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ORIGINAL PAPER

Equality of average and steady-state levels in some nonlinear models of biological oscillations

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Abstract Nonlinear oscillatory systems, playing a major role in biology, do not exhibit harmonic oscillations. Therefore, one might assume that the average value of any of their oscillating variables is unequal to the steady-state value. For a number of mathematical models of calcium oscillations (e.g. the Somogyi–Stucki model and several models developed by Goldbeter and co-workers), the average value of the cytosolic calcium concentration (not, however, of the concentration in the intracellular store) does equal its value at the corresponding unstable steady state at the same parameter values. The average value for parameter values in the unstable region is even equal to the level at the stable steady state for other parameter values, which allow stability. This holds for all parameters except those involved in the net flux across the cell membrane. We compare these properties with a similar property of the Higgins–Selkov model of glycolytic oscillations and two-dimensional Lotka–Volterra equations. Here, we show that this equality property is critically dependent on the following conditions: There must exist a net flux across the model boundaries that is linearly dependent on the concentration variable for which the equality property holds plus an additive constant, while being independent of all others. A number of models satisfy these conditions or can be transformed such that they do so. We discuss our results in view of the question which advantages oscillations may have in biology. For example, the implications of the findings for the decoding of calcium oscillations are outlined. Moreover, we elucidate interrelations with metabolic control analysis.

Keywords Calcium oscillations · Chaotic dynamics · Glycolytic oscillations · Lotka–Volterra equations · Metabolic control analysis

This paper is dedicated to the memory of the late Reinhart Heinrich, who was the academic teacher of S.S. and, to a great extent, also of M.M.

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Introduction

Nonlinear oscillations play an important role in many biological processes (cf. Goldbeter 1996; Heinrich and Schuster 1996; Hofbauer and Sigmund 1998). For example, oscillations in the concentration of intracellular calcium ions are of relevance in regulating several cellular processes. Since the 1980s, Ca^{2+} oscillations are the subject of intense experimental (e.g. Woods et al. 1986; Berridge et al. 1998; Dupont et al. 2000) and theoretical studies (cf. Goldbeter 1996; Schuster et al. 2002; Falcke 2004). In several types of non-excitable cells such as hepatocytes, oocytes and pancreatic acinar cells, these oscillations occur due to an exchange of Ca^{2+} between the cytosol and intracellular stores such as the endoplasmic reticulum (ER). Ca^{2+} oscillations are often triggered by the binding of an

external agonist, such as some hormones or ATP, resulting in the formation of inositol 1,4,5-trisphosphate (IP_3). IP_3 binds to Ca^{2+} channels in the ER, promoting the release of Ca^{2+} from this internal store. This process is furthermore amplified by a positive feedback of cytosolic Ca^{2+} on channel opening, named calcium-induced calcium release (CICR) (cf. Goldbeter 1996; Kummer et al. 2000). As this and some other processes involved obey nonlinear kinetics, Ca^{2+} oscillations are a nonlinear phenomenon. From the theory of differential equations, it follows that nonlinear kinetics is a necessary prerequisite for obtaining stable limit-cycle oscillations, attracting neighbouring trajectories.

In linear systems, by contrast, oscillations with constant amplitude are marginally stable rather than representing a limit cycle. That means that a small deviation in the initial values leads to a different amplitude. For constant-amplitude oscillations in linear systems, each variable averaged over one or several oscillation periods equals the value at the steady state (which is then marginally stable as well) because the oscillations are simple sinus functions. Nonlinear oscillations can show this property as well, if they involve special symmetries, like in a pendulum. In nonlinear systems without symmetry, however, this equality often does not hold (cf. Ritter and Douglas 1970).

Somewhat surprisingly, Lotka–Volterra systems involving linear and bilinear terms do fulfill this equality property (cf. Hofbauer and Sigmund 1998; Walter 2000; Stucki and Urbanczik 2005). The question arises for which type of biological nonlinear oscillations the equality between average values and steady-state values is fulfilled. We have analyzed this and found examples both of models showing this equality property and of models not showing it. By discussing with several peers (including Reinhart Heinrich), we have realized that some had been aware of this. However, as neither the structural reasons for this property, nor case studies for calcium and glycolytic oscillations seem to have been published before we feel that time is more than ripe to do so. Here, we deal with this issue in a relatively general way and show interrelations to approaches such as metabolic control analysis.

For discussing the biological relevance of the equality property, the following points are of interest: The property does not hold in a model of oscillatory peroxidase activity, where the average level of reactive oxygen species (ROS) is much lower than the steady-state level at the same parameter values (Hauser et al. 2001; Olsen et al. 2003). In that case, the steady state is stable because the system shows hard excitation. ROS are used in some cell types for signalling and have a harmful effect in oxidizing several cell compounds. ROS are also used for killing bacteria by leukocytes, so that a fine-tuned concentration is necessary. Oscillatory dynamics offers the possibility of employing harmful substances as second messengers by maintaining them at very low average concentrations (Hauser et al. 2001). Similarly, Ca^{2+} is harmful to the cell at higher concentrations because Ca^{2+} salts then precipitate. Thus, signal transmission via the decoding of Ca^{2+} oscillations is favourable to occur at a low average concentration of Ca^{2+}. It is now commonly accepted that the conversion of the oscillatory signal into a nearly stationary output in non-excitable cells works by Ca^{2+}-dependent phosphorylation of proteins. The theoretical prediction of this mechanism by Goldbeter et al. (1990) and Dupont and Goldbeter (1992, 1998) was confirmed experimentally by De Koninck and Schulman (1998). A crucial feature in this process is how the protein activity depends on calcium. If this dependence was linear, then the equality between average and steady-state values would imply that for the decoding, it would not matter if the signal is transmitted by oscillations or by an adjustable stationary level of calcium. We will come back to this point in the "Discussion" section.

In "Methods", the examined mathematical models of Ca^{2+} oscillations are presented. In "The equality property", the equality property is tackled analytically and numerically. In the respective sub-sections, a comparison is made with the Higgins–Selkov model of glycolytic oscillations (Higgins 1964; Selkov 1968) and with Lotka–Volterra models, and interrelations to metabolic control analysis are outlined. "Discussion" is devoted to discussing the results and giving an outlook on further studies.

Methods

Let Z denote the concentration of that substance, Z, for which we want to examine whether equality between average and steady-state concentrations holds. In the case of Ca^{2+} oscillations, free cytosolic Ca^{2+} will usually be that variable. Let us consider the following features:

(a) The system is open in the sense that there is an exchange of Z across the cell membrane (or, in general, across the model boundaries).
(b) The net Z flux across the cell membrane is a linear function of Z with a positive additive constant (positive with respect to the influx).
(c) The net flux mentioned in condition (b) is independent of all other concentration variables in the model (e.g. Ca^{2+} in the intracellular stores and Ca^{2+} bound to proteins).

Condition (a) implies that there is no linear conservation relation with non-negative coefficients (e.g. no conserved sum) for the various Ca^{2+} species in the model. It is fulfilled for many models of Ca^{2+} oscillations in non-excitable cells presented in the literature, e.g. Somogyi and Stucki (1991), Goldbeter et al. (1990), Dupont and

Goldbeter (1993), and Borghans et al. (1997) as well as for the Higgins–Selkov oscillator (see Sect. 3.2). It is not for the models proposed by Marhl et al. (1997, 1998a, 1998b, 2000) and Fall and Keizer (2001), which do involve a conserved sum. The latter models give rise to oscillations as well. With respect to the flow of energy, they are open since ATP is consumed.

Condition (c) is fulfilled in most models of Ca^{2+} oscillations. It appears to be trivial that Ca^{2+} in intracellular stores can hardly affect the fluxes in question because the membrane surrounding the stores separates it from these processes and Ca^{2+} bound to proteins cannot normally cross the cell membrane due to the size of proteins. However, it has been observed experimentally that depletion of intracellular Ca^{2+} stores can cause the activation of the so-called capacitative Ca^{2+} entry, occurring through store-operated Ca^{2+} channels (Berridge 1995; Parekh and Putney 2005). It has long remained elusive how this activation works. Recently, Roos et al. (2005) and Liou et al. (2005) (see also Putney 2005; Draber et al. 2005) have found a protein, STIM1, present both in the plasma membrane and ER membrane and possibly transmitting that signal within homoaggregates. Alternatively, store-operated Ca^{2+} entry mediated by a diffusible signal, produced by depleted Ca^{2+} stores, has been suggested (Randriamampita et al. 1993; Thomas and Hanley 1995; Bolotina and Csutora 2005). Store-operated Ca^{2+} entry has also been analysed by mathematical models (Li et al. 1997; Wiesner et al. 1996; Kowalewski et al. 2006). In the present paper, however, we focus on models fulfilling the above three conditions and, in addition, analyse some models including conservation relations and examine nonlinear efflux functions.

Feature (b) appears to be a critical condition. It needs not always be fulfilled because also Michaelis–Menten, Hill or other nonlinear rate laws are possible, and are indeed used in some models (Höfer 1999; Kummer et al. 2000). A justification of this feature may arise from the low concentration of cytosolic Ca^{2+}, the peak height of which is limited for several reasons by about 1 μM (cf. Goldbeter 1996). When the substrate concentration is much lower than the half-saturation constant, the Michaelis–Menten kinetics can be simplified to a linear kinetics and so can the Hill kinetics when the substrate concentration is comparable with the half-saturation constant.

Models that do fulfil all of the three conditions are, for example, the minimalist model proposed by Somogyi and Stucki (1991) and the model presented by Borghans et al. (1997), which was analysed further by Houart et al. (1999) and Rozi and Jia (2003). While the former model is two-dimensional and gives rise to regular oscillations, the latter model is three-dimensional and can give rise to regular as well as irregular (complex) oscillations depending on parameter values.

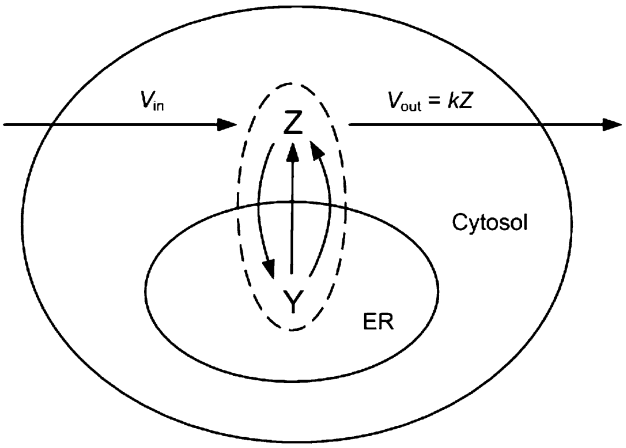

Fig. 1 Scheme of the processes described by the class of Ca^{2+} oscillation models fulfilling conditions (a)–(c). The ellipses indicate membranes. Abbreviations: Z, cytosolic calcium; Y, calcium in the ER or other intracellular store, V_{in} rate of Ca^{2+} influx into the cell, V_{out} rate of Ca^{2+} efflux out of the cell

The Somogyi–Stucki model is given by the following equations (see also Fig. 1):

$$\frac{dZ}{dt} = V_{in} - V_2 + V_3 + k_f Y - kZ \tag{1}$$

$$\frac{dY}{dt} = V_2 - V_3 - k_f Y \tag{2}$$

with V_{in} = const., and counting both concentrations in terms of the cytosol volume.

Cytosolic Ca^{2+} changes due to the influx (V_{in}) from, and efflux (kZ) to, the extracellular medium. Furthermore, Ca^{2+} is pumped into (V_2) and released from (V_3) the intracellular store (here the ER), besides a passive leak efflux ($k_f Y$). Analogously to that leak and the efflux kZ, also the pumping rate V_2 into the ER is assumed to be linear, $k_2 Z$. In contrast, the CICR from the ER is described by a nonlinear, Hill-like equation

$$V_3 = \frac{k_3 Y Z^4}{K^4 + Z^4}. \tag{3}$$

The stimulation level of the cell is quantified by the rate constant k_3, which is linked to the level of IP_3, influencing the channel activity. In a certain parameter range, this model gives rise to spike-like oscillations (Somogyi and Stucki 1991; for mathematical analyses, see also Heinrich and Schuster 1996; Schuster and Marhl 2001).

An additional agonist-dependent influx of Ca^{2+} into the cell is considered by the "one-pool model" (Dupont and Goldbeter 1993), given by Eq. (4):

$$V_{in} = V_0 + V_1 \beta. \tag{4}$$

The influx V_{in} consists of V_1, denoting the maximum rate of stimulus-induced Ca^{2+} influx, and a constant V_0. The parameter β represents the stimulation level of the cell by

an agonist and varies between 0 and 1. This parameter β either describes the dependence of the input into the cell (Dupont and Goldbeter 1993) or of the efflux from a special internal store ("two-pool model" proposed by Goldbeter et al. 1990) on the stimulation.

Similar as in the Somogyi–Stucki model, the IP_3 concentration is considered constant and therefore the agonist influence is expressed indirectly, in V_3 or V_{in}, by the degree of saturation of the IP_3 receptor, β. The models of Goldbeter et al. (1990) and Dupont and Goldbeter (1993) are based on the general governing Eqs. (1) and (2) for the entire system, with the detailed kinetics for V_2 and V_3 involving more nonlinear terms (for the equations and a detailed description, see the cited papers).

Borghans et al. (1997) proposed a more complicated model, also based on the CICR mechanism. It differs from the model proposed by Dupont and Goldbeter (1993) in that the IP_3 concentration is considered as a variable, A, and a third differential equation is included for that variable, describing the Ca^{2+}-stimulated degradation of IP_3 by an IP_3-3-kinase. The stimulation level of the cell is included as a rate constant in the IP_3 dynamics. The release of Ca^{2+} from the internal stores into the cytosol (V_3) is activated by cytosolic Ca^{2+} and IP_3 (for the equations and a detailed description, see Borghans et al. 1997). The dynamic behaviour of the model of Borghans et al. (1997) has been investigated in more detail by Houart et al. (1999). They found not only simple periodic spiking, but also complex Ca^{2+} oscillation patterns such as bursting, chaos and quasi-periodicity.

In our analysis, the differential equations were solved by using the software MADONNA (University of Berkeley, CA) with the Rosenbrock (stiff) integration method (Figs. 2, 4 and 7). Bifurcation diagrams for chaotic system states (Figs. 3 and 5) were obtained by integrating the differential equations via a fourth order Runge–Kutta procedure by a program written by one of the authors (M.P.).

Results

The equality property

We now analyse the models reviewed in Sect. 2. Summing up Eqs. (1) and (2) gives:

$$\frac{d(Y+Z)}{dt} = V_{in} - kZ. \tag{5}$$

So, cytosolic Ca^{2+} and internal store Ca^{2+} can be regarded together as virtually one pool, which is replenished by the influx V_{in} and depleted by the efflux kZ, as diagrammed in Fig. 1. Due to Eq. (5), the concentration of cytosolic Ca^{2+} at the (possibly unstable) steady state, at which $dZ/dt = dY/dt = 0$, reads:

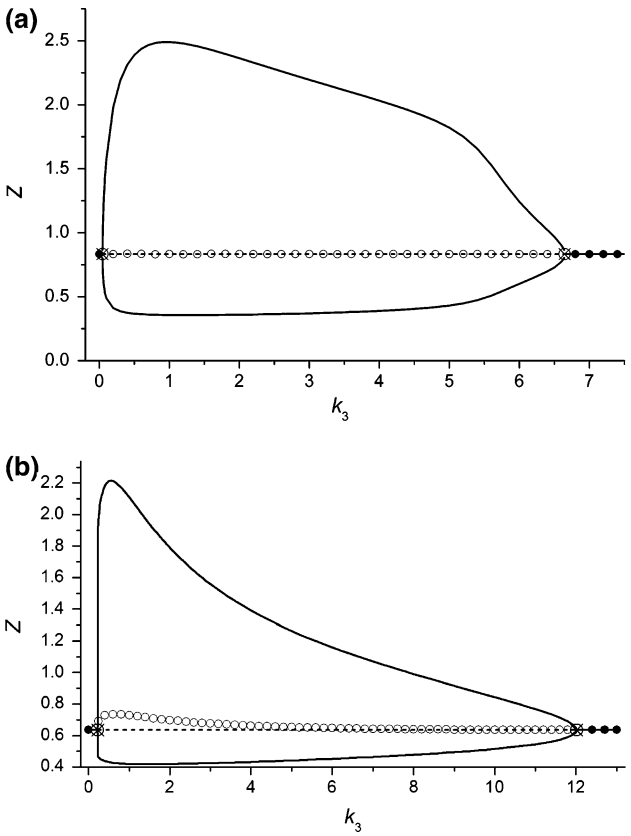

Fig. 2 Comparison of steady-state and average levels of cytosolic Ca^{2+} for the Somogyi–Stucki model by numerical calculations. **a** Original model. **b** Variant in which the efflux is a Hill function, $V_{max} \times Z^3/(K_m^3 + Z^3)$, of the cytosolic Ca^{2+} concentration. *Filled (empty) circles* average values in the steady-state (oscillatory) regime; *solid (dashed) horizontal line* stable (unstable) steady state; *curved lines* envelope of oscillations. Parameter values: $V_{in} = 1$, $k = 1.2$, $k_2 = 2$, $K = 1$, $k_f = 0.01$, $V_{max} = 3$, $K_m = 0.8$

$$Z_{ss} = \frac{V_{in}}{k}. \tag{6}$$

Now we calculate the average cytosolic Ca^{2+} concentration of the oscillating signal. For periodic oscillations, integration of Eq. (5) over one period T gives:

$$\int_0^T \left(\frac{d(Y+Z)}{dt}\right) dt = \int_0^T V_{in}\, dt - \int_0^T kZ\, dt = 0, \tag{7}$$

as $Z(T) = Z(0)$ and $Y(T) = Y(0)$. Thus, Eq. (7) simplifies to:

$$\int_0^T Z dt = \int_0^T \frac{V_{in}}{k} dt = \frac{V_{in}}{k} T. \tag{8}$$

Dividing the integral of Z over one period T by that period gives the average concentration $\langle Z \rangle$ of the oscillating Ca^{2+}. Due to Eq. (6), this is equal to its steady-state concentration:

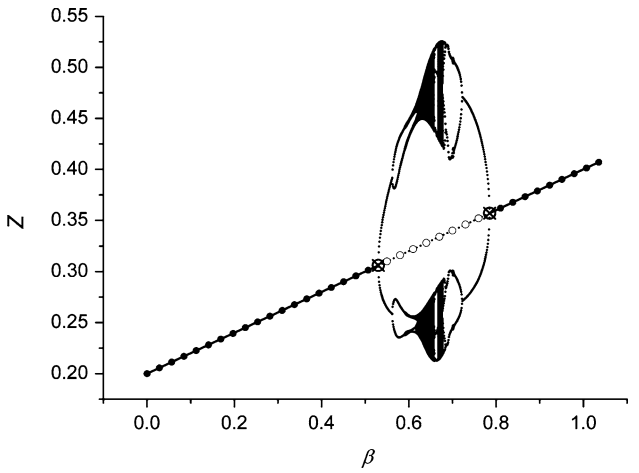

Fig. 3 Illustration of the equality property for the Borghans et al. (1997) model with parameter values from Houart et al. (1999) by numerical calculations. *Filled (empty) circles* average values in the steady-state (oscillatory) regime; *solid (dashed) straight line* stable (unstable) steady state; *curved lines* local maxima and minima of oscillations. Parameter values are the same as in Houart et al. (1999), Table 1, set "Chaos". The averages were computed over $t = 10^5$ min. Convergence was observed from 10^4 min on

$$\langle Z \rangle = Z_{ss}. \tag{9}$$

This completes the proof of the equality property under study. It can be seen that the positive additive constant mentioned in condition (b) (here denoted V_{in}) is necessary because otherwise, both the steady-state and average concentrations would be zero or even negative so that limit-cycle oscillations would be impossible. Interestingly, the average value in the oscillatory regime is not only equal to the value at the unstable steady state for the same parameter values, but also equal to the steady-state value for (other) parameter values that allow the steady state to be stable. This is because the steady state concentration is independent of k_3 (Fig. 2a). In fact, this is the case for all parameters except those entering the equation for the steady-state value (Eq. 6), that is, except V_{in} and k (see also Fig. 3).

For Ca^{2+} in the ER, the equality property does not, however, hold. The above proof of the equality property does not work for that concentration. Numerical simulations (not shown) indicate that the average Ca^{2+} concentration in the endoplasmic reticulum is higher than the steady-state value.

For models having the equality property (9) and in which the input rate is a linear function of a stimulus parameter, β (cf. Eq. (4)), as in the models of Goldbeter et al. (1990), Dupont and Goldbeter (1993), and Borghans et al. (1997), a linear relation between β and <Z> follows directly from Eqs. (6) and (9):

$$\langle Z \rangle = \frac{1}{k}(V_1\beta + V_0). \tag{10}$$

This linear relationship can be seen by comparing Fig. 5a, b in Rozi and Jia (2003).

To illustrate the equality property (Eq. 9) numerically, the average level, $\langle Z \rangle$ was calculated for the Somogyi–Stucki model (by integrating Eqs. 1–3) and the Borghans model with parameter values from Houart et al. (1999) (Figs. 2a and 3, respectively). To investigate whether the equality property is dependent on the linearity of the net flux, we now analyse two variants of the Somogyi–Stucki model in which a nonlinear term instead of the linear Ca^{2+} efflux out of the cell (kZ in Eq. 1) is taken. When a quadratic efflux term of the form kZ^2 is assumed, an equation analogous to Eq. (5) results in a steady state concentration of

$$Z_{ss} = \sqrt{\frac{V_{in}}{k}}, \tag{11}$$

while averaging leads to

$$\langle Z^2 \rangle = \frac{V_{in}}{k}. \tag{12}$$

This means that the average of the squared concentration, $\langle Z^2 \rangle$ is equal to the square of the steady-state concentration in the modified model. For oscillatory regimes, this equality does not entail the equality property (9). This is supported by numerical results (not shown), indicating that the average value is lower than the steady-state value.

As experimental data indicate a Hill kinetics for the Ca^{2+} extrusion (Camello et al. 1996; Carafoli 1991), we now choose a Hill kinetics term:

$$V_{out} = \frac{V_{max}Z^3}{K_M^3 + Z^3} \tag{13}$$

A Hill coefficient of 3 was assumed referring to the results of Camello et al. (1996). The steady state value of cytosolic Ca^{2+} is then obtained as

$$Z_{ss} = \sqrt[3]{\frac{V_{in}}{V_{max} - V_{in}}K_M} \tag{14}$$

while calculating the average results in

$$\frac{V_{in}}{V_{max}} = \left\langle \frac{Z^3}{K_M^3 + Z^3} \right\rangle \tag{15}$$

As the term in brackets is a nonlinear function of Z and the average of a nonlinear function is not generally equal to the function of the average, the equality property does not generally hold. Numerical calculations indicate that the average Ca^{2+} concentration depends on the position of the K_m value between base-line and maximal peak height of the oscillation and the shape of the peaks (sharp or sinusoidal). Therefore, in the case of Hill kinetics, the average Ca^{2+} concentration can be higher or lower than the steady-state value. These qualitative differences are due to the

effect of the convex and concave part of the Hill function. The parameter values in Fig. 2b were chosen such that the experimentally observations were qualitatively mimicked. The oscillation shows a spike-like pattern in the predominant part of the parameter range, which resembles experimentally obtained peak shapes (e.g. Somogyi and Stucki 1991). For most k_3 values in Fig. 2b, the base-line of the oscillations is closer to the K_m value than the maximal peak height. With the diagrammed parameter values, the average cytosolic Ca^{2+} concentration is slightly higher than the steady state value in some parameter range and nearly equal to it in another.

In Fig. 3, the linear dependency between Z_{ss} and $\langle Z \rangle$ on the agonist concentration β can be clearly seen (see Eqs. 4, 6 and 10). In the above mentioned models, Z_{ss} and $\langle Z \rangle$ are linearly dependent on V_{in} and its components, that is, the "leak influx" V_0 and agonist concentration β. An increase of the average value with increasing agonist concentration (Woods 1986) or external Ca^{2+} concentration (Somogyi and Stucki 1991) has indeed been found in experiments.

Our above proof of Eq. (9) applies to every type of regular oscillation in the considered class of models, including folded limit cycles and periodic bursting. Care has to be taken when the oscillations are irregular (quasi-periodic or chaotic) because a period, T, cannot then be defined. Nevertheless, numerical calculations over a large time span show that the equality property also holds in that case (Fig. 3). Based on the ergodicity (recurrent behaviour) of the trajectory in chaotic attractors and the boundedness of the attractors (cf. Thompson and Stewart 2002), a theoretical explanation of that observation can be given. If we consider initial values within the attractor, the Y, Z and A values return to an arbitrarily close proximity of these values: $|Y(\hat{T}) - Y(0)| < \varepsilon$ and analogously for Z and A (Fig. 4). Importantly, the (approximate) recurrence time, $\hat{T}$, is not generally the same for repeated returns to a close proximity of the initial values. Now, one can choose a very large time span, T, that is the sum of sufficiently many "recurrence times". In Fig. 4, the trajectory during two of such consecutive "recurrence times" is shown for illustration. In the numerical computation of the average, we used a time span sufficiently long that convergence could be observed. Thus, Eqs. (7, 8) can be applied with an arbitrarily small error and the equality property practically holds also in the case of irregular oscillations. Clearly, this is not a strict mathematical proof, which we leave for future work.

We now examine the model proposed by Marhl et al. (2000). We mentioned above that it does not satisfy condition (a) because it involves a conservation relation for Ca^{2+}. We here analyse both the original model and a slightly modified variant that does satisfy condition (a).

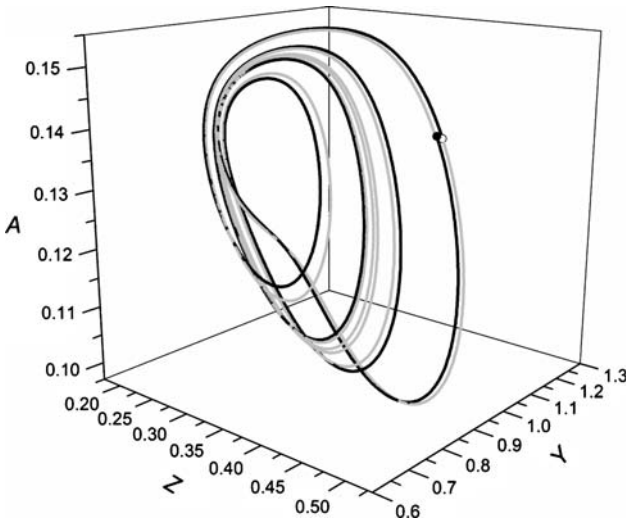

Fig. 4 Three-dimensional plot of two successive parts of the trajectory, which come very near to the initial point. The trajectory shows the numerical solution of the Houart et al. (1999) model in the chaotic regime. Parameter values: $\beta = 0.675$; all other parameter values are the same as in Houart et al. (1999). *Filled circle* initial point; empty circle, point on the trajectory that is located very close to the initial point at the end of the first "orbit" (*black curve*). The end point of the second "orbit" (*grey curve*) is so close to the initial point that it cannot be distinguished in the diagram. The two "orbits" are passed within about 5 min (*black curve*) and about 7 min (*grey curve*), respectively

We open the model in the sense that we additionally introduce a Ca^{2+} exchange between the cytosol and the extracellular space in a way that the net transmembrane flux is linearly dependent on the Ca^{2+} concentration in the cytosol. The modified equation for cytosolic Ca^{2+} reads:

$$\frac{dZ}{dt} = V_{ch} + V_{leak} - V_{pump} + V_{m\_out} - V_{m\_in} - V_{pr\_on} + V_{pr\_off} + V_{in} - kZ \tag{16}$$

where the fluxes V_i are in part highly non-linear functions (for their expressions and the remaining system equations, see the original paper by Marhl et al. (2000), where the symbol J was used instead of V). The only difference to the original model (Marhl et al. 2000) is that additional terms V_{in} and kZ denoting a constant Ca^{2+} influx into the cell and an efflux out of the cell, respectively, are included in Eq. (16). Consequently, if V_{in} and k are unequal to zero, the total concentration of Ca^{2+} in the cell is no longer constant. Thus, instead of calculating the concentration of the Ca^{2+} bound to the cytosolic proteins, Y_3, by the use of the conservation relation for the total concentration of Ca^{2+} in the cell (see Marhl et al. 2000), we need to take into account a differential equation for Y_3:

$$\frac{dY_3}{dt} = k_+ Z \cdot Pr - k_- Y_3 \quad (17)$$

Y_1, Y_2 and Y_3 stand for the Ca^{2+} levels in the ER, mitochondria and Ca^{2+}-protein complexes, respectively. Pr denotes the free protein concentration.

The system structure is analogous to that presented in Fig. 1. However, this system is somewhat more complex since the cytosolic Ca^{2+} is linked to three different compartments: the ER, mitochondria and proteins. So, Ca^{2+} in all the compartments can be regarded together as virtually one pool, which is replenished by the influx V_{in} and depleted by the efflux kZ of Ca^{2+} out of the cell. Mathematically, instead of the sum of two variables taken in Eq. (5), we need to take into account an appropriate linear combination of all four variables Z, Y_1, Y_2 and Y_3 to show that the average level of cytosolic Ca^{2+} indeed equals the level at the steady state. We take the following expression, which is analogous to Eq. (5):

$$\frac{d\left(\frac{\rho_{ER}}{\beta_{ER}} Y_1 + \frac{\rho_m}{\beta_m} Y_2 + Y_3 + Z\right)}{dt} = V_{in} - kZ \quad (18)$$

ρ_{ER} and ρ_m denote the ER/cytosol and mitochondria/cytosol volume ratios, respectively. β_{ER} and β_m are the corresponding buffer capacities. By analogous considerations as above, calculating the steady-state concentration of cytosolic Ca^{2+} (see Eq. 6) and the average cytosolic Ca^{2+} concentration of the oscillating signals (see Eqs. 7–8), we obtain Eq. (9).

In Fig. 5, we compare the original model (Marhl et al. 2000) with the modified model including influx and efflux. Note that in the oscillatory region of the original model, average and steady-state cytosolic Ca^{2+} concentrations are unequal to each other, notably $\langle Z \rangle < Z_{ss}$, while in the modified model, the equality property holds.

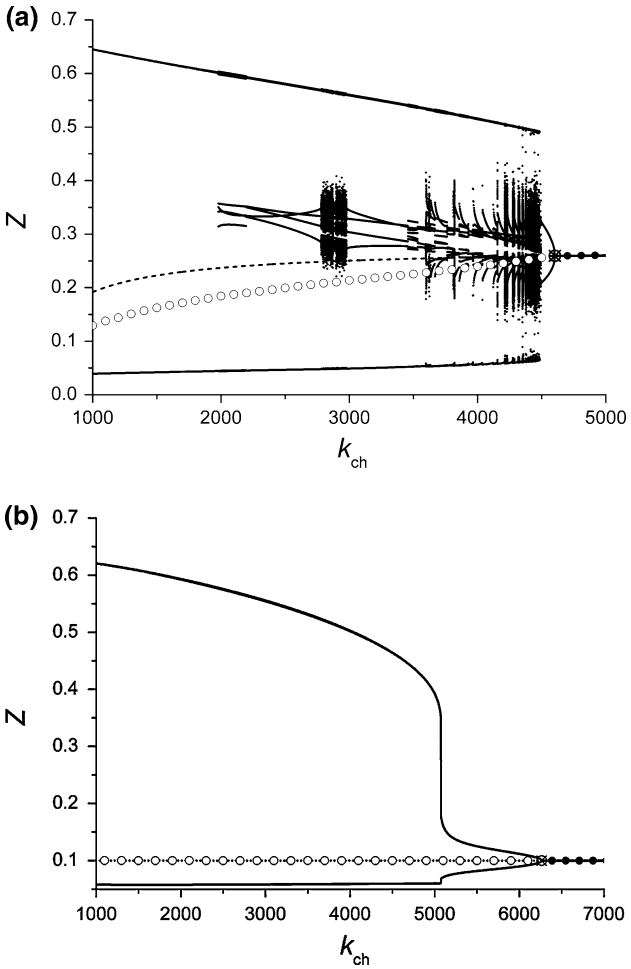

Fig. 5 Comparison of steady-state and average levels of cytosolic Ca^{2+} for (**a**) the model proposed by Marhl et al. (2000) and (**b**) the modified model including a Ca^{2+} exchange across the cell membrane (cf. Eqs. 16 and 17) by numerical calculations. *Filled (empty) circles* average values in the steady-state (oscillatory) regime; *dashed line* unstable steady state; *solid lines* local maxima and minima of oscillations or stable steady state; k_{ch} rate constant of calcium channel. Parameter values: V_{in} = 1.0 μMs^{-1}, k = 10 s^{-1} (all other parameter values are the same as in Marhl et al. (2000)). The averages were computed over $t = 10^5$ s. Convergence was observed from 10^4 s on

Comparison with the Higgins–Selkov oscillator

The Higgins–Selkov model of glycolytic oscillations (Higgins 1964; Selkov 1968) is characterized by a constant influx leading to Y, a positive feedback of the product Z on its own production (autocatalysis) and a linear efflux of Z (Fig. 6). Y and Z denote fructose-6-phosphate and fructose-1,6-bisphosphate, respectively. In comparison to subsequent models of glycolytic oscillations (e.g. Wolf and Heinrich 2000; Madsen et al. 2005), the Higgins–Selkov model is a minimalist model showing the essential features. It is worth noting that the exact mechanism of the positive feedback in glycolytic oscillations is still under investigation (Madsen et al. 2005).

The system equations read

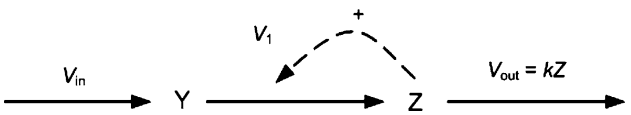

Fig. 6 Reaction scheme of the Higgins–Selkov oscillator. In the case of glycolytic oscillations, Y and Z denote fructose-6-phosphate and fructose-1,6-bisphosphate, respectively

$$\frac{dY}{dt} = V_{in} - V_1 \quad (19)$$

$$\frac{dZ}{dt} = V_1 - kZ \quad (20)$$

The rate laws for influx and efflux are the same as in the

Somogyi-Stucki model. For the interconversion step, the following nonlinear rate law is used in the Higgins–Selkov model

$$V_1 = k_1 Y Z^2 \tag{21}$$

In a suitable parameter range, this model gives rise to limit-cycle oscillations (Higgins 1964; Selkov 1968; see also Heinrich and Schuster 1996). The model fulfils all the three conditions (a)–(c) stated in Sect. 2. There is an important difference to the Ca^{2+} models, though. While, in the latter, the reactions forming and consuming Y represent a sort of dead-end, the interconversion step in the Higgins–Selkov model belongs to a straight pathway involving also the influx and efflux. Thus, the throughput flux brings about the turnover of both substances. Nevertheless, the net flux (influx minus efflux) is a linear function of Z also here because the input flux is constant (note that the first reaction is irreversible) and the output reaction obeys a linear rate law. Moreover, Y and Z can again be regarded virtually as one pool with respect to influx and efflux.

Summing up Eqs. (19) and (20) leads to the same steady-state concentration as in the Ca^{2+} models fulfilling the three conditions, Eq. (6). Thus, Z_{ss} is again dependent on influx and efflux rates only. Integration over one period leads to Eq. (7) and finally to the equality property, Eq. (9), for periodic oscillations.

Numerical calculations illustrate this equality, as shown in Fig. 7. It can be seen that, in total, three types of dynamic behaviour can be observed: a stable steady state with positive concentrations in some parameter range, stable limit-cycle oscillations in another parameter range, and a case where the trajectory runs to $Z \rightarrow 0$ and $Y \rightarrow$ infinity. The latter behaviour can occur for all parameter values but its basin of attraction gets smaller and smaller as k_1 increases (proof not given here). In the parameter region where neither the steady state nor the limit cycle is stable, the latter type of asymptotic behaviour is the only one. Then, the equality property does not hold because one variable tends to infinity.

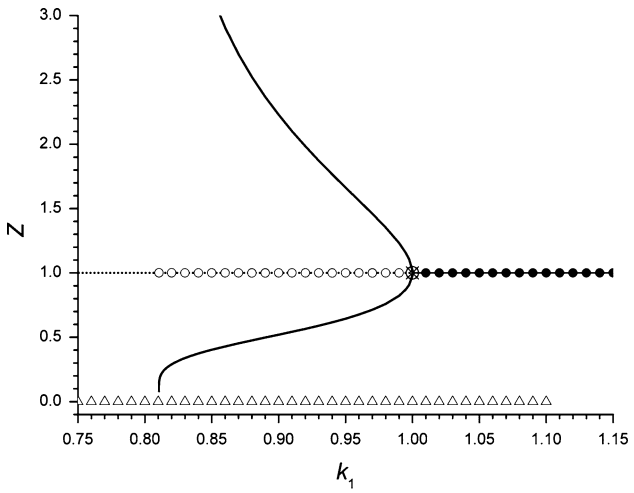

Fig. 7 Illustration of the equality property for the Higgins–Selkov model by numerical calculations. *Empty triangles* stable asymptotic solution where $Z \rightarrow 0$ and $Y \rightarrow$ infinity; *empty circles* average values in the case of limit cycles; *filled circles* average values in the case of stable steady state. *Solid (dashed) horizontal line* stable (unstable) steady state; *curved lines* envelope of oscillations. In the region where two different asymptotic behaviours are possible, the outcome depends on the initial values. Parameter values: $V_{in} = 1$, $k = 1$, initial values of Z were below or equal $Z_{ss} = 1$ (smallest: $Z_0 = 0.5$), $Y_0 = 1$

Interrelations with metabolic control analysis

A necessary prerequisite for the equality property (9) to hold is that the steady-state value of the variable under consideration is determined uniquely by the influx and efflux reactions. That is, the transport reactions across the membrane of the intracellular stores in the Ca^{2+} models and the reaction between Y and Z in the Higgins–Selkov oscillator must not have any influence on this value and, hence, on the steady-state efflux, kZ. Otherwise, the nonlinear kinetics of these processes would affect the average value. In metabolic control analysis (cf. Heinrich and Schuster 1996; Fell 1997), a situation where some processes do not have any influence (control) on the fluxes of some other processes or on some steady-state concentrations is called "control insusceptibility" (Schuster and Schuster 1992; Heinrich and Schuster 1996; for a related study, see Teusink and Westerhoff 2000). This situation can occur for several reasons, such as (1) irreversibility of some reaction, (2) complete saturation of some enzyme, (3) a special stoichiometric structure, or (4) quasi-equilibrium enzymes (for case (4), see Kholodenko et al. 1998). That the steady state is unstable in the case of oscillations does not imply any problems since the control coefficients are formally defined also for unstable states as long as the Jacobian matrix has full rank (Reder 1988).

In the Higgins–Selkov oscillator, the first reaction is assumed to be irreversible. In any unbranched reaction chain with the first reaction being irreversible and without feedback on that reaction, the reactions downstream of the first one do not exert any flux control (Heinrich and Rapoport 1974). Accordingly, in the Higgins–Selkov oscillator, the second reaction is not able to exert any control on the steady-state flux. Moreover, it cannot control the concentration Z either due to Eq. (6), while it can control Y. If the first reaction were reversible, Y would affect the input rate, so that the second reaction would gain control over Z, and the equality property would no longer hold.

In the case of Ca^{2+} oscillation models, the special network structure rather than the irreversibility of the input

reaction is of importance. The equality property would not be violated if the input reaction were reversible as long as the rate of the backward reaction is linearly dependent on Z. This is because both the influx and efflux reactions in the Ca^{2+} oscillation models connect to Z, in contrast to the Higgins–Selkov oscillator.

To analyse the network properties, it is convenient to consider the stoichiometry matrix, **N** (cf. Reder 1988; Heinrich and Schuster 1996). In this matrix, the stoichiometric coefficients are gathered such that the rows and columns of the matrix correspond to substances and reactions, respectively. The scheme of Ca^{2+} fluxes shown in Fig. 1 has the following matrix **N** (with the first row corresponding to Z),

$$\mathbf{N} = \begin{pmatrix} 1 & -1 & -1 & 1 & 1 \\ 0 & 0 & 1 & -1 & -1 \end{pmatrix}. \tag{22}$$

For analysing the properties of a network at steady state, it is useful to consider the nullspace matrix, **K**, to the stoichiometry matrix, defined by

$$\mathbf{NK} = \mathbf{0} \tag{23}$$

(cf. Reder 1988). For the matrix given in Eq. (22), a possible choice of the nullspace matrix is

$$\mathbf{K} = \begin{pmatrix} 1 & 0 & 0 \\ 1 & 0 & 0 \\ 0 & 1 & 1 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{pmatrix} \tag{24}$$

Control insusceptibility can occur if the nullspace matrix can be block-diagonalized (Schuster and Schuster 1992):

$$\mathbf{K} = \begin{pmatrix} \mathbf{K}_1 & 0 & \cdots \\ 0 & \mathbf{K}_2 & \cdots \\ \vdots & \vdots & \ddots \end{pmatrix} \tag{25}$$

The matrix given in Eq. (24) has such a structure, with the influx and efflux corresponding to one block and the fluxes across the ER membrane corresponding to another one. Therefore, the fluxes through the subsystems corresponding to the blocks can be changed independently of each other. For example, the cyclic flux through the Ca^{2+} pump and the Ca^{2+} channel in the ER membrane (and possibly through the leak) in a steady state can be changed without affecting the efflux kZ. Thus, the concentration Z_{ss} is independent of the properties of the nonlinear processes within the cell. However, this is not a sufficient condition. A second necessary condition concerns the so-called link matrix, **L**. This matrix, which expresses the conservation relations (if any), is defined in the following way (Reder 1988). The rows of **N** are rearranged such that a maximum number of linearly independent rows are situated at the top:

$$\mathbf{N} = \begin{pmatrix} \mathbf{N}_0 \\ \mathbf{N}' \end{pmatrix}, \tag{26}$$

where $\mathbf{N}_0$ has the same rank as **N**. Then,

$$\mathbf{N} = \mathbf{L}\,\mathbf{N}_0. \tag{27}$$

When the system does not involve any conservation relations (like the Somogyi–Stucki model), **L** is simply the $n \times n$ identity matrix, with n denoting the number of substances. In order for control insusceptibility to occur, also the link matrix must be block-diagonalizable,

$$\mathbf{L} = \begin{pmatrix} \mathbf{L}_1 & 0 & \cdots \\ 0 & \mathbf{L}_2 & \cdots \\ \vdots & \vdots & \ddots \end{pmatrix} \tag{28}$$

which is trivially fulfilled if it is the identity matrix. Condition (28) is not fulfilled for the model by Marhl et al. (2000), which is a closed system involving binding of Ca^{2+} to proteins (for a related model, see Fall and Keizer 2001). That system (in the unmodified form without influx and efflux) has the stoichiometry matrix

$$\mathbf{N} = \begin{pmatrix} -1 & 1 & 1 & -1 & 1 & -1 & 1 \\ 1 & -1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 \\ 0 & 0 & 0 & 0 & 0 & -1 & 1 \end{pmatrix}, \tag{29}$$

where the rows have been arranged such that the first three rows are linearly independent. The five rows then correspond, in that order, to the substances Z, Y_1, Y_2, Y_3 and Pr. The link matrix reads

$$\mathbf{L} = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \\ -1 & -1 & -1 \\ 1 & 1 & 1 \end{pmatrix} \tag{30}$$

This matrix implies the conservation relations

$$Z + Y_1 + Y_2 + Y_3 = \text{const.}, \tag{31a}$$

$$-Z - Y_1 - Y_2 + Pr = \text{const.}, \tag{31b}$$

Relation (31b) can be replaced by the simpler relation $Y_3 + Pr =$ const., which is the sum of Eqs. (31a) and (b). The link matrix given in Eq. (30) cannot be block-diagonalized. Thus, the kinetic parameters of the processes within the cell do control the concentration Z. This can be understood by considering the conservation relation. If, for example, the binding of Ca^{2+} to proteins is accelerated, the steady state value of Y_3 increases, which withdraws part of Z. In contrast, in the open model (including V_{in} and the efflux kZ), Z can be replenished from the outside.

Control insusceptibility of a subsystem A by a subsystem B due to network structure requires a third condition,

which is related to condition (c): The metabolites participating only in the reactions belonging to the subsystem B (like Y in the example systems) should not affect the reaction rates in subsystem A (see Schuster and Schuster 1992, or Heinrich and Schuster 1996, for a mathematical formulation). This condition is fulfilled for most models of Ca^{2+} oscillations. However, as mentioned in Sect. 2, store-operated calcium entry (Berridge 1995; Parekh and Putney 2005) would violate that condition.

The analysis in this section allows one to devise more complicated models for which the equality property (9) holds. For example, there can be intracellular stores in addition to the ER and mitochondria. The exchange fluxes across the membranes of these stores would imply additional diagonal blocks in the nullspace matrix.

Comparison with Lotka–Volterra models

A very famous model giving rise to oscillations is the two-dimensional Lotka–Volterra system describing predator-prey dynamics (cf. Hofbauer and Sigmund 1998; Murray 2002):

$$\dot{x} = x(a - by) \tag{32a}$$

$$\dot{y} = y(-c + ex) \tag{32b}$$

where x and y denote the population numbers of prey and predator, respectively. The nontrivial steady state given by

$$x_{ss} = c/e, \tag{33a}$$

$$y_{ss} = a/b \tag{33b}$$

is a centre surrounded by marginally stable oscillations. That is, these are no limit cycles because they do not attract neighbouring trajectories.

For system (32a, b), conditions (b) and (c) are not fulfilled. Although one could form a linear combination of these two equations such that the bilinear terms cancel each other, the resulting equation would still depend on both x and y. Nevertheless, system (32a,b) has the remarkable property that the average values equal the steady-state values. This can be proved as follows (cf. Hofbauer and Sigmund 1998; Walter 2000; Stucki and Urbanczik 2005). The proof is based on a transformation of the variables by dividing Eqs. (32a) and (b) by x and y, respectively

$$\frac{\dot{x}}{x} = \frac{\mathrm{d}}{\mathrm{d}t}\log x = a - by \tag{34a}$$

$$\frac{\dot{y}}{y} = \frac{\mathrm{d}}{\mathrm{d}t}\log y = -c + ex \tag{34b}$$

Multiplication of Eq. (34a) by dt and integration over one period T gives

$$\int_{x(0)}^{x(T)} \mathrm{d}\log x(t) = \int_0^T (a - by)\mathrm{d}t \tag{35}$$

$$\log x(T) - \log x(0) = aT - b\int_0^T y(t)\mathrm{d}t. \tag{36}$$

Since $x(T) = x(0)$, this results in

$$\frac{1}{T}\int_0^T y(t)\mathrm{d}t = \frac{a}{b}, \tag{37}$$

which implies, due to Eq. (33b),

$$\langle y \rangle = y_{ss}. \tag{38}$$

An analogous proof can be given for the average of x, resulting in $\langle x \rangle = x_{ss}$.

Provided that the steady state is unique and no variable tends to zero or infinity, this proof can be generalized to Lotka–Volterra equations for more than two populations (Theorem 5.2.3 in Hofbauer and Sigmund 1998) and the related replicator equations, which involve bilinear and trilinear terms (Schuster et al. 1981; Theorem 7.6.4 in Hofbauer and Sigmund 1998). Stucki and Urbanczik (2005) have shown that the equality also holds for a minimal model of the oscillator proposed by Willamowski and Rössler (1980), which is a special three-dimensional Lotka–Volterra model.

Here, we generalize the proof, in the two-dimensional case, to the following equations:

$$\dot{x} = f(x)(a - by) \tag{39a}$$

$$\dot{y} = g(y)(-c + ex) \tag{39b}$$

where $f(x)$ and $g(y)$ are arbitrary (possibly nonlinear) functions of x respectively y. They only have to fulfil the very weak condition that they should not have any zero in the interval of x and y given by the minimum and maximum values of the oscillation. Under this condition, we can divide the equations by these functions:

$$\frac{\dot{x}}{f(x)} = a - by \tag{40a}$$

$$\frac{\dot{y}}{g(y)} = -c + ex \tag{40b}$$

Now, the proof runs as above, with log$x$ replaced by $\int [f(x)]^{-1}\mathrm{d}x$. By contrast, replacing the terms $a - by$ or $-c + ex$ on the right-hand sides of Eq. (39a, b), respectively, by nonlinear functions entails that the equality property is no longer fulfilled (cf. Walter 2000).

It is worth mentioning the correspondence between the terms $a - by$ and $-c + ex$ in the Lotka–Volterra equations

and the term $V_{in} - kZ$ in the Ca^{2+} models. Both are linear functions, which is a prerequisite for the proof of the property in question.

Although Lotka–Volterra models obey a nonlinear conservation relation (Hofbauer and Sigmund 1998), condition (a) is satisfied because these models describe open systems. For the original equations, conditions (b) and (c) seem not to be fulfilled; however, by the variable transformation shown above, leading to log$x$ and log$y$, the latter two conditions can, in a sense, be considered fulfilled because the right-hand sides of Eq. (34a, b) are linear functions. However, the term involving x occurs on the right-hand side of the equation for y and vice versa, while the term $V_{in} - kZ$ occurs on the right-hand side of the equation for $Y + Z$ in the Ca^{2+} and glycolysis models.

Discussion

Nonlinear oscillatory systems do not exhibit harmonic oscillations. Therefore, unless special symmetries are present, one might assume that the average value of any oscillating variable is unequal to the value of that variable at the corresponding unstable steady state at the same parameter values, where "corresponding" means that the steady state is a continuation of that state from which the oscillation emerged in a bifurcation. Here, we have shown that, for several models of intracellular Ca^{2+} oscillations in non-excitable cells such as the Somogyi–Stucki and Dupont-Goldbeter "one-pool model", these two values do equal each other for cytosolic calcium (Eq. 9). They do not, however, equal each other for the Ca^{2+} concentration in the intracellular store. The equality property is due to the existence of a Ca^{2+} net flux across the cell membrane, which is linearly dependent on the cytosolic Ca^{2+} concentration and independent on the other concentrations, as phrased above in conditions (a)–(c). The equality property even holds for some models giving rise to chaotic oscillations.

We have shown that the equality property holds for one variable (corresponding to fructose-1,6-bisphosphate) in the Higgins–Selkov model of glycolytic oscillations. Also for an extended model of glycolytic oscillations (Wolf and Heinrich 2000), numerical simulations show that the property holds for some of the variables (J. Wolf personal communication). We have also shown that this property is linked to the phenomonon of control insusceptibility (Sect. 3.3). A potential application is that, whenever the equality property is observed for a real system with unknown network structure, conclusions about that structure can be drawn. About concentration control insusceptibility, less is known (see Teusink and Westerhoff 2000, for some results) than about about zero flux control. It will be interesting to extend metabolic control analysis in that direction in the future.

Under conditions (a)–(c), the average level in the oscillatory situation is not only equal to the level at the (in reality non-observable) unstable steady state with the same parameter values, but also equal to the steady-state level for (other) parameter values that allow the steady state to be stable. This holds for all parameters except those involved in the net flux across the cell membrane because the steady-state concentration only depends on these parameters. This reasoning is important because, in evolution, the organisms must change parameter values to switch from a stationary regime to an oscillatory regime. Since the average Ca^{2+} or metabolite concentration in the oscillatory regime—compared to its steady state value—depends on the type of the influx and efflux kinetics, our results may lead to hypotheses why special kinetics of the processes involved may have evolved.

In particular, for the models by Goldbeter et al. (1990), Dupont and Goldbeter (1993), and Borghans et al. (1997), we have derived that the equality property implies a linear dependency between the level of stimulation β and the average concentration of the oscillating calcium $\langle Z \rangle$.

Our results are interesting in view of the frequently posed question which advantages oscillations may have in biology (Heinrich and Schuster 1996; Dupont and Goldbeter 1998; Gall et al. 2000). It has been suggested that lowering some concentrations could be an advantage of oscillations. For example, lowering the average ROS concentration by oscillations has indeed a beneficial effect by protecting the peroxidase from inactivation (cf. Hauser et al. 2001; Olsen et al. 2003). Also in some oscillatory Ca^{2+} systems, a concentration lowering is observed, for example, in the model of Marhl et al. (2000) (see Fig. 5a). However, in systems in which the three above-mentioned conditions are (approximately) fulfilled, changing the average concentration is not an effect of oscillations. Nevertheless, systems showing the equality property can lower the average (and steady-state) concentration by reducing the influx or activating the efflux.

As alluded to in the Introduction, the equality property has implications for the decoding of calcium oscillations (for modelling the decoding, see Gall et al. 2000; Dupont et al. 2003; Schuster et al. 2005; Marhl et al. 2006; Marhl and Grubelnik 2007). As outlined above, there is a tendency to keep the Ca^{2+} concentration low. In order that Ca^{2+} binding proteins can be activated without increasing the average Ca^{2+} level, it is favourable that Ca^{2+} oscillates and the activity of the decoding protein depends nonlinearly on Ca^{2+} in such a way that short spikes contribute much to protein activation (Dolmetsch et al. 1998, Gall et al. 2000). Thus, spike-like oscillations can activate these proteins in a frequency-dependent manner with an average

Ca^{2+} level equal, or even lower, than the steady-state level. It has been argued that this is a reason why Ca^{2+} oscillates rather than that its steady-state concentration is varied for signal transduction (Gall et al. 2000; Dupont et al. 2003). Experiments show that Ca^{2+} oscillations enhance the NFAT-dependent transcription compared to a constant Ca^{2+} signal with the same average at low Ca^{2+} stimulation levels (Dolmetsch et al. 1998). This was ascribed to the highly nonlinear dependence of the activity of the transcription factor NFAT on the Ca^{2+} concentration. In this study we conclude that, in model systems where the equality property is valid, the effect of oscillations is not a change in the average Ca^{2+} concentration, but may rather be that a more efficient protein activation can be achieved.

It is of course interesting to derive conditions under which the average concentration is lower than the steady-state value and conditions under which it is higher. Our numerical results (shown only partly here) indicate that the average concentration is lower (higher) than the steady-state value whenever the kinetics of the efflux is a convex (concave) function. It will be the subject of a sequel paper to prove this analytically, for example, by Jensen's inequality (cf. Rudin 1987).

One might argue that a linear rate law for the efflux in systems showing Ca^{2+} oscillations is not very realistic, because experimental data are indicative of a Hill kinetics for the export out of the cell in non-excitable cells (Camello et al. 1996; Carafoli 1991). However, since a Hill kinetics has both a convex and concave part, the two effects may approximately cancel each other in a case where the K_m value has an appropriate intermediate value between base-line and maximal peak height of the oscillation. This was indicated by numerical simulations, shown in Fig. 2b. Condition (b) is then approximately fulfilled, so that equality is likely to be approximately valid.

It has been known earlier that a similar equality property holds for the variables in Lotka–Volterra models (cf. Hofbauer and Sigmund, 1998) although conditions (b) and (c) are not fulfilled. Here, we have generalized this result, in the two-dimensional case, to equations in which the variables x and y on the right-hand sides of Eqs. (32a) and (b), respectively, are replaced by any (possibly nonlinear) functions of x respectively y not having any zero in the interval of x and y given by the minimum and maximum values of the oscillation.

The oscillations in two-dimensional Lotka–Volterra systems are marginally stable, that is, they do not represent limit cycles. In that sense and also in view of the equality property, these systems behave like linear systems. By contrast, Ca^{2+} oscillations represent limit cycles. There is some mathematical interrelation between Lotka–Volterra models and the models of Ca^{2+} oscillations fulfilling the condition (b) given in Sect. 2 because both involve linear terms, $a - by$ and $-c + ex$ respectively $V_{in} - kZ$. The proof for the classical Lotka–Volterra equations as well as for the modified equations given above runs via a transformation to linear equations. Thus, some intrinsic linearity is used.

It cannot, however, be concluded that the equality property holds for all nonlinear oscillations (Ritter and Douglas 1970). Here, we have examined two example models for which it does not hold: the Somogyi–Stucki model with quadratic kinetics or Hill kinetics for the efflux reaction and the model of Marhl et al. (2000), for which condition (b) and (a), respectively, are not fulfilled. Also in systems in which condition (c) is not satisfied, such as in the presence of store-operated Ca^{2+} entry, the equality property is not likely to hold. Several mathematical models examined store-operated Ca^{2+} entry in Ca^{2+} transients (Wiesner et al. 1996) and Ca^{2+} oscillations, in particular considering voltage-gated fluxes (Li et al. 1997) and a diffusible messenger (Kowalewski et al. 2006). It is worthwile analysing the relation between average and steady-state Ca^{2+} levels in the case of store-operated Ca^{2+} in future studies.

It is an interesting question why, at least in some cells, an interaction between Ca^{2+} in the stores and Ca^{2+} flux across the cell membrane appears to have emerged during evolution. It might be possible that control insusceptibility is avoided by evolution. A similar observation concerns sugar metabolism. Flux control insusceptibility would occur if fructose-2,6-bisphosphate did not directly affect any enzyme in the glycolytic pathway. However, it is an effector of phosphofructokinase-1 (cf. Schuster and Schuster 1992).

The equality property is also interesting for the following reason. Several approaches in theoretical biology start from the assumption of steady state, e.g. metabolic control analysis (cf. Heinrich and Schuster 1996; Fell 1997), metabolic pathway analysis (cf. Papin et al. 2004) and flux balance analysis (cf. Palsson 2006). As far as fluxes are concerned, it is known that many results of these analyses can be generalized to oscillations because, in this case, the steady-state equation $\mathbf{NV} = \mathbf{0}$ holds for the average fluxes (for some extensions of metabolic control analysis to oscillations see, e.g. Heinrich and Schuster 1996, and Reijenga et al. 2002). In view of the above results, we can conclude that also for concentration variables, many results of these analyses can be generalized to oscillation models if the equality property is fulfilled.

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