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ORIGINAL PAPER

Superstatistics analysis of the ion current distribution function: Met_3PbCl influence study

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Abstract A novel analysis of ion current time series is proposed. It is shown that higher (second, third and fourth) statistical moments of the ion current probability distribution function (PDF) can yield new information about ion channel properties. The method is illustrated on a two-state model where the PDF of the compound states are given by normal distributions. The proposed method was applied to the analysis of the SV cation channels of vacuolar membrane of *Beta vulgaris* and the influence of trimethyllead chloride (Met_3PbCl) on the ion current probability distribution. Ion currents were measured by patch-clamp technique. It was shown that Met_3PbCl influences the variance of the open-state ion current but does not alter the PDF of the closed-state ion current. Incorporation of higher statistical moments into the standard investigation of ion channel properties is proposed.

Keywords Superstatistics · Patch clamp · Organolead · Time series

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Introduction

The patch-clamp method was introduced by Neher and Sackmann (1976). It enables one to measure the current through an individual channel and obtain its characteristics. The method can be used in two main configurations: whole cell or small patch of membrane. The general interest of research is to observe current–voltage characteristics (e.g., Kusnier et al. 2006; Basavappa et al. 1995) or the time dependence of current characteristics (e.g., Schaefer et al. 2003) and eventually the response of ion channels to impulses (e.g., Dallman et al. 1998). The second approach is to consider the patch-clamp measurements as a time series and investigate its statistical properties, e.g. Markovian properties or the features of the open/closed-state distribution (Grzywna et al. 1998; Siwy et al. 2002; Borys and Grzywna 2008; Nelson 2002; Mercik et al. 2000). However, most works concentrate on channel state dynamics (open–close) and do not take into account other statistical properties of the ion current. It should be stressed that the current measured for a single ion channel cannot be considered as a typical macroscopic variable for which we could expect that fluctuations would result from measurement imperfections. In contrast, ion current fluctuations are an internal feature of the system and should be carefully analysed. The ion current through an ion channel has to be considered as a stochastic variable and all of its parameters analysed. In view of the above considerations, not only mean values must be considered as an independent variable; also other parameters such as variance (or standard deviation) should be taken into account in such analysis. Herein we will assume that the ion current PDF is described by a normal distribution and therefore that both the mean and variance of the ion current are independent variables defining the investigated distribution. In this regard, the influence of external factors on ion channel properties can affect not only the voltage–current characteristics but also other characteristics of the PDF such as its variance, skewness or kurtosis. Herein, analysis of the statistical properties of a two-state system where the currents of the open and closed states are described by a Gaussian PDF is performed. The results are compared with experimental data, and it is shown that organolead influences not only the mean value of the ion current but also higher statistical characteristics of the ion current PDF. A lead compound was investigated because lead and its compounds, originating from natural sources as well as from human activity, pollute our environment. Lead compounds are usually toxic to humans, animals and plants. Especially dangerous are organic derivatives of lead. Despite the knowledge of lead toxicity, it is broadly used in various industries, which results in serious hazard to life and health of living organisms (see e.g, Craig 2003 and references therein; Carruyo et al. 2008; Day et al. 1996; Andreottola et al. 2008; Bragadin et al. 2007; White et al. 2007; Silbergeld 2003; Cairney et al. 2002; Lespes et al. 2009; Michalzik et al. 2007). Despite strong political effort, e.g. in removing lead from fuel (Needleman 2000), lead compounds are still present in our environment (Juberg et al. 1997; Needleman 1998; Ye and Wong 2006), and sufficient exposure to lead can exert severe and lasting effects on human health, affecting e.g. the peripheral and central nervous system, joints, muscles, kidneys and the haematopoetic system (Papanikolaou et al. 2005; Philip and Gerson 1994). Special care should be taken in the case of lead poisoning in children, because of its long-lasting effects (Nagpal and Brodie 2009; Lidsky and Schneider 2003; Maqusood Ahamed 2007). Therefore the influence of lead on biological tissues is an important subject. Before the discussed compounds enter living organisms they have to cross the biological barrier of the cell membrane. These compounds can modify or destroy the membrane and finally influence the whole organism. Research on the influence of organolead compounds on cell membrane function has shown various effects: erythrocyte haemolysis (Kleszczyńska and Sarapuk 2001), change of membrane fluidity and its mechanical properties (Sarapuk et al. 2001), modification of lipids and membrane proteins (Przestalski et al. 2000), influence of ion channels and membrane pores (Bragadin et al. 2007) and others. These effects depend on the compounds analysed and their concentration. Especially intriguing is the influence of low concentrations of organolead compounds, which are considered safe. Therefore, in this study, the influence of the chosen organolead compound (Met_3PbCl) at low concentration on cell membrane was investigated. The vacuolar membrane of *Beta vulgaris* was selected, because the structure and function of this membrane are well known (Coyaud et al. 1987; Hedrich et al. 1986; Miedema et al. 2003; Hedrich and Neher 1987), and it can be used as a model membrane. Plant cells contain central vacuoles filling more than 90% of the cell volume. With growth they become a significant warehouse of Ca^{2+} ions. The basic ionic currents in vacuolar membranes of higher plants are conducted by nonselective slow activated cationic channels (SV) and fast activated channels (FV). The latter are responsible for ionic conductivity of tonoplast under physiological conditions and, unlike SV channels, they are active at physiological concentration of cytoplasmic Ca^{2+} and blocked by calcium ions, while increasing their concentration (above 1 μM) in cytoplasm (Hedrich and Neher 1987; Tikhonova et al. 1997; Pottosin and Schönknecht 2007). It was found that they are also sensitive to the redox potential (Carpaneto et al. 1999) and other physiological factors (Allen and Sanders 1997). Sensitivity of SV channels to Ca^{2+} ion concentration is related to their involvement in Ca^{2+} homeostasis of cells (Pantoja et al. 1992).

In the conditions most commonly used in research applying the patch-clump technique (symmetrical K^+ ion concentration and millimolar lumen concentration of Ca^{2+}), SV channels are activated at positive membrane potentials. It is worth noticing that in vitro (in patch-clamp experiments) SV channels are activated by positive (non-physiological) membrane voltages, whereas in vivo these channels are, in theory, completely deactivated.

The aim of the present study is to determine the effect of an organolead compound (Met_3PbCl) on vacuolar SV channels. The starting point of these investigations was the assumption that disturbance of ion channels can explain the influence of low concentrations of lead compounds on cell membrane function. Herein, we show that lead compounds can affect the ion current PDF of the channel membrane.

Materials and methods

Mathematical model

In patch-clamp experiments, ion current time series are registered. They consist of recordings of current in the open and closed states. These sets can be considered as a mixture of measurements performed in the closed and open states. Such experiments can be modelled by using superstatistics, i.e. a composition of normal and binomial distributions. In this section, statistical parameters such as the mean, standard deviation, skewness and kurtosis of these superstatistics are investigated. In the theoretical analysis, it is assumed that the observed signal is a combination of open and closed states. The probability distributions of the current measured in these states are given by normal distributions (m_1, σ_1) and (m_2, σ_2). Open and closed states occur with probability p_1 and p_2, respectively. It is assumed that open and closed states are statistically independent. The properties of the model system are investigated by Monte Carlo (MC) simulations. In every MC step, two time series of length $N = 10^6$ data points are sampled from the normal distributions (m_1, σ_1) and (m_2, σ_2), respectively, than the statistical parameters mean (M), variance (σ^2), skewness (S) and kurtosis (K) are calculated. The procedure is repeated $n = 10^2$ times. Finally, estimators and confidence intervals of M, σ^2, S and K are obtained. The following three cases are considered in detail:

1. The open and closed states are equally probable and the distribution functions of these states differ only in their mean values, i.e. $m_2 - m_1 \neq 0$;
2. The open and closed states are equally probable but the distribution functions of these states differ in their variance, i.e. $\sigma_2 - \sigma_1 \neq 0$, and have a constant shift in expected values;
3. The parameters defining the probability distribution functions differ by a constant value and the influence of the changes in probability of open and closed states is investigated.

Description of the experiment

Experiments were carried out using patch-clamp technique. The currents passed through several channels were recorded (*Beta vulgaris*) in outside-out (i.e. cytoplasmic side out) configuration. Vacuoles were directly extruded into the recording chamber (about 1 mL in volume) by cutting a slice of fresh tissue and rinsing the surface with bathing solution. The control bath solution was: 100 mM KCl, 2 mM $MgCl_2$, 0.1 mM $CaCl_2$, 5 mM MES (4-Morpholineethanesulfonic acid), 5 mM Tris and 400 mM sorbitol, pH 7.5 (adjusted by 0.1 N NaOH), osmolarity 656 mOsm. The standard pipette solution was: 100 mM KCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM MES (Morpholineethanesulfonic acid), 5mM Tris and 340 mM sorbitol, pH 5.5, 587 mOsm. Membrane polarity was consistent with the Bertl convention (Bertl et al. 1992). Electrical measurements were carried out using an EPC-7 Plus amplifier (List-Medical-Electronic Darmstadt, Germany); experimental data were stored using PatchMaster software (HEKA Electronic, Lambrecht, Germany). Signal was probed every 500 µs and filtered by a five-pole Bessel filter at cut-off frequency of 1 kHz. The Bessel filter was an integral part of the EPC-7 Plus amplifier. Transmembrane voltage was controlled in voltage clamp conditions. The voltage, stepped every 10 mV in the range (−50, 100 mV), was constant during several seconds. The micropipettes were formed from Kimax-51 glass capillaries. The tips of micropipettes were polished and coated with Sylgard. The resistance of micropipettes was in the range 1.8–4 MΩ, and seal resistance 8−26 GΩ. Concentration of Met_3PbCl in the bathing solution was 0.1 mM. The exchange of incubated media was carried out by continuous perfusion of the measuring chamber (1 mL/s) using an infusion pump. All experiments were carried out at room temperature (22 ± 1°C). For data analysis the ion current recordings were normalised by subtracting the zero offset of the current signal (the source of the offset was mainly leak currents and nonstability of the electrochemical potential).

Data analysis

In the experiment, the current through an ion channel was measured at membrane potentials from −50 to 100 mV at steps of 10 mV. The experiment was repeated 13 times with control bath solution and 9 times with bath solution containing 0.5 mM Met_3PbCl. The time series registered during the patch-clamp measurements were verified against the presence of more than one active channel; the histograms of registered signals were obtained, and cases with more than two maxima were rejected. After inspection of the data, analysis was carried out on 11 time series for the control bath solution and 5 time series for the solution with 0.1 mM Met_3PbCl. Each time series registered at a given membrane potential consisted of 15,990 data points. The statistical parameters of selected time series and their confidence intervals were obtained by bootstrap method (Davison and Hinkley 1997). The calculations were performed by using Octave 3.0.1 software. Finally, the time series for which the open state of the ion channel was clearly seen were chosen for further analysis; these were time series registered at membrane potential U of 70, 80, 90 and 100 mV. The chosen time series were filtered, and open- and closed-state currents were separated. The value of the threshold current was obtained by the algorithm proposed by Mercik et al. (1999). Then, the obtained statistical parameters of the open and closed states were analysed.

Results

Mathematical model

The statistical parameters (M, σ^2, S and K) in the case of superstatistics obtained as a superposition of normal and binomial distribution are given by Eqs. 1–5.

$$M = p_1 m_1 + p_2 m_2, \quad (1)$$

$$\sigma^2 = p_1(\sigma_1^2 + m_1^2) + p_2(\sigma_2^2 + m_2^2) - (p_1 m_1 + p_2 m_2)^2, \quad (2)$$

$$S = \frac{m_1^3 p_1[1 - 3p_1 - p_1^3 - p_1^2(p_2 - 3)]}{\sqrt{(p_1(m_1^2+\sigma_1^2) + p_2(m_2^2+\sigma_2^2) - (p_1 m_1 + p_2 m_2)^2)^3}} - \frac{3m_1^2 m_2 p_1 p_2[1 + p_1^2 + p_1(p_2 - 3)]}{\sqrt{(p_1(m_1^2+\sigma_1^2) + p_2(m_2^2+\sigma_2^2) - (p_1 m_1 + p_2 m_2)^2)^3}} - \frac{3m_1 p_1[m_2^2 p_2(1 + p_2(p_1 + 3) + p_2^2) + (p_1 + 1)\sigma_1^2 + p_2\sigma_2^2]}{\sqrt{(p_1(m_1^2+\sigma_1^2) + p_2(m_2^2+\sigma_2^2) - (p_1 m_1 + p_2 m_2)^2)^3}} - \frac{m_2 p_2[m_2^2(3p_2 + (p_1 - 3)p_2^2 + p_2^3 - 1) + 3(p_1\sigma_1^2 + (p_2 - 1)\sigma_2^2)]}{\sqrt{(p_1(m_1^2+\sigma_1^2) + p_2(m_2^2+\sigma_2^2) - (p_1 m_1 + p_2 m_2)^2)^3}}, \quad (3)$$

$$\begin{aligned} K = {} & \frac{m_1^4 p_1[1 - 7p_1 + 12p_1^2 + p_1^4 + p_1^3(p_2 - 7)]}{(p_1(m_1^2+\sigma_1^2) + p_2(m_2^2+\sigma_2^2) - (p_1 m_1 + p_2 m_2)^2)^2} \\ & + \frac{4m_1^3 m_2 p_1 p_2[6p_1 + p_1^3 + p_1^2(p_2 - 7) - 1]}{(p_1(m_1^2+\sigma_1^2) + p_2(m_2^2+\sigma_2^2) - (p_1 m_1 + p_2 m_2)^2)^2} \\ & + \frac{m_2^4 p_2[1 - 7p_2 + 12p_2^2 + (p_1 - 7)p_2^3 + p_2^4]+}{(p_1(m_1^2+\sigma_1^2) + p_2(m_2^2+\sigma_2^2) - (p_1 m_1 + p_2 m_2)^2)^2} \\ & + \frac{6m_2^2 p_2(2p_2 - 1)(p_1\sigma_1^2 + (p_2 - 1)\sigma_2^2)}{(p_1(m_1^2+\sigma_1^2) + p_2(m_2^2+\sigma_2^2) - (p_1 m_1 + p_2 m_2)^2)^2} \\ & + \frac{4m_1 m_2 p_1 p_2 m_2^2(6p_2 + (p_1 - 7)p_2^2 + p_2^3 - 1)}{(p_1(m_1^2+\sigma_1^2) + p_2(m_2^2+\sigma_2^2) - (p_1 m_1 + p_2 m_2)^2)^2} \\ & + \frac{4m_1 m_2 p_1 p_2[(6p_1 - 3)\sigma_1^2 + 3(2p_2 - 1)\sigma_2^2]}{(p_1(m_1^2+\sigma_1^2) + p_2(m_2^2+\sigma_2^2) - (p_1 m_1 + p_2 m_2)^2)^2} \\ & + \frac{6m_1^2 p_1[m_2^2 p_2(2p_2 + p_1^2 p_2 + p_1(2 - 7p_2 + p_2^2) - 1)]}{(p_1(m_1^2+\sigma_1^2) + p_2(m_2^2+\sigma_2^2) - (p_1 m_1 + p_2 m_2)^2)^2} \\ & + \frac{6m_1^2 p_1[(2p_1 - 1)((p_1 - 1)\sigma_1^2 + p_2\sigma_2^2)]}{(p_1(m_1^2+\sigma_1^2) + p_2(m_2^2+\sigma_2^2) - (p_1 m_1 + p_2 m_2)^2)^2} \\ & - \frac{3[p_1^2\sigma_1^4 + (p_2 - 1)p_2\sigma_2^4 - p_1(\sigma_1^4 - 2p_2\sigma_1^2\sigma_2^2)]}{(p_1(m_1^2+\sigma_1^2) + p_2(m_2^2+\sigma_2^2) - (p_1 m_1 + p_2 m_2)^2)^2}. \end{aligned} \quad (4)$$

It is worth stressing that Eqs. 1–5 are functions of the parameters defining the superstatistics i.e. p_1, m_1, σ_1, p_2, m_2 and σ_2. It is important to note that the mean value (Eq. 1) depends only on p_1, m_1, p_2 and m_2 and so does not depend on the variance of the compound signals, i.e. on σ_1 and σ_2. Therefore, standard analysis of ion current is not capable of registering changes in the variance of compound signals. Only investigation of higher statistical moments, such as variance, skewness and kurtosis, allows observation and detection of changes in the ion current distribution. Under the assumption that only two states are observed, the probabilities p_1 and p_2 have to satisfy Eq. 5.

$$p_1 + p_2 = 1. \quad (5)$$

Equations 1–5 are in fact a set of nonlinear equations in six variables. Therefore, in order to solve them, one needs an additional assumption, e.g. based on the investigated system properties. If such information is provided, Eqs. 1–5 can be solved directly by algebraic or numerical methods.

MC simulations illustrate the above considerations. The following three cases were considered:

I. The parameters p_1, p_2, m_1, σ_1 and σ_2 are fixed, and the value of m_2 is varied;
II. The parameters p_1, p_2, m_1, m_2 and σ_1 are constant, and σ_2 is changed;
III. The parameters p_1, m_1, m_2, σ_1 and σ_2 are constant, and p_2 is changed.

The algorithm of the MC simulations was as follows:

1. For the given set of parameters (p_1, m_1, σ_1), (p_2, m_2 and σ_2), a time series of $N = 10^6$ elements is generated;
2. Statistical parameters (M, σ, S and K) are calculated and stored;
3. Steps 1–2 are repeated 10^2 times and ensembles of statistical parameters are collected;
4. Estimators and statistical error of M, σ, S and K for the analysed superstatistics are calculated;
5. The parameters of the superstatistics are changed and steps 1–4 are repeated.

Simulation results (I)

The parameters of the superstatistics were: $p_1 = p_2 = 0.5$, $m_1 = 0$, $\sigma_1 = \sigma_2 = 1$ and $m_2 \in (0, 100)$ with step size of 1. The results (i.e. M, σ_2, S and K as functions of shift in mean values) are presented in Figs. 1 and 2. The mean value and standard deviation depend linearly on the difference between the expected values of compound signals (Fig. 1). Skewness does not depend on the value of m_2 and is close to zero (Fig. 2). The kurtosis is asymptotically decreasing from 0 to −2 as m_2 approaches 100. These findings are in agreement with Eqs. 1–5, where the mean value depends linearly on m_1 and m_2. Standard deviation is a linear function of m_2. Skewness is close to zero, and kurtosis decreases asymptotically from 0 to −2 as m_2 increases.

Simulation results (II)

At this stage, the role of standard deviation is analysed. The superstatistics parameters were chosen as follows: $p_1 = p_2 = 0.5$, $m_1 = 0$, $m_2 = 1$, $\sigma_1 = 1$ and $\sigma_2 \in (0, 100)$. The results, i.e. the estimators of M, σ, S and K as functions of σ_2, are presented in Figs. 3 and 4. The mean value (Fig. 3) does not depend on the difference in standard deviation. These findings are in agreement with Eq. 1, where M depends only on the expected values of the compound signals. The standard deviation of the total

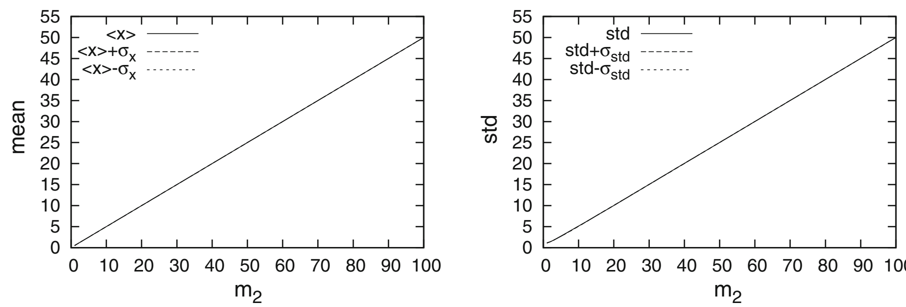

Fig. 1 Mean value (*left*) and standard deviation (*right*) as functions of m_2. Simulation assumptions were: $p_1 = p_2 = 0.5$, $m_1 = 0$ and $\sigma_1 = \sigma_2 = 1$. The length of the generated time series was $N = 10^6$. *Solid line*: mean value of compound signal; *dashed lines*: lower and upper bounds of the 70% confidence interval

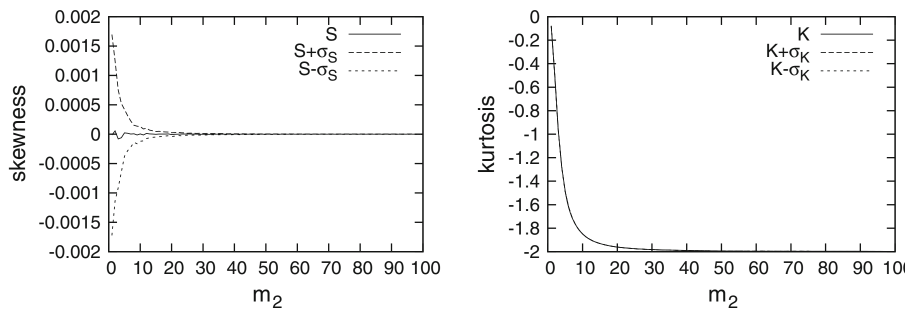

Fig. 2 Skewness (*left*) and kurtosis (*right*) as functions of m_2. Simulation assumptions were: $p_1 = p_2 = 0.5$, $m_1 = 0$ and $\sigma_1 = \sigma_2 = 1$. The length of the generated time series was $N = 10^6$. *Solid line*: mean value of compound signal, *dashed lines*: lower and upper bounds of the 70% confidence interval

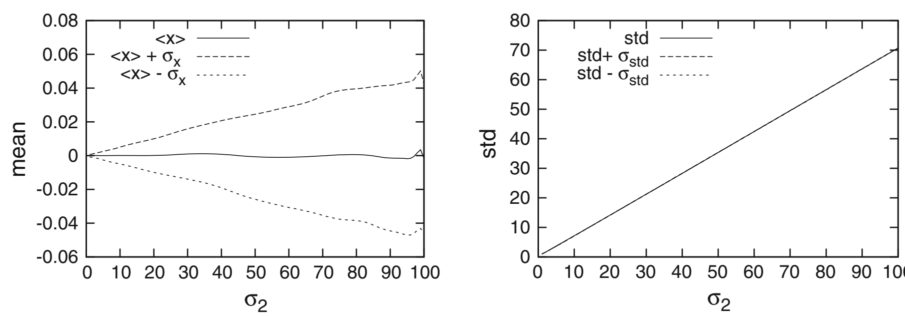

Fig. 3 Mean value (*left*) and standard deviation (*right*) as functions of σ_2. Simulation assumptions were: $p_1 = p_2 = 0.5$, $m_1 = 0$, $m_2 = 1$ and $\sigma_1 = 1$. The length of the generated time series was $N = 10^6$. *Dashed lines*: lower and upper bounds of the 70% confidence interval

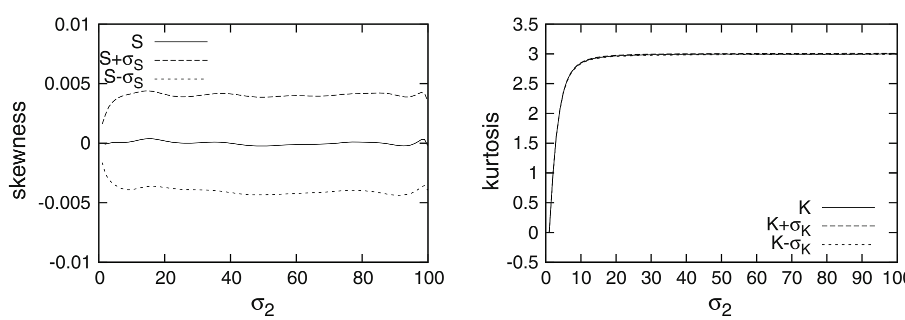

Fig. 4 The skewness (*left*) and kurtosis (*right*) as functions of σ_2. The simulation assumptions were: $p_1 = p_2 = 0.5$, $m_1 = 0$, $m_2 = 1$ and $\sigma_1 = 1$. The length of the generated time series was $N = 10^6$. *Dashed lines*: lower and upper bounds of the 70% confidence interval

signal as a function of the difference of standard deviation of compound signals is presented in Fig. 3. It is linearly increasing as σ_2 grows. The skewness (Fig. 4) is asymptotically decreasing as σ_2 grows. This is a result of domination of the signal with significantly larger variance, so the skewness asymptotically approaches the skewness of the normal distribution. In the case of kurtosis analysis, a similar situation is observed: the kurtosis approaches the kurtosis of the normal distribution (Fig. 4).

Simulation results (III)

In the final simulated case, the influence of probability shift on the statistical properties of the compound signal is investigated. The parameters of simulations were the following: in the closed state: $m_1 = 0$, $\sigma_1 = 1$; and three cases for the open state: (1) $m_2 = 10$, $\sigma_2 = 10$, (2) $m_2 = 10$, $\sigma_2 = 5$, (3) $m_2 = 5$, $\sigma_2 = 5$. The results are presented in Figs. 5 and 6. The mean value presented in

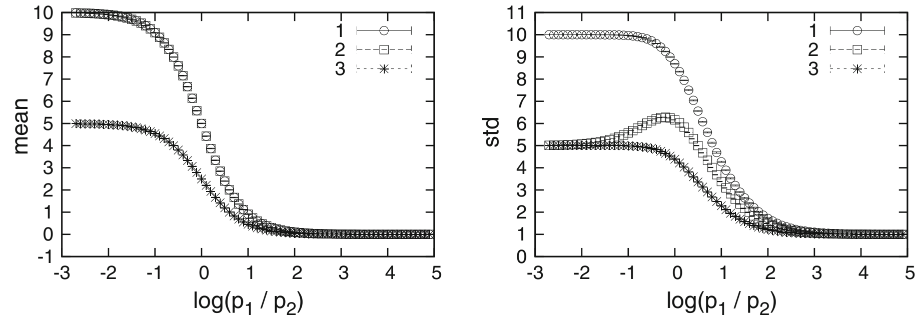

Fig. 5 Mean value (*left*) and standard deviation (*right*) of compound signal as functions of probability of open and closed channel state ratio on semilog scale. The simulation parameters were: time series length $N = 10^6$, $m_1 = 0$, $\sigma_1 = 1$; and, for line 1, $m_2 = 10$, $\sigma_2 = 10$; for line 2, $m_2 = 10$, $\sigma_2 = 5$; and for line 3, $m_2 = 5$, $\sigma_2 = 5$

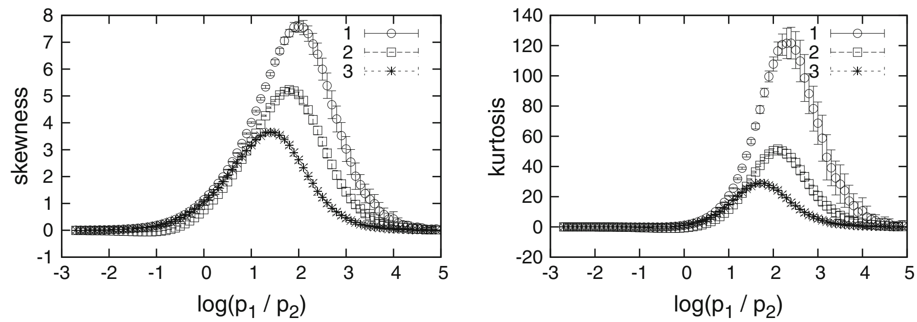

Fig. 6 Skewness (*left*) and kurtosis (*right*) of compound signal as functions of probabilities of open and closed channel state ratio on semilog scale. The simulation parameters were: time series length $N = 10^6$, $m_1 = 0$, $\sigma_1 = 1$; and, for line 1, $m_2 = 10$, $\sigma_2 = 10$; for line 2, $m_2 = 10$, $\sigma_2 = 5$; and for line 3, $m_2 = 5$, $\sigma_2 = 5$

Fig. 5 depends on the ratio of open- and closed-state probabilities. If one of the signals is dominant, the mean value asymptotically approaches the mean of that dominant signal. A similar situation is observed in the case of standard deviation analysis (Fig. 5), where the standard deviation of the compound signal asymptotically approaches the standard deviation of the dominant signal. It is worth noting that, for some combinations of parameters (e.g. $m_1 = 0$, $\sigma_1 = 1$, $m_2 = 10$, $\sigma_2 = 5$), the standard deviation is not a monotonic function of the p_1/p_2 ratio but exhibits a maximum at $p_1/p_2 = 1$. The skewness and kurtosis (Fig. 6) for the signal where one of the components is dominant asymptotically go to zero. The position and height of the maximum depend on the distribution parameters (m_1, σ_1, m_2, σ_2).

Experimental data

The examples of the registered ion current at membrane potential $U \in (50,\ 100\ \text{mV})$ and the corresponding ion current histograms are shown in Figs. 7 and 8, respectively. The standard *I*–*V* characteristic of the open-state ion current is presented in Fig. 9. Circles and asterisks denote the medians open-state current in the control solution and in the presence of Met_3PbCl, respectively. The lower and upper ends of the whiskers correspond to the first and third percentiles of the open-state current. The linear function was fitted to the measured data. Taking into account single channel recordings we have shown that the conductance of a single channel (calculated as a slope of *I*–*V* characteristics) is about 75 pS, which is in good agreement with other measurements (for 100 mM symmetrical K^+ concentration) (Coyaud et al. 1987; Schulz-Lessdorf and Hedrich 1995).

The statistical parameters (M, σ, S and K) as functions of the membrane potential are presented in Figs. 10–13. The mean value of the ion current is presented in Fig. 10. The measurement performed in the control bath solution is denoted by a dashed line, and in the presence of Met_3PbCl by a dotted line. The mean value of the ion current decreases almost linearly with increasing membrane potential.

In the next step the standard deviation (σ) of the ion current was calculated. The results are presented in Fig. 11. The standard deviation of the ion current at membrane potential in the range $U \in (-30,\ 50\ \text{mV})$ has the same value (within the confidence interval) for experiments in the control solution and in the presence of Met_3PbCl. Significant differences are observed for membrane potential greater than 50 mV, the regime in which the open state of the channel is active. Skewness and kurtosis are presented in Figs. 12 and 13. As in the case of mean value and standard deviation, addition of Met_3PbCl does not change the skewness or kurtosis of the ion current for membrane potential $U \in (-30,\ 50\ \text{mV})$, the range in which the closed state is dominant. The influence of Met_3PbCl is better seen in the kurtosis than in the skewness, e.g. for membrane potential $U = 60$ mV, the presence of Met_3PbCl in the solution increases the kurtosis by 60% while the skewness is increased by only 8%. The results obtained by analysis of the mean, standard deviation, skewness and kurtosis

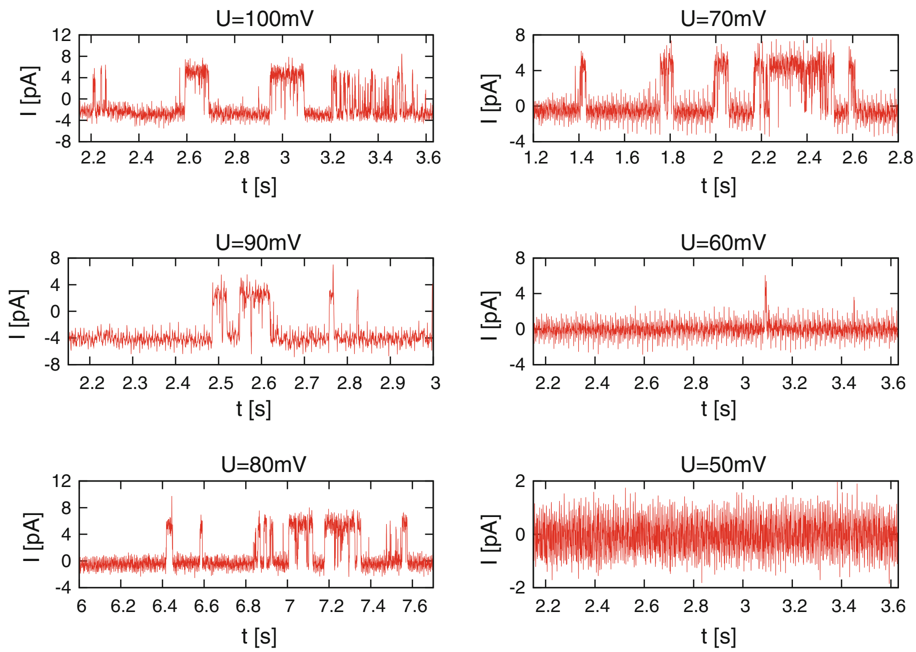

Fig. 7 Example ion current registered at membrane potential U = 100, 90, 80, 70, 60 and 50 mV in the control solution. The offset current has been subtracted

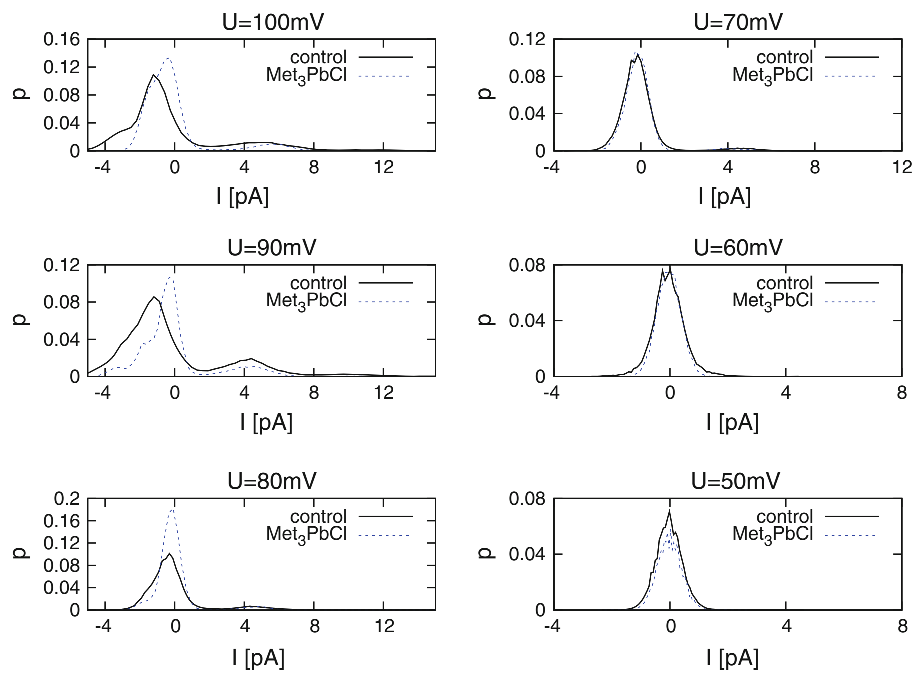

Fig. 8 Histogram of the ion current at membrane potential U = 100, 90, 80, 70, 60 and 50 mV. *Solid line* control solution, *dashed line* in the presence of 0.1 mM Met_3PbCl

suggest that Met_3PbCl influences the statistics of the open-state ion current distribution. Therefore, the ion current time series at membrane potential $U \in (70, 100$ mV) were chosen for further analysis. The ion current time series were transformed into dwell (open–close) time series by applying the algorithm defined in Mercik et al. (1999). Then the probabilities of the open and closed states were calculated. The results are presented in Table 1. The presence of organolead compounds led to significant lowering of the probability of the open state of the channel. For the external voltage considered, the ratio between the probability of the open state in the control solution and in the presence of Met_3PbCl is approximately 2.

Next, the results of the dwell time analysis and calculated statistical parameters of the ion current time series (M, σ, S and K) were used in order to solve the set of Eqs. 1–5 analytically. The parameters of the open- and closed-state current distributions are presented in Tables 2 and 3. The main changes induced by the presence of organolead are observed in the open-state characteristics.

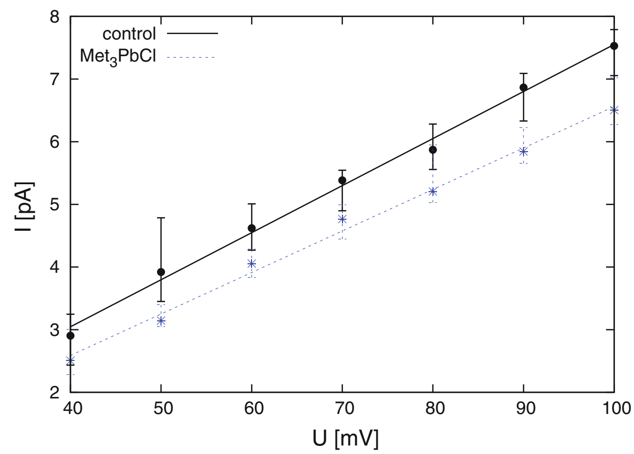

Fig. 9 *I–V* characteristics recorded in control solution (*black dots*) and with 0.1 mM Met_3PbCl (*blue asterisks*), and the linear function fitted to the data (*solid* and *dashed lines*, respectively)

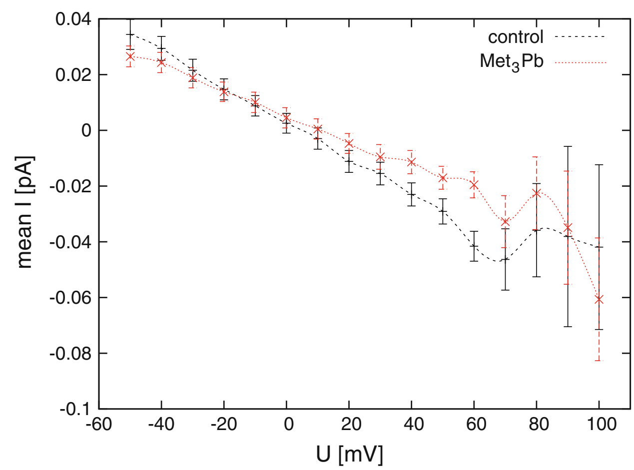

Fig. 10 Mean value of the ion current with *error bars* of the measured ion current as a function of membrane potential: *dashed line* in control solution, *dotted line* in the presence of 0.1 mM Met_3PbCl

The mean value of the ion current in the open state is increasing (by 0.2–0.7 pA, except for the case $U = 90$ mV), while the standard deviation of the open-state current is decreasing (by 0.4–1.5 pA, except for the lowest membrane potential considered). Also the closed-state current distribution is influenced by the organolead, however the changes are not as significant as in the case of the open-state channel parameters. For the closed state, the mean value of the current is slightly increasing (by 0.1–0.3 pA), while the standard deviation is decreasing.

The interesting observation is that the presence of Met_3PbCl changes not only the mean value of the current but also its standard deviation and hence the variance of the open-state ion current probability distribution function (Tables 1, 2 and 3).

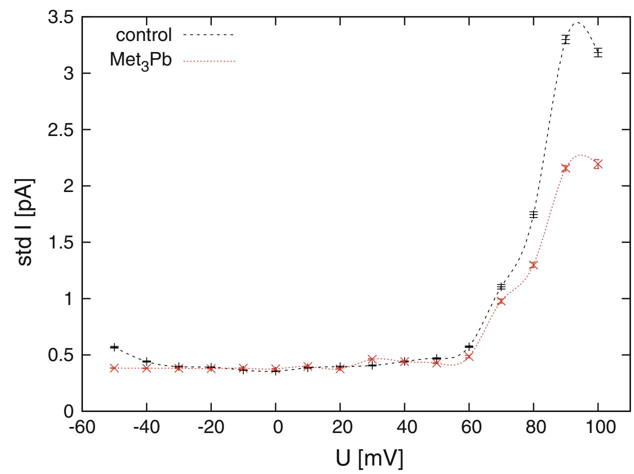

Fig. 11 Standard deviation of the ion current with *error bars* of the measured ion current as a function of membrane potential: *dashed line* in control solution, *dotted line* in the presence of 0.1 mM Met_3PbCl

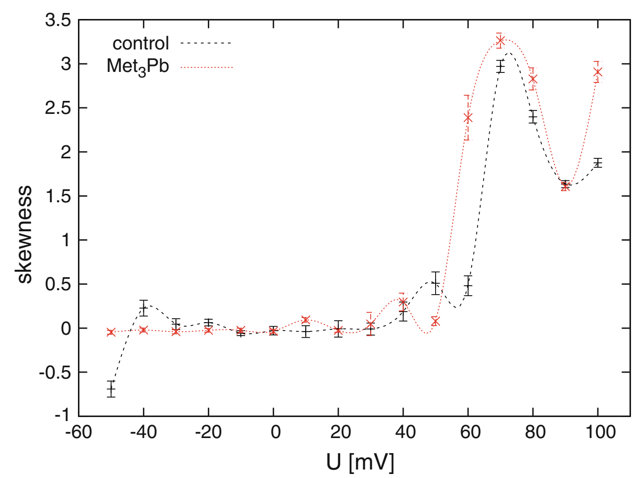

Fig. 12 Skewness of the ion current with the *error bars* of the measured ion current as a function of membrane potential. The *dashed line* in control solution, *dotted line* in the presence of 0.1 mM Met_3PbCl

Discussion

The activity of ion channels is crucial to cell biology. To a first approximation, it seems that the mean current of ion channels is crucial to the cell. Therefore, most works (Lee and Sung 2002; Chung and Kuyucak 2002; Nekouzadeh and Rudy 2007; Pandey et al. 2007; Vora et al. 2005; etc.) concentrate on the features of the mean value. However the ion current, due to its origin, is a statistical process; therefore all of its parameters should be analysed. Herein, higher statistical moments were investigated. It was shown by MC simulations that higher statistical moments are sensitive to parameters other than the mean value of the probability distribution function. It has been shown that the

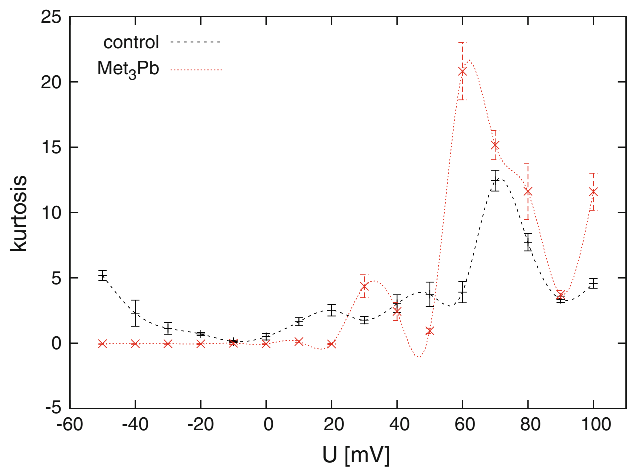

Fig. 13 Kurtosis of the ion current with the *error bars* of the measured ion current as a function of membrane potential. *Dashed line* in control solution; *dotted line* in the presence of 0.1 mM Met_3PbCl

Table 1 Probability of open state of the ion current in the case of control solution and in the presence of Met_3PbCl

U	70 (mV)	80 (mV)	90 (mV)	100 (mV)
Control	0.175	0.175	0.191	0.187
Met_3PbCl	0.097	0.097	0.100	0.095

Table 2 Mean value and standard deviation of ion current in open and closed states in the control solution

U (mV)	Closed state		Open state	
	M (pA)	σ (pA)	*M* (pA)	σ (pA)
100	−0.714	1.868	2.870	5.337
90	−0.691	2.170	2.733	5.253
80	−0.434	0.751	1.840	3.233
70	−0.329	0.088	1.290	2.188

Table 3 Mean value and standard deviation of ion current in open and closed states in the presence of Met_3PbCl

U (mV)	Closed state		Open state	
	M (pA)	σ (pA)	*M* (pA)	σ (pA)
100	−0.444	1.188	3.577	4.743
90	−0.330	1.654	2.625	3.750
80	−0.243	0.702	2.031	2.847
70	−0.206	0.436	1.581	2.287

mean value of the composed signal is sensitive only to the expected values of the component signals. Only higher statistical moments, i.e. the standard deviation, skewness or kurtosis, are able to reflect changes in the variance of compound processes. The theoretical investigations were verified by analysis of experimental data. The influence of Met_3PbCl on the ion current of *Beta vulgaris* vacuolar membrane was analysed. It was shown that presence of Met_3PbCl in the solution influences not only the mean value of the ion current but also higher statistical moments, especially the standard deviation and kurtosis. This influence was clearly seen at high values of membrane potential ($U > 60$ mV), at which the open state of the ion channel is active. This finding suggested that Met_3PbCl influences the open state of the ion channel. The calculated statistical features of the observed ion currents were substituted into Eqs. 1–5 and the equation solved. It was shown that the main changes were exhibited in the standard deviation of the open-state time series. It is clear that the observed changes are mainly restricted to the open-state ion current. This may suggest that Met_3PbCl modifies the ion channel, altering the PDF of the ion current. The question of correlation between the changes of ion current variance and membrane dysfunction remains open at this stage of research. However, due to the toxicity of organolead compounds, one can suspect that the observed changes in variance of the ion current distribution are a manifestation of Met_3PbCl toxicity. The present work shows that analysis of higher statistical moments of ion current can yield new information about ion channel properties. In this context, the interesting question arises of the kind of changes in the ion channel that can cause alteration of the variance of the probability distribution function. It also shows the need for development of the theory of ion channels towards a description in which all significant parameters of the probability distribution function are incorporated in the analysis.

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