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Importance of cell variability for calcium signaling in rat airway myocytes

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ABSTRACT

Calcium signaling controls several essential physiological functions in different cell types. Hence, it is not surprising that different aspects of Ca^{2+} dynamics are in the focus of in-depth and extensive investigations. Efforts concentrate on the development of proper theoretical models that would provide a unified description of Ca^{2+} signaling. Remarkably, experimentally recorded Ca^{2+} signals exhibit a rather large diversity, which can be observed irrespective of the cell type, measuring techniques, or the nature of the signal. Our goal in the present study therefore is to present a theoretical explanation for the variability observed in experiments, whereby we focus on caffeine-induced Ca^{2+} responses in isolated airway myocytes. By employing a stochastic model, we first test whether the observed variability can be attributed to intrinsic fluctuations that are a common feature of biochemical reactions that govern Ca^{2+} signalization. We find that stochastic effects, within ranges that correspond to actual conditions in the cell, are far too modest to explain the large diversity observed in experimental data. Foremost, we reveal that only cell variability in theoretical modeling can appropriately describe the observed diversity in single-cell responses.

1. Introduction

Mathematical modeling is a well-established method when studying complex biological systems. The method is useful and the predictive power of theoretical models is in many cases indispensable for catalyzing further experimental studies [1,2]. Modeling of Ca^{2+} signaling, as other cellular processes, consists in the mathematical description of the functional properties of the components of the cellular system, and the analysis of the predictions of the model about the behavior of the cell system. Validation of the model basically consists in the comparison of the predicted output of the model system with the experimental data. Though some scientific literature has been devoted to cellular diversity in several cell types [3–7], the experimental data that are typically considered are the mean values calculated from several recordings, with no or little interest in interindividual variability. Nevertheless, biological systems are under permanent influences of several internal and external protuberances and the variability in biological systems has to be taken into account. Interindividual variability is actually, as well as the central tendency usually estimated by the mean value, a physiological property, which can be quantified by the standard deviation. As a consequence, a realistic model should account not only for the mean tendency but also for the individual dispersion.

Additionally, one of the limitations of the theoretical modeling is the lack of precise experimental data for the parameters that describe the components of the system, so that they may be chosen in a given range. Hence, the biological relevance of the values chosen for the parameters should also be addressed. The problem is the fact that the correspondence between the model predictions and the experimental data may critically depend on the parameter values incorporated in the model. In that sense, in some cases, mathematical models are considered as “hypersensitive” to changes in model parameters. The general idea is that the cell, as a biological system, exhibits a relative robustness, i.e., its behavior is not deeply affected by small changes in biological parameters. If not, the biological system, which is always under permanent influences of several internal and external perturbances, would be unable to maintain its functional integrity. It is hence admitted that, to be realistic, the model should reflect this biological robustness and hence exhibits itself a relative robustness.

Usually, the robustness of the system is evaluated by the sensitivity analysis, which is a means to acquire insight about the importance of model parameters. Perhaps the most famous example is the metabolic control analysis, which represents in general a systematic method for analyzing the control of steady states. It quantifies the extent to which any parameter, but more notably all molecular processes, controls any steady-state variable within a metabolic pathway [8,9]. Methods, developed in the framework of metabolic control analysis have

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afterward been successfully applied to the control analysis of various phenomena in biological systems, including non-steady states and oscillations [10–15]. However, in the modeling of signaling processes, it is difficult to objectify a correspondence between the effect of such arbitrary changes and experimental biological measurements.

The cellular dynamics results from a huge body of biochemical reactions, which are all subjects to thermal noise, and belongs in reality to non-deterministic processes, which require special treatments [16–18]. In the last decade a lot of stochastic models have been developed for describing and studying different cellular processes. In the modeling of gene expression and circadian rhythms, for example, where the number of reacting particles in the cell is very low (only few hundred in some cases), the implementation of stochastic algorithms has been emphasized and realized by several authors [19–23]. Furthermore, recent experimental and theoretical investigations indicate that Ca^{2+} signaling is clearly subjected to stochastic dynamics as well [24–29]. Consequently, stochastic modeling of intracellular Ca^{2+} signaling pathways is gaining increasingly more attention [30–36]. Recent findings indicate that the stochastic nature of Ca^{2+} signaling arises from the stochastic behavior of ion channels in the membranes. Local random opening and closing of these channels introduces stochasticity into global Ca^{2+} responses [28,37,38]. Moreover, it has been observed that the Ca^{2+} release channels are spatially organized in clusters [39,40]. Such an organization can on one hand enhance the sensitivity of the Ca^{2+} signaling pathway in the presence of internal fluctuations by weak stimulation levels (coherence resonance) [31,41], and on the other hand it can explain the intrinsic irregularity of Ca^{2+} oscillations by supra-threshold agonist concentrations observed in experiments [27,42].

Nevertheless, it is difficult to surely identify and to characterize perturbances to which biological systems are submitted. However, we have an objective measurement of their impact, since it is reasonable to consider that the observed cell-to-cell variability is the consequence of these changes. Hence, the cell-to-cell variability is a critical biological parameter that should be taken into account in the modeling of the functional properties of the components of the system and in the sensitivity analysis of the cellular system.

The aim of this study is to elucidate a rather large cell-to-cell variability in calcium responses as observed experimentally. First, we determine whether cell-to-cell variability can be explained by non-deterministic molecular processes. For that purpose, we have developed a stochastic version of a model of Ca^{2+} signaling we have already published [43], and we compare the individual cell response pattern and interindividual cell response dispersion predicted by the stochastic model with original Ca^{2+} response traces and observed cell-to-cell variability.

Second, we propose a “reverse” sensitivity analysis. In contrast to the well-known sensitivity analysis, where the model's behavior is studied in the context of variations in model parameters, we scrutinize the inversed problem. In particular, since the scattering of the output calcium signals is known from experiments, we determinate the range within a given parameter should vary, so that the subsequent variations in the predicted output correspond to the observed cell-to-cell variability of experimental recordings. In addition, we propose an extended version of such reverse sensitivity analysis, where all parameters are varied simultaneously and thus the overall dispersion of parameters is evaluated. In this way the actual variability of model parameters is estimated, at which theoretical predictions concur with experimental observations.

This study is based on the calcium response to caffeine stimulation in airway myocytes, a biological cellular system for which we have both a theoretical model that includes the major components involved in Ca^{2+} homeodynamics, and a large set of experimental measurements. The conclusions are extended to the general question of cellular Ca^{2+} signaling, on the basis of bibliographical data on the Ca^{2+} response to various agonists in different cell types.

2. Materials and methods

2.1. Experimental

2.1.1. Cell preparation and fluorescence measurement of $[Ca^{2+}]_i$

Measurements of the concentration of calcium ions ($[Ca^{2+}]_i$) in freshly isolated cells were performed as previously described [43]. Here summarizing briefly, rat tracheae were obtained from male Wistar rats 10–15 weeks old, weighing 300–400 g. Animals were sacrificed by CO_2 exposure, heart and lungs were removed en bloc, and the trachea was rapidly dissected out. The muscular strip located on the dorsal face of the trachea was further dissected, the epithelium-free muscular strip was cut into several pieces and the tissue was then incubated overnight (14 h) in low-Ca^{2+} (200 μM) physiological saline solution (PSS; composition given below) containing 0.5 mg ml^{-1} collagenase, 0.35 mg ml^{-1} pronase, 0.03 mg ml^{-1} elastase and 3 mg ml^{-1} bovine serum albumin at 4 °C. After this time, the muscle pieces were triturated in a fresh enzyme-free solution with a fire polished Pasteur pipette to release cells. Cells were stored for 1 to 3 h to attach on glass coverslips at 4 °C in PSS containing 0.8 mM Ca^{2+} and used on the same day. In control experiments, immunocytochemistry was performed using monoclonal mouse anti-smooth muscle α-actin antibodies and FITC-conjugated anti-mouse IgG antibodies to verify that the isolated cells obtained by dissociation were smooth muscle cells (data not shown). Cells were loaded with indo-1 by incubation in PSS containing 1 μM indo-1 acetoxymethylester for 25 min at room temperature and then washed in PSS for 25 min. Coverslips were then mounted in a perfusion chamber and continuously superfused at room temperature. A single cell was illuminated at 360 ± 10 nm. Emitted light from that cell was counted simultaneously at 405 nm and 480 nm by two photomultipliers (P100, Nikon). $[Ca^{2+}]_i$ was estimated from the 405/480 ratio using a calibration for indo-1 determined within the cells. Caffeine (5 mM) was applied to the tested cell by a pressure ejection from a glass pipette located close to the cell. No changes in $[Ca^{2+}]_i$ were observed during test ejections of PSS (data not shown). Each record of $[Ca^{2+}]_i$ response to caffeine was obtained from a different cell. Cytosolic calcium concentrations ($[Ca^{2+}]_i$) are expressed as mean ± SD, for the number of cells indicated in the text.

2.1.2. Solutions, chemicals and drugs

PSS contained (in mM): 130 NaCl, 5.6 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 11 glucose, 10 Hepes, pH 7.4 with NaOH. Collagenase (type CLS1) was from Worthington Biochemical Corp. (Freehold, NJ, USA). Bovine serum albumin, elastase, pronase and caffeine (CAF) were purchased from Sigma (Saint Quentin Fallavier, France). Indo-1 AM was from Calbiochem (France Biochem, Meudon, France). Indo-1 AM was dissolved in dimethyl sulphoxide in which the maximal concentration used in our experiments was <0.1% and had no effect on the resting value of the $[Ca^{2+}]_i$ nor on the variation of the $[Ca^{2+}]_i$ induced by caffeine (data not shown).

2.1.3. Bibliographical data

Data obtained from the literature were taken from publications selected via Pubmed database. To be selected, the articles should give original data on intracellular Ca^{2+} concentration at rest and upon stimulation that triggers $[Ca^{2+}]_i$ increase. Data should be presented as mean ± standard deviation (SD) and/or standard error of the mean (SE), with the number of measurements. Usually, SD is not given in the publications. In this case, it has been recalculated from SE and the sample size (n) using the formula $SD = SE \times \sqrt{n}$. SD was calculated as the absolute value, and as percent of the mean value.