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Research Article

Distinctive Features of Criticality in the Operation of Membrane Na^+/K^+-ATPases

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Abstract

Based on the data of mass spectrometry of metals in the hair of 947 healthy individuals and 954 liquidators of the Chernobyl accident, the authors of the article propose to use some markers enabling to distinguish between the subcritical and critical phases in Na/K homeostasis of epidermal cells.

The main criterion for phase separation was the numerical values of Na and K at the C_{thr} point (the beginning of the critical phase), from which linearization begins on the Pareto distribution plot on a double logarithmic scale. The plotting for Na and K and location of the C_{thr} point was performed based on the results of hair spectrometry from 10,000 healthy individuals aged 20 - 45 years.

In the authors' opinion, the following indicators can serve as additional distinguishing features of these phases: a) the cluster character of quantitative shifts in the metal-ligand homeostasis of metals such as Na, K, Al, Cd, Cr, Cu, Fe, Li, Pb, V and Zn, against the background of oxidative/nitrosative stress; b) reliable positive Na-K and negative K-Zn bonds (Pearson) according to the data of mass spectrometry of metals; c) an increase in the numerical values of the synchronization index - an integral indicator of the tightness of linear connections (pairwise correlations) between metals.

Keywords: Nitric Oxide; Metal-Ligand Homeostasis; Self-Organized Criticality; Epidermis

Introduction

Previous publications on the problem of metal ligand homeostasis (MLH) in the epidermis provide evidence enabling us to recognize MLH as a phenomenon of self-organized criticality (SC-phenomenon). We consider the following to be the most convincing among them:

The presence of a power-law relationship (confirmed graphically on a double logarithmic scale) between the content of metals in the epidermis (hair) and the number of individuals in certain intervals of numerical values for metals such as Na, K, Ca Al, Cd, Cr, Cu, Fe, Li, Pb, V and Zn [1-3];

Reliable linear relationship between the concentration values of sodium (Na) and potassium (K) indicating the synchronous operation of membrane Na^+/K^+-ATPases [4];

Conjugate (cluster) nature of quantitative shifts in MLH against the background of oxidative/nitrosative stress [5].

In our opinion, the working hypothesis regarding the ability of membrane ATPases, which are responsible for the traffic of metals, to self-organize (transition to a critical state) upon oxidative/nitrosative modification of these enzymes by reactive oxygen species (ROS) and reactive nitrogen species (RNS) has both theoretical and practical significance [1].

On the one hand, it is becoming obvious that MLH is related to the processes of cellular bioenergetics occurring at the membrane level, and on the other, an opportunity opens up for identifying new indicators (markers) of criticality in the operation of membrane pumps [1].

These markers, if their practical suitability is proven, could be used to verify the critical state along with the already known method of revealing a power-law relationship as a criterion of criticality (the method of approximating a straight line in a double logarithmic scale) [2].

In this regard, we need the following indicators to clarify their diagnostic significance:

Synchronization index (SI)- an integral assessment of the tightness of the linear relationship (pairwise correlations) between the quantitative values of metals in the biosubstrate [6];

Unidirectional shifts in the concentration values of metals observed under oxidative/nitrosative stress [5] and/or in different age groups [3];

Some pair correlations taking the highest nominal values in the critical phase (for example, K-Na and K-Zn bonds) [4].

To solve this problem, we used the results of quantitative spectrometry of metals (Na, K, Al, Cd, Cr, Cu, Fe, Li, Pb, V and Zn) in the epidermis (hair) obtained earlier at the Center for Biotic Medicine (Moscow) in 947 healthy subjects and 954 liquidators of the Chernobyl accident [5].

The spectrometric data in each of the groups were divided into two categories depending on the probable belonging of Na and K homeostasis to the subcritical or critical phase. The criterion for the division was the numerical values of these metals at the point C_{thr} (the beginning of the critical phase). From this point on, the linearization of the Pareto distribution plot on a logarithmic scale begins. Plotting for Na and K and identification of the C_{thr} point were done based on the results of hair spectrometry from 10,000 healthy individuals aged 20 - 45 years. The research results are presented in this work.

Materials and Methods

Using inductively coupled plasma mass spectrometry (ICP-MS) on a NexION 300D device (Perkin Elmer Inc., Shelton, CT, USA) at the Center for Biotic Medicine (Moscow), a quantitative analysis of hair was made for the following metals: Na, K, Al, Cd, Cr, Cu, Fe, Li, Pb, V and Zn. The studies were carried out in 947 healthy subjects (238 men and 709 women aged 2 to 86 years) and 954 liquidators of the Chernobyl accident - Moscow residents (213 women and 741 men aged 37 to 82 years). In both groups, the medians (*Me*) of the concentration values of the indicated metals were found depending on the MLH phase and the formatting method (by Na or K).

The sampling of biomaterial was carried out with mandatory and voluntary consent of the subjects in accordance with the ethical standards set forth in the 1964 Helsinki Declaration and its later amendments.

In addition, we analyzed the results of atomic emission spectrometry of hair for the content of Na and K, obtained from 10,000 healthy Moscow residents (5,000 men and 5,000 women aged of 20 - 45 years) to reveal the power-law relationship between the content of metals in the derivative of the epidermis and the amount individuals (SC-feature). For this, the probability density of the power-law distribution (PDF) was estimated and this distribution was fitted using the linearization method (Pareto). In our previous study we used a similar mathematical approach [2].

The point of the beginning of linearization C_{thr} located on the Pareto plots for Na and K served as a criterion for the distribution of subjects in both groups (practically healthy and emergency responders) into two categories: with subcritical and critical modes of operation of membrane Na^+/ K^+-ATPases.

Within these modes (subcritical and critical), the degree of synchronization (criticality) of operating membrane pumps was determined based on the results of the analysis of the tightness of the connection (Pearson) between the levels of metals in the biosubstrate (pair linear correlations). For this, the synchronization index (SI) was found - the area under the curve consisting of the numerical values of the coefficient *r* (without taking into account the sign), located on the graph in ascending order [6].

Matlab software package was used for statistical data processing.

Results and Discussion

In order to reveal the power-law relationship between the content of metals (Na, K) in the epidermis and the number of individuals in a given range of numerical values, as well as to find the C_{thr} point (the beginning of linearization), Pareto plots were built in a double logarithmic scale, on which you can see the probability density of the power-law distribution (PDF).

PDF is determined by the following formula:

$$p_{k,m}(x) = \frac{km^k}{x^{k+1}}.$$

Below is an "ideal" case fitting over the random numbers with Pareto distribution in order to judge the quality of a fitting (See figure 1).

Figure 1: The "ideal" case of random fit with the Pareto distribution (RND). The blue curve is the density estimate from spectrometric data. The red curve is the fitting of the power-law distribution by the linearization method. RMSE is the root mean square error. C_{thr} is the lowest concentration from which the fitting begins.

To obtain a normalized histogram, the column boundaries were selected uniformly on a logarithmic scale (30 columns for 10,000 measurements). At the top of the figures are indicated: the name of the metal, RMSE is the root mean square error, since C_{thr} is the lowest concentration from which the fitting begins. Concentration values lower than C_{thr} are not taken into account. The calculation of the distribution density parameters is presented in table 1.

Metal	k	m
K	2.9309	1.9422
Na	3.3278	4.6004
RND	5.0046	0.90352

Table 1: Calculated parameters of the density of the power-law distribution (PDF).

Figure 2 and 3 show the estimates of the density and linearization of the power-law distribution according to the spectrometry data for sodium (Figure 2) and potassium (Figure 3).

Figure 2: Estimation of density and linearization of power distribution for sodium (Na); designations are the same as in figure 1.

The points of the beginning of linearization (C_{thr}) for Na and K (Figure 1 and 2) separate on the graph two fundamentally different phases in the operation of membrane Na^+/ K^+-ATPases: subcritical and critical. The transition of the critical phase to the supercriti-

Figure 3: Estimation of density and linearization of power distribution for potassium (K); designations are the same as in figure 1.

cal one (which is admissible but poorly confirmed graphically) can only be assumed.

However, taking into account the presumably small size of this phase which is unlikely to significantly affect the final result, we considered all values of Na and K that exceeded C_{thr} to be "critical" (it is possible, however, that some of them may belong to the supercritical phase).

The use of C_{thr} as a "dividing" criterion made it possible to single out individuals with Na- and K-homeostasis in the critical ([Na] and [K] > C_{thr}) or subcritical ([Na] and [K] < C_{thr}) phase from the total number of observations, which was a prerequisite for the comparative analysis of the mentioned phases.

This allowed the entire array of spectrometry data (Na, K, Al, Cd, Cr, Cu, Fe, Li, Pb, V and Zn) in both groups (healthy and emergency responders) to be formatted depending on the level of Na and K in the biosubstrate (in ascending order of this indicator). As a result, groups of healthy people with subcritical and critical phases of Na- and K-homeostasis and similar groups among the liquidators of the accident were formed.

Depending on the phase (subcritical or critical) of sodium or potassium homeostasis, a correlation analysis (Pearson) of the numerical values of metals (pairwise correlations) was made and the synchronization indices (SI) were calculated according to the method we proposed earlier [6]. In addition, the medians (*Me*) of the concentration values of metals in the critical and subcritical phases of Na- and K-homeostasis were found. The results are shown in table 2-5.

Metal	Formatting of sodium (Na)	
	Subcritical phase of MLH [Na] < 183.5 mcg/g Me (mcg/g) (n = 466)	Critical phase of MLH [Na] > 183.5 mcg/g Me (mcg/g) (n = 481)
Na	83.3	466
K	34	252.7
Al	4.2	7.2
Cd	0.01	0.03
Cr	0.33	0.46
Cu	13.9	13.5
Li	0.01	0.03
Fe	12.1	16
Pb	0.43	0.71
V	0.04	0.06
Zn	190.6	168.8
SI (synchronization index)	5.1	6.4
r_{Na-K}	0.1	0.6
r_{K-Zn}	-0.08	-0.3

Table 2: The median (Me) metal level in hair, synchronization index (SI), Na-K and K-Zn correlations (Pearson) in different phases of Na-homeostasis in healthy subjects.

Metal	Formatting of potassium (K)	
	Subcritical phase of MLH [K] < 50.2 mcg/g Me (mcg/g) (n = 345)	Critical phase of MLH [K] > 50.2 mcg/g Me (mcg/g) (n = 602)
Na	77,9	348
K	4,6	198,6
Al	3,04	7,5
Cd	0,01	0,03
Cr	0,3	0,46

Cu	14,1	13,5
Li	0,01	0,02
Fe	10,57	16,2
Pb	0,35	0,7
V	0,03	0,056
Zn	195,1	169,2
SI (synchronization index)	4,3	6,6
r_{Na-K}	0,18	0,59
r_{K-Zn}	- 0,08	- 0,27

Table 3: Median (Me) of metal levels in hair, synchronization index (SI), Na-K and K-Zn correlations (Pearson) in different phases of K-homeostasis in healthy subjects.

Metal	Formatting of sodium (Na)	
	Subcritical phase of MLH [Na] < 183.5 mcg/g Me (mcg/g) (n = 206)	Critical phase of MLH [Na] > 183.5 mcg/g Me (mcg/g) (n = 748)
Na	116.4	597.5
K	60.1	289.6
Al	15.4	18.01
Cd	0.12	0.16
Cr	0.72	0.8
Cu	9.5	10.1
Li	0.02	0.04
Fe	14.2	20.1
Pb	0.47	0.88
V	0.08	0.08
Zn	184.4	163.4
SI (synchronization index)	4.7	9.4
r_{Na-K}	0.05	0.69
r_{K-Zn}	-0.17	-0.42

Table 4: Median (Me) of metal levels in hair, synchronization index (SI), Na-K and K-Zn correlations (Pearson) in different phases of Na homeostasis among accident liquidators.

Metal	Formatting of potassium (K)	
	Subcritical phase of MLH [K] < 50.2 mcg/g Me (mcg/g) (n = 123)	Critical phase of MLH [K] > 50.2 mcg/g Me (mcg/g) (n = 831)
Na	115.8	512.3
K	32.5	247.5
Al	13.8	17.8
Cd	0.09	0.16
Cr	0.7	0.8
Cu	9.4	10.02
Li	0.027	0.036
Fe	14	19.5
Pb	0.47	0.83
V	0.08	0.08
Zn	187.5	166.3
SI (synchronization index)	7.9	8.9
r_{Na-K}	0.26	0.69
r_{K-Zn}	0.1	-0.43

Table 5: Median (Me) of the level of metals in hair, synchronization index (SI), Na-K and K-Zn correlations (Pearson) in different phases of K-homeostasis in liquidators of the accident.

Based on the results of the comparative analysis in healthy subjects and Chernobyl accident liquidators, it can be concluded that there are differences between the subcritical and critical phases of MLH regardless of belonging to the indicated groups and the formatting method (by Na or K).

We are talking about such indicators as SI, r_{Na-K} and r_{K-Zn}, which in the critical phase were higher than in the subcritical phase (See table 2-5). These data may serve as additional confirmation of the synchronous operation of Na^+/K^+-ATPases (and, possibly, other ATPases from the P-type family involved in metal transport) during the critical period of MLH. In the subcritical period, such synchronization was not observed. It should be noted that synchronization (in terms of the SC theory) is a special case of a critical state.

If (depending on the phase) we compare the medians (*Me*) of the concentration values of other metals (Al, Cd, Cr, Cu, Li, Fe, Pb, V, and Zn), then the combined (cluster) nature of the shifts in *Me* values is clearly revealed (See table 2-5). So, in the critical phase, both in healthy people and in liquidators of the accident (regardless of the formatting method), the content of Al, Cd, Cr, Li, Fe, Pb, V in the biosubstrate was higher, and Cu and Zn were lower than in the subcritical one. The noted exceptions among the accident liquidators: the level of copper (Cu) in the critical phase was higher than in the subcritical phase, and the level of vanadium (V) remained unchanged regardless of the phase and method of formatting (See table 4 and 5).

The found quantitative shifts of metals can hardly be classified as random, since already in the first works on the MLH problem [5] it was found that the level of metals in the accident liquidators (based on hair spectrometry data) significantly differed from the control in accordance with the specified algorithm: [Na]↑, [K]↑, [Al]↑, [Cd]↑, [Cr]↑, [Cu]↓, [Li]↑, [Fe]↑, [Pb]↑, [V]↑, [Zn]↓.

It is significant that the value of SI in the general group of the accident liquidators, as it turned out later, was higher in comparison with the control (6.13 vs 2.17) [6].

It is known that ROS production in liquidators of the Chernobyl accident exceeds normal values [7], which can lead to activation of membrane ATPases and an increase in transmembrane traffic of metals. At the same time, such changes in MLH may be associated not only with an increased level of ROS (oxidative stress), but also with a probable increase in the production of RNS (nitrosative stress) [8].

To test this hypothesis, a comparative analysis of the level of metals (mass spectrometry) in the epidermis (hair) and the magnitude of the EPR signal of nitroxide in the same substrate (EPR analysis) was made in 58 liquidators of the Chernobyl accident and 58 practically healthy subjects [3].

With a significantly higher (compared to healthy) EPR signal of nitroxide in the hair of the liquidators of the accident (34.1 units vs 19.1 units), the shifts in MLH in all metals (except for vanadium) had the same direction as in critical phase of MLH (compared to subcritical) and / or in the general group of the accident liquidators (n = 954) compared with healthy (n = 947): [Na]↑, [K]↑, [Al]↑, [Cd]↑, [Cr]↑, [Cu]↓, [Li]↑, [Fe]↑, [Pb]↑, [V]↑, [Zn]↓.

In the same work [3], we were able to show the influence of a person's age on quantitative changes in the MLH of the epidermis. Interest in this comparison is due to the fact that increased production of ROS and RNS, recognized activators of Na^+/K^+-ATPase (and, possibly, other ATPases from the P-type family) should be expected in the extreme age categories.

Therefore, 3 groups of practically healthy people were formed with 100 persons per group (1 to 19 year-olds, 20 to 33 year-olds and 58 to 85 year-olds). The metal content in the hair (mass spectrometry), SI and the tightness of the K-Zn bond (Pearson) were determined.

The quantitative shifts of MLH in the extreme age groups (vs average) completely coincided in their direction and corresponded to the already known algorithm: [Na]↑, [K]↑, [Al]↑, [Cd]↑, [Cr]↑, [Cu]↓, [Li]↑, [Fe]↑, [Pb]↑, [V]↑, [Zn]↓. Besides, a negative K-Zn bond was clearly detected and the SI values were noticeably higher (12.6 and 12.05 vs 9.5 resp.) in the extreme age groups (vs average).

The results obtained do not contradict the concept of self-organized criticality, according to which membrane ATPases (P-type) are the main participants in ATP-dependent metal traffic, being oscillators, are capable of self-organization or transition to a critical (synchronous) mode of operation. The role of activators of this process can be played by ROS and RNS, which can bind to the SH-groups of cysteine in the protein molecule of ATPases (oxidation and/or S-nitrosylation).

As the production of ROS and RNS (oxidative/nitrosative stress) increases and the total number (density) of activated pumps on the cell membrane increases, take place the self-triggering of the synchronous (critical) mode of operation of the membrane Na+/K+-ATPases and, possibly, of other ATPases from of the P-type family.

Conclusion

Distinctive signs of criticality (synchronization) in the operation of membrane Na^+/K^+-ATPases (providing for the traffic of Na and K) can be as follows:

Conjugated (cluster) nature of quantitative shifts in MLH of such metals as Na, K, Al, Cd, Cr, Cu, Fe, Li, Pb, V and Zn, against the background of oxidative/nitrosative stress.

High level of Na-K correlation (Pearson) and moderately expressed linear negative K-Zn relationship according to the data of mass spectrometry of metals in the biosubstrate (hair).

Positive (upward) shifts in the numerical values of the synchronization index (SI) - an integral indicator of tightness in linear relationships (pairwise correlations) between the concentration values of metals in the biosubstrate.

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