values of $|V_o/q|$ in the physiological range of 100–140 mM.

We experimentally measured the potential across the membrane on superficial cells of the lumbrical muscle of the mouse. The cells were impaled with fine tipped micro electrodes in a normal control medium [14,15]. We changed $[\text{K}^+]_o$ and waited until V_m relaxed to a new steady state value. After this value was recorded, $[\text{K}^+]_i$ was changed again. The change to a medium with a different $[\text{K}^+]_i$ is too slow to induce an action potential that may ultimately drive the cell to another steady state. Generally, after changing $[\text{K}^+]_o$, the relaxation to a new V_m was straightforward and a matter of minutes. Fig. 3 shows the result of an experiment with one cell. $[\text{K}^+]_o$ was decreased in a stepwise fashion from the physiological 5.7 mM down to 0.76 mM. Subsequently $[\text{K}^+]_o$ was increased, decreased, and then increased again to 5.7 mM. In the region between 1.5 and 3.5 mM there is an obvious bistability and, in the course of the experiment, we appear to cycle through a hysteresis loop. The experiment was repeated nine times with

identical type cells. Whenever a good seal around the electrode was established, the same bistability was found between the same values of $[\text{K}^+]_o$. The experiment of Fig. 3 was selected for modeling, because it was the most ‘stable’ one, i.e. in this experiment the final point ($[\text{K}^+]_o$, V_m) coincided most closely with the initial point from before the system was moved through the hysteresis loop. However, also in other successful experiments V_m was found never to vary more than 5% between the initial and final point of the entire experiment.

Fig. 2 shows the data points from the experiment in Fig. 3 together with the curves that result from the theoretical model. Parameters for the model were chosen in a range consistent with what has been reported in the literature. The upper stable branch represents the depolarized solution in which the IRK channels are largely closed. The lower branch represents the hyperpolarized solution. For the hyperpolarized solution the potassium permeability P_K is approximately 25 times as large as for the depolarized solution. The dynamically unstable segment that connects the two curves was not drawn. When $[\text{K}^+]_o$ is in the bistable region, it depends on the history whether the system will be on the hyperpolarized or on the depolarized branch.

In heart cells hysteresis has been observed in voltage clamp experiments by Gadsby and Crane-field [11]. These authors, furthermore, made mention of the bistability. Data obtained by Brismar and Collins [16] on glial cells point in a similar direction.

It is well known that also for chloride the transmembrane electric potential is more or less balanced out by the chemical potential [2]. At rest a significant fraction of the chloride channels is open and, as with potassium, this contributes to the stabilization of the membrane potential. A more complete and quantitatively accurate description should thus involve chloride as well as many more types of pumps, transporters and channels. It is nevertheless remarkable that a simple model, involving only the Na,K-ATPase, Na^+ channels, and K^+ channels, can account for the steady states of the living cell. We have presented a model in which we included the empirically observed V_m and $[\text{K}^+]_o$ dependence of the permeability of the

IRK. The model explains the electrophysiological behavior of the cell at low $[K^+]_o$, i.e. the bistability of the membrane potential. The experiments and the model, furthermore, show that the membrane potential and the fluxes and permeabilities are interdependent in a non-trivial way and the effect of a pharmacological agent on one component of the model can easily be misinterpreted and taken as an effect on another component.

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