SODIUM-POTASSIUM PUMP AND BIOELECTROGENESIS IN TEACHING PHYSIOLOGY: MISCONCEPTIONS AND NEW DIDACTIC APPROACH

Petar Raychev
University of Sofia (Bulgaria)

Abstract. Teaching and conceptualizing basic mechanisms underlying generation of resting membrane potential (RMP) is one of the starting and critically important points in all modern physiology courses and textbooks for students of medicine. Mastering concept on RMP and bioelectrogenesis is a basic precondition for students' introduction into almost all fields of physiology, and particularly in physiology of excitable structures and sensory systems. Sodium-potassium pumps can be regarded as cells' power plants which generate and maintain RMP. However, it is a strange paradox, taking into account importance of the subject, that one of the most widely-spread and persisting misconception among the students is just about the contribution of electrogenic effect (EE) of sodium-potassium pump (Na^+/K^+ pump) to the RMP. Fighting such critical misconceptions requires development and practical implementation of effective and specifically addressed didactic solutions in teaching-learning process of physiology.In the present report is proposed a new, compatible with active learning strategies, didactic model. It is based on quantifying specific contribution of electrogenic and non-electrogenic mechanisms of Na^+/K^+ pump to RMP. Implementation of the model can be done through Goldman-Hodgkin-Katz (GHK) and Mullins-Noda (MN) equations or through equations based on Millman theorem (also known as chord-conductance equations, CC). Newly developed model enables teaching staff to apply active learning strategies in this relatively complicated and abstract but very important part of physiology. Additional advantage of the model is that it allows demonstration, in a relatively simple and intuitive way, compatibility of MN equation with classical pump-leak model and limitations of GHK equation in conceptualizing bioelectrogenic processes in normal cells at steady state.

Keywords: medical education; active learning; resting membrane potential; electrogenic effect; sodium-potassium pump; Goldman-Hodgkin-Katz equations; Mullins-Noda equation; bioelectrogenesis.

Introduction

Modern physiology is an integrative science based on a fast evolving knowledge with developed deductive infrastructure. In such dynamic environment teaching physiology for students of medicine is a challenging task. Achieving high-quality educational 'product' requires permanent efforts from lecturers and instructors towards optimization of traditional didactic models and constant search for innovative approaches based on reflection and reevaluation of content, structure and pedagogical implementation of academic curriculum.

One of the key topics in physiology, as a science and fundamental academic discipline in medical education, is about Na^+/K^+ pump and its role in generation of RMP and maintaining osmotic equilibrium of the cells. Discovery of Na^+/K^+ pump in the middle of 20th century was one of the peak achievements in the life-sciences at that time (Skou, 1957) which dramatically expanded our knowledge in many fields of physiology (e.g., excitable structures, sensory systems, excretion processes, etc.). Comprehending role of Na^+/K^+ pump in bioelectrogenesis is a critical precondition for mastering concepts for a broad range of physiological and pathophysiological processes. So, this topic is one of the immutable starting points in modern courses and textbooks of physiology (Guyton & Hall, 2010; Hall, 2016; Sherwood, 2016; Costanzo, 2018).

Despite the fundamental importance of the subject in the system of physiology knowledge, between students permanently arise critical misconceptions about Na^+/K^+ pump and its role in bioelectrogenesis. Perhaps most widespread and persistent among them concerns EE of Na^+/K^+ pump and its specific contribution to RMP. The essence of EE is in removing one positive charge from the cell during each working cycle of Na^+/K^+ pump on the prize of one molecule ATP (Fig. 1). After mastering the concept of EE many students erroneously assume that EE is the main (or even the only) cause for RMP. This misconception is diagnosed easily by instructors through its logical consequence that if there was no EE of Na^+/K^+ pump and it exchanged equal number of ions in both directions (for example 1:1 or 2:2) RMP wouldn't exist. If this misconception is not revealed and corrected on time, later it prevents (or strongly hinders) understanding mechanisms underlying bioelectrogenesis of the cells (e.g., the role of specific chemical and electrochemical gradients of the ions and selective permeability of the cell membrane to them).

Subsequently, such misunderstanding will also negatively affect attempts of the students to grasp mechanisms involved in generation and dynamics of different types of graded potentials and action potentials.

In such challenging points in teaching-learning process of physiology, involving more generalized and/or abstract content, applying active learning strategies looks promising direction (Burdo & O'Dwyer, 2015; Modell, 1996) in the quest for optimal and effective didactic solutions.

Aims

Aim of the present report is to provide innovative and suitable for teaching purposes (it means relatively simple, intuitive, and compatible with good pedagogical

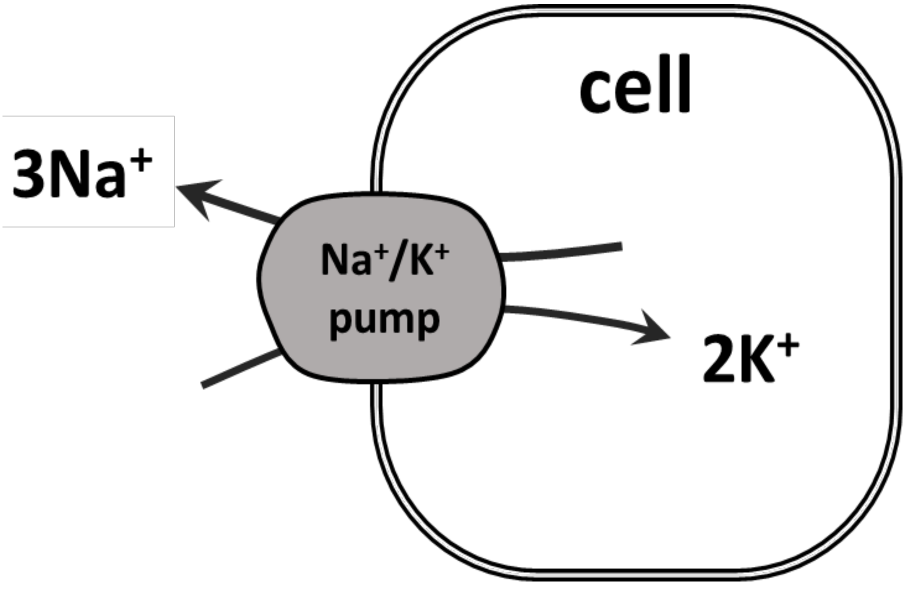

Figure 1. Electrogenic effect of Na^+/K^+ pump. In each working cycle Na^+/K^+ pump exchanges three Na^+ for two K^+ on the prize of one molecule ATP (not shown). The net effect is pulling out one positive charge from the cell and prevalence of negative charges inside. It is very easy and intuitive for students to conclude from this model that EE is the main (or even only!) mechanism responsible for RMP.

practices) alternative(s) to traditional schemes of teaching subject on the role of Na^+/K^+ pump in bioelectrogenesis. In particular, newly proposed didactic model aims to prevent arising of some critical misconceptions about the contribution of electrogenic and non-electrogenic effects of Na^+/K^+ pump to RMP of the normal cell.

In order to be effective in achieving aforementioned goals and useful in teaching-learning process, newly proposed didactic model is designed to have following special features: (1) it uses simple, well-known to students and convenient for teaching purposes mathematical toolkit; (2) it is based on (or cumulative to) the knowledge acquired from university and pre-university courses of physics, biophysics, and cell biology; (3) it is compatible with established good teaching and training practices in medical physiology (in particular active learning methods); (4) it provides clear, illustrative and easy to understand results thereby helping students more efficiently to master concept on the role of Na^+/K^+ pump in bioelectrogenesis.

Methods for quantitative evaluation of electrogenic effect of SPP in teaching process

For teaching purposes, contribution of EE of Na^+/K^+ pump to the RMP (in mV) can be demonstrated and quantified by consecutive calculations of RMP values with (E_{+ee}) and without (E_{+ee}) taking into account EE in the following way:

$$EE = E_{+ee} - E_{-ee} \tag{1}$$

To enhance illustrative power of the result EE could be expressed in percentages (EE%):

$$EE\% = \frac{EE}{E_{+ee}} 100 \tag{2}$$

On the ground of established traditions and modern tendencies in teaching-learning practices in physiology for medical students (Raychev, 2019), two main approaches for quantification of EE can be proposed.

The first one I will name 'thermodynamic' because it is based on equations which have generic links and mathematical similarity to the Nernst equation. Nernst equation (especially in the form at 310 K or 37^0 C) is routinely introduced in almost all modern physiology courses and textbooks for medical students (e.g., Conti 2004, Costanzo 2018, Guyton & Hall 2010) and in teaching practice is widely used for conceptualizing mechanisms of transmembrane ion movements and equilibrium potentials (E_x):

$$E_X = \frac{61}{z} \, lg \frac{[X]_e}{[X]_i} \tag{3}$$

where $[X]_e$ and $[X]_i$ are extracellular and intracellular concentrations of ion X having electrical charge z. Thermodynamic approach is based on two equations = famous GHK equation (Goldman, 1943; Hodgkin & Katz, 1949) and very similar to it in shape (but not in its essence!) and almost unknown among physiologists MN equation (Mullins & Noda, 1963).

The second approach is based on chord-conductance (CC) equation which is based on Millman (1940) theorem and well known to students (from university and pre-university courses of physics) Ohm's law and first law of Kirchhoff.

Thermodynamic approach

In thermodynamic approach contribution of EE of Na^+/K^+ pump to RMP can be quantified through combined application of aforementioned GHK and MN equations. Both can be used separately to figure out RMP but not EE. From physiological perspective essential difference between them is that GHK equation quantifies RMP only on the ground of concentration gradients of the ions and selective permeability of the cell membrane while MN equation counts also EE of Na^+/K^+-pump. It is worthy to recall that at the time of publication of GHK equation (Hodgkin & Katz, 1949) Na^+/K^+ -pump (Skou, 1957) was not known in science. Therefore, its indirect non-electrogenic contribution (Eq. (1)) to RMP can be calculated using following form of GHK (at 37^0 C):

$$E_{-ee} = 61\ lg\frac{P_{K^+}[K^+]_e + P_{Na^+}[Na^+]_e}{P_{K^+}[K^+]_i + P_{Na^+}[Na^+]_i} = 61\ lg\frac{[K^+]_e + \frac{P_{Na^+}}{P_{K^+}}[Na^+]_e}{[K^+]_i + \frac{P_{Na^+}}{P_{K^+}}[Na^+]_i} \qquad (4)$$

where $[K^+]$ $[K^+]$ and $[Na^+]$ $[Na^+]$ are extracellular (index *e*) and intracellular (index *i*) molar concentrations of the ions while $P_{K^+} P_{K^+}$ and $P_{Na^+} P_{Na^+}$ are their membrane permeabilities.

Figuring out EE_{+ee} from (1) can be done in a similar way EE_{-ee} , but this time on the ground of MN equation:

$$E_{+ee} = 61\ lg\frac{rP_{K^+}[K^+]_e + P_{Na^+}[Na^+]_e}{rP_{K^+}[K^+]_i + P_{Na^+}[Na^+]_i} = 61\ lg\frac{r[K^+]_e + \frac{P_{Na^+}}{P_{K^+}}[Na^+]_e}{r[K^+]_i + \frac{P_{Na^+}}{P_{K^+}}[Na^+]_i} \qquad (5)$$

On the ground of E_{-ee} and E_{+ee} , 'pure' contribution of EE of Na^+/K^+-pump to RMP can be figured out by Eqs. (1) and (2). Coefficient r (Mullins & Noda, 1963), which reflects stoichiometry of Na^+/K^+-pump (r=3/2=1,5), is the only formal difference between Eq. (4) and Eq. (5).

Application of thermodynamic approach for teaching purposes can be illustrated in the following way. Let us assume that $[K^+]_e[K^+]_e$ and $[Na^+]_e[Na^+]_e$ are 4 and 142 mM/l. Respective figures for $[K^+]_i[K^+]_i$ and $[Na^+]_i[Na^+]_i$ are 140 and 10 mM/l. We also assume that $P_{K^+} = 25.P_{Na^+} P_{K^+} = 25.P_{Na^+}$. All values of the variables are in their typical physiological ranges. Substituting in Eq. (4) we have:

$$E_{-ee} = 61\ lg\frac{4 + \frac{1}{25}142}{140 + \frac{1}{25}10} = -71\ mV \qquad (6)$$

EE contribution to RMP is taken into account through MN Eq. (5):

$$E_{+ee} = 61\ lg \frac{1{,}5.4 + \frac{1}{25}.142}{1{,}5.140 + \frac{1}{25}.10} = -77\ mV \tag{7}$$

Contribution of EE of Na^+/K^+-pump to RMP is -6 mV (Eq, (1)). Expressed in percentages (Eq. (2)) it is about 7,5%. Both results clearly demonstrate minimal, although not negligible, portion of EE in total value of RMP.

Equations based on Millman theorem (chard-conductance equations)

In modern teaching-learning process of physiology for medical students it has become a common practice introducing concepts of RMP and bioelectrogenesis to be done by CC equation instead of traditional GHK equation (Raychev, 2019). From teaching perspective, this alternative approach offers some important didactic advantages. Most prominent among them are simplified mathematical form and the fact that law of Ohm and first law of Kirchhoff are well known to the students. Introducing and conceptualizing bioelectrogenic mechanisms of Na^+/K^+-pump through familiar and intelligible basic concepts and ideas of physics, chemistry and mathematics makes teaching-learning process much more integrated and effective. Classical assumptions of GHK model for constancy of ion currents across the cell membrane are also met in CC-equation for Na^+ and K^+ and RMP is figured out in the following way:

$$E_{-ee} = \frac{g_{K^+}E_{K^+} + g_{Na^+}E_{Na^+}}{g_{K^+} + g_{Na^+}} = \frac{E_{K^+} + \frac{g_{Na^+}}{g_{K^+}}E_{Na^+}}{1 + \frac{g_{Na^+}}{g_{K^+}}} \tag{8}$$

where $E_{K^+}E_{K^+}$ and $E_{Na^+}E_{Na^+}$ are Nernstian equilibrium potentials (3) and g_{K^+} g_{K^+} and $g_{Na^+}g_{Na^+}$ are conductances (reciprocals of resistance in classical form of Ohm's law) of the respective ions. EE can be counted in a way similar to that in MN equation (Raychev, 2019; Baldissera, 2005):

$$E_{+ee} = \frac{1{,}5g_{K^+}E_{K^+} + g_{Na^+}E_{Na^+}}{1{,}5g_{K^+} + g_{Na^+}} = \frac{1{,}5E_{K^+} + \frac{g_{Na^+}}{g_{K^+}}E_{Na^+}}{1{,}5 + \frac{g_{Na^+}}{g_{K^+}}} \tag{9}$$

Similar to thermodynamic approach, EE contribution to RMP can be quantified substituting values from Eqs. (8) and (9) in Eqs. (1) and (2).

Let us take the values for K^+ and Na^+ concentrations from previous example (6) and use them in (3). Calculated equilibrium potentials ($E_{K^+} E_{K^+}$, $E_{Na^+} E_{Na^+}$) are -94 mV and -70 mV respectively. Let's assume that ratio $g_{Na^+} g_{Na^+} / g_{K^+} g_{K^+}$ is 1/25. After substituting values in Eq. (8) calculated RMP is:

$$E_{-ee} = \frac{-94 + 0{,}04.70}{1 + 0{,}04} = -88\ mV \tag{10}$$

Taking into account EE in RMP can be done using Eq. (9). This time RMP is about -90 mV. Pure contribution of EE to RMP is 2 mV, Eq. (1), which constitutes only 2,2 % of RMP, Eq. (2). As in a previous example, figures again unequivocally demonstrate minimal portion of EE in total value of RMP.

Discussion

Bioelectrogenesis is one of the most problematic but critically important topic in teaching-learning process of physiology. Introducing this relatively abstract and complicated field of physiology to students via active-learning strategies based on well-known concepts from physics, chemistry and mathematics enables instructors and lecturers to apply more flexible and effective didactic models in their practice. Quantifying specific contribution of EE and non-electrogenic mechanism of Na^+/K^+-pump to RMP allows students to acquire some 'hands-on' experience on the subject and creates promising new paradigm in this area of teaching-learning practice of physiology.

A reasonable didactic strategy for Integration of thermodynamic approach in teaching process can be illustrated by the following simple linear algorithm: (1) Students consider GHK equation (4) and identify physiologically important factors determining value of RMP (ion chemical gradients, characterized by concentrations of Na^+ and K^+, and selective ion permeability of the cell membrane); (2) RMP is calculated using GHK equation and values of the variables which are typical for normal cells at steady-state (e.g. neurons or muscle cells); (3) MN equation is introduced. Its advantages over GHK equation are discussed (in particular taking into account contribution of EE of Na^+/K^+-pump to RMP); (4) Using the same values of the parameters as in step 2, RMP is calculated again but this time through MN-equation; (5) Specific contribution of EE to RMP is evaluated on the ground of Eqs. (1) and (2).

Similar algorithm with analogous steps can be implemented on the base of CC, Eqs. (8) and (9).

Above presented algorithm must not be perceived as a rigid unchangeable structure but as a flexible and expandable didactic approach which is able to include (and to be included) in different teaching-learning methods and strategies.

It can be modified by supplementing additional steps and by expanding one or more of the steps in greater details. For instance, in steps 2 and 4 RMP can also be calculated at ratio $P_{Na^+}P_{Na^+}/P_{K^+}P_{K^+}$ = 1/30 and observed differences to be analyzed. Step 1 can be expanded by making comparison between Nernst equation and GHK equation and pointing out fundamental differences between them (equilibrium nature of the systems with single ion in the first case, and poly-ion non-equilibrium systems in the second one). In an additional step 6 RMP can be counted on the ground of MN equation assuming $P_{Na^+} = P_{K^+}P_{Na^+} = P_{K^+}$ (precondition for maximal contribution of EE to RMP).

Quantified contribution of EE to RMP, figured out by both methods (thermodynamic and one based on CC equation), provides suitable didactic illustration of minimal, but not fully negligible, role of EE in bioelectrogenesis. Discrepancy between two methods in figuring out values for RMP and EE contribution can be explained by the fact that in CC equations (and Ohm's law respectively) the effect of chemical gradients on ions currents is not taken into account (Hille, 1992). From didactic perspective, these differences are negligible and both approaches can be used successfully for teaching purposes.

Thermodynamic and Millman-based approach have some specific advantages and limitations in teaching practice which must be considered.

Major advantage of thermodynamic approach is that it is based on equations which mathematical expression is very similar to the Nernst equation – probably the best known equation among physiologists and widely used in their teaching practice. GHK equation itself is also well known and is presented in most of the modern textbooks of physiology for students of medicine (Raychev, 2019). Although MN equation is not popular in physiology circles, its mathematical shape is almost identical to those of GHK (compare (4) and (5)) making it suitable for use in teaching-learning process. In fact, if assumption is made for Na^+/K^+-pump without EE (r=1), MN equation would have the same expression as those of GHK. Taking into account that MN equation is compatible with pumps-leaks model, a more precise and consistent didactic approach could be implemented if quantification of EE is based only on MN equation at different values for r. When r=1,5 in (5) RMP is calculated with EE. If r=1 RMP is calculated without contribution of EE.

A major limitation of thermodynamic approach, from perspective of medical students and their educational background, is associated with its relatively complicated mathematical shape and more abstract thermodynamic concepts and ideas behind it.

In this respect a Millman theorem-based (CC-equation) approach looks more attractive and better fitted for teaching purposes. Its mathematical machinery is more simplified despite the presence of Nernstian equilibrium potentials for Na^+ and K^+. Even more important is the fact that conceptual framework behind these equations is based on Ohm's law and First law of Kirchhoff which are well known to students from their pre-university and university courses of physics. As a consequence, it is much easier calculations (and mathematical 'language' behind them) to be interpreted and discussed by students. For instance, products of $g_{K^+} E_{K^+} g_{K^+} E_{K^+}$ and $g_{Na^+} E_{Na^+} g_{Na^+} E_{Na^+}$ (Eqs. (6) and (7)) can be interpreted as K^+ and Na^+ currents across the cell membrane when RMP=0 mV. This exemplifies how well known concepts from physics can be integrated in the teaching process of physiology helping students to master complex ideas in physiology.

As a beneficial side effect, quantification of EE offers additional advantage in teaching process. Although fully incompatible with the pump-leak model of a normal living cell at steady-state, equations based on the assumption of ions flux steady state (GHK Eq. (4) and CC Eq. (8)) continue to dominate teaching the topic about bioelectrogenesis in modern lectures and textbooks of physiology (Raychev, 2019). Conceptualizing bioelectrogenesis with concomitant use of equations which count, Eqs. (5) and (9), and which not count EE can serve as an excellent starting point for discussion on advantages and limitations of the models of living cells on which they are based.

Conclusion

An innovative didactic model for introduction the topic of the Na^+/K^+-pump and its role in bioelectrogenesis to students of medicine is proposed. Compared to traditional teaching schemes, newly described model is characterized by adding new content to the topic (MN equation and form of the CC-equation counting EE) and alternative methods and structure on which teaching process is based. Presented approach is aimed to address specific and persistent problematic point in physiology teaching associated with wide spread misconceptions among the students of medicine about the role of Na^+/K^+-pump (in particular its EE) in generating RMP.

Deeply rooted misunderstanding about the leading role of EE of Na^+/K^+-pump in RMP generation can be explained by the simplicity and deductive power of the model on which it is based. Its compatibility with well-known concepts of physics, chemistry and cell biology which students acquire during their university and pre-university education also plays important role. Superficial and inadequate presentation by the lecturers, instructors and authors of textbooks of bioelectrogenesis, a critically important but relatively complicated and abstract topic, can be pointed out as another source of confusion and misunderstanding between students.

Quantification of EE and its specific contribution towards the RMP provides an alternative and promising way for improving effectiveness in mastering concepts on bioelectrogenesis with long-lasting results.

From didactic perspective of particular importance is the fact that equations on which quantification is based serve as an intellectual tool for active involvement of the students in teaching-learning process by participation in their 'linguistic' analysis, doing calculations and discussing and interpreting results. Integrating active-learning strategies in mastering concepts on more complicated and abstract topics from physiology as RMP and bioelectrogenesis is a promising and attractive direction for constant attempts of lecturers, instructors and textbook authors to improve quality of teaching and training process.

Discussions on differences between equations of GHK and MN (or different forms of CC equation) can be further beneficial in defining steady-state of normal living cells and in introducing pump-leak model. By getting firm grasp on bioelectrogenesis, students acquire fundamental knowledge for mastering different types of ''passive'' membrane potentials (e.g., graded potentials and action potentials) and physiology of excitable structures.

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✉ **Dr. Petar Raychev**
Department of Chemistry, Biochemistry, Physiology and Pathophysiology
Faculty of Medicine
University of Sofia
1, Kozyak St.
1407 Sofia, Bulgaria
E-Mail: petar.raychev@gmail.com