Control over action potential, calcium peak and average fluxes in cyclic quasi-steady-state ion transport system in cardiac myocytes: in silico studies.

Jaroslaw Dzbek and Bernard Korzeniewski

Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

Correspondence: B. Korzeniewski, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, ul. Gronostajowa 7, 30-387 Kraków, Poland, tel. (48 12) 664 63 73, fax. (48 12) 664 69 02, e-mail: benio@mol.uj.edu.pl

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Abstract

Metabolic Control Analysis was originally developed to deal with the steady-state systems. In the present theoretical study the control analysis is applied to the cyclic quasi-steady-state system of ion transport in cardiac myocytes. It is demonstrated that the metabolic control of particular components (channels, exchangers, pumps) of the system over such quasi-steady-state variables as action potential amplitude, action potential duration, area under calcium peak and average fluxes through particular channels during one oscillation period can be defined and calculated. It is shown that the control over particular variables in the analyzed, periodical system is distributed among many (potentially all) components of the system. Nevertheless, some components seem to exert much more control than other components, and different variables are controlled to the greatest extend by different channels. Finally, it is hypothesized that the Na\(^+\) and K\(^+\) transport system exerts a significant control over the Ca\(^{2+}\) transport system, but not inversely.

Abbreviations: I\(_X\): total current (flux) through system element X (in pA); Na: Na channel; NaK: sarcolemmal Na\(^+/K^+\)-ATPase; K1: inward rectifier channel; bNSC: background non-selective cation channel; Kr: delayed rectifier channel, rapid component; CaL: L-type Ca\(^{2+}\) dependent channel; NaCa: sarcolemmal Na\(^+/Ca^{2+}\) exchanger; Ks: delayed rectifier channel, slow component; CaT: T-type Ca\(^{2+}\) channel; Cab: sarcolemmal Ca\(^{2+}\) leakage; KATP: ATP-sensitive K\(^+\) channel; Kp: non-specific, voltage-dependent outward (plateau) channel; l(Ca): Ca\(^{2+}\) activated background cation channel; to: transient outward current channel; RyR: ryanodine Ca\(^{2+}\) channel; SRU: Ca\(^{2+}\)-ATPase SR pump; SRT: transcompartmental SR Ca\(^{2+}\) transport; SRL: SR Ca\(^{2+}\) leakage.
Introduction

Metabolic Control Analysis (MCA) [1–3] has appeared to be an immensely useful quantitative tool for analysing the dynamic behaviour of biochemical systems. It was used in a great number of experimental and theoretical studies concerning the control of metabolic pathways (see [4–9] just for a few examples). In its original form MCA concerns steady-state system properties. Its most fundamental idea is based on the ratio of the relative change \( \frac{\text{d}Y}{Y} \) in some variable \( Y \) caused by a small relative change \( \frac{\text{d}X}{X} \) in some parameter/variable \( X \), to the latter change:

\[
\frac{\partial Y}{\partial X} \tag{1}
\]

In principle these changes are infinitesimal, but in practice sufficiently small finite changes can be considered. Several coefficients have been defined within MCA. Perhaps the most important and most frequently used is the flux control coefficient (FCC) expressed as a relative change in the flux \( J \) through a given system divided by a relative change in a given enzyme concentration/activity \( E_i \) that caused the change in the flux:

\[
C^J_{Ei} = \frac{\partial J/J}{\partial E_i/E_i} \tag{2}
\]

The flux control coefficient for a given enzyme is a global parameter; its value depends on the kinetic properties of the whole system and not only on the properties of this enzyme. The values of FCCs can be different in different steady states. Usually, in linear pathways, the value of the flux control coefficient of a given enzyme is between 0 (no control over flux) and 1 (entire control over flux). It was demonstrated that in linear metabolic pathways the values of FCCs for all components of the system sum up to unity (this is the so-called summation property):

\[
\sum_{i=1}^{n} C^J_{Ei} = 1 \tag{3}
\]

However, this property does not (have to) concern branched pathways. Metabolic Control Analysis demonstrated that the control over the flux is distributed among many different steps and therefore there are no rate-limiting steps. Flux control coefficients may be attributed not only to (single) enzymes, but also to metabolic blocks (groups of enzymes), carriers (e.g. ATP/ADP carrier) and physical processes (e.g. proton leak through the inner mitochondrial membrane).

Another important coefficient is the concentration control coefficient (CCC) defined as the ratio of the relative change in a given metabolite concentration \( M_j \) to the relative change in a given enzyme concentration/activity \( E_i \) that caused the change in the metabolite concentration:

\[
C^{M_j}_{Ei} = \frac{\partial M_j/M_j}{\partial E_i/E_i} \tag{4}
\]
The values of MCC can be either positive or negative. In a given metabolic system the sum of the values of all concentration control coefficients equals zero (this is the so-called connectivity property):

$$\sum_{j=1}^{n} C_{Ei}^{Mj} = 0$$  \hspace{1cm} (5)

Yet another coefficient defined within MCA is the elasticity coefficient expressing the sensitivity of a given enzymatic reaction rate $v_k$ to the concentration of a given metabolite $M_j$:

$$\epsilon^k_{Mj} = \frac{\partial v_k}{\partial M_j} / \frac{v_k}{M_j}$$  \hspace{1cm} (6)

Generally the values of flux control coefficients of particular components of some system are inversely proportional to the values of their elasticity coefficients. Some other coefficients, for instance the response coefficient, have been defined within MCA. However, they are not directly related to the present theoretical study.

As it was mentioned above, Metabolic Control Analysis is traditionally applied to steady-states. It also was shown, that MCA framework is not straightforwardly applicable to transient and oscillatory systems [13-16]. However, several theoretical approaches have been defined in order to tackle problems arising in the analysis of such systems [13–17]. It has been demonstrated that in quasi-steady-state systems with a constant oscillation period (imposed through e.g. external stimulation) it is possible to define variables whose unique relative changes can be calculated and analyzed under the framework of MCA [15-16]. In such systems, variable values return periodically to the initial value, average fluxes during one cycle can be determined and such variables as the amplitude of, duration of, and area under the oscillation (pulse) of some metabolite concentration (or thermodynamic force) can be defined.

A good example of such a cyclic quasi-steady-state system is the ion circulation and action potential generation in isolated cardiac myocytes. In this system heart cells are periodically stimulated electrically with a constant stimulation interval. This stimulation increases transiently some ion channel conductivities, generates an ion movement across the cell membrane and sarcoplasmatic reticulum membrane, and thus induces changes in the membrane potential. Several kinetic models of this system have been developed [10–12] that are generally similar, although differ in details. The elements of the system taken into account explicitly in the model developed by Matsuoka and co-workers [12], used in the present study, are presented in Fig. 1. The system is composed of several pumps and ion channels that conduct Na⁺, K⁺ and/or Ca²⁺ currents.

The most important events occurring in the system during one oscillation period are as follows. External electrical stimulation causes a very high, but very short activation of Na channel, what leads to an intensive short-term inward sodium current $I_{Na}$. This is related to a sudden increase in the membrane potential from about -85 mV (resting potential) to over +40 mV (action potential). As a result, K1 channel closes, allowing to generate a plateau phase in the membrane potential. Increase of the membrane potential above -30 mV activates CaL channel and causes an influx of Ca²⁺ ions from the extracellular space to cytosol ($I_{CaL}$). Increased Ca²⁺ concentration promotes calcium-induced calcium release (CICR).
from sarcoplasmic reticulum (SR). This flux occurs through RyR channels (I_{RyR} current) with its amplitude being about two orders of magnitude higher than that of the I_{CaL}; nevertheless RyR channels are quickly deactivated and the ratio of total Ca$^{2+}$ entry through RyR and CaL channels is 15:1. The high membrane potential causes also a slow, time-dependent activation of K (Ks + Kr) channels and an increase in K$^+$ ion efflux from the cell (I_{Ks} + I_{Kr}). As a result the membrane potential decreases, K1 channel is activated and the decrease in the membrane potential is further accelerated. Finally, the ATP-driven Na$^+$/K$^+$ pump (NaK) and Na$^+$/Ca$^{2+}$ exchanger (NaCa) bring the system back to the initial resting state. The changes in the membrane potential result from the ion (charge) movement across the cellular membrane and their magnitude is dependent on the capacitance of the membrane. 400 ms after a previous external stimulation the next stimulation is applied and the whole cycle is repeated. Fig. 2 shows the simulated time course of the membrane potential and cytosolic Ca$^{2+}$ concentration during one cycle. Of course, the calcium pulse is crucial for the stimulation of the myocyte contraction (driven by actomyosin-ATPase) and at least some components of the ATP-producing system. The above description of the behaviour of the system is a generalized one and the detailed description can be found in [10–12].

In the present theoretical study the metabolic control exerted by particular components of the ion transport system in cardiac myocytes (channels, pumps, exchangers) over the amplitude and duration of the action potential, area under the calcium peak and average fluxes through particular components is analyzed. It is found that: 1. the control is distributed between different steps; 2. some steps tend to have more control than other steps; and 3. different steps have the greatest control over different variables. Generally, it is demonstrated that Metabolic Control Analysis is very useful in describing not only steady-states, but also cyclic quasi-steady-states in biochemical/biophysical systems.
Theoretical procedures

In the computer simulations performed in the present study the model of ion transport in isolated cardiac myocytes developed previously by Matsuoka and co-workers [12] was used without any modifications. Within this model the dependences of the activities of particular elements of the system (channels, pumps, exchangers) on ion concentrations, membrane potential and time are expressed in the form of kinetic equations. The changes of ion concentrations and membrane potential over time are described as a set of ordinary differential equations. The authors of the model validated it successfully by a comparison of computer simulations with experimental data concerning the action potential time course, internal Ca\(^{2+}\) concentration transient and sarcomere length changes occurring during the contraction event. They studied in the theoretical way the effect of large-scale changes in some selected channel activities on the shape of the action potential and the magnitude of some selected currents; however, they did not perform the control analysis of the system and did not deal with the variables analyzed in the present study.

The discussed model was implemented by us in the Fortran programming language. In order to check that no errors were made during the implementation several system properties, including those presented in Fig. 2, were simulated and compared with the simulations presented in the original paper [12]. The complete description of the model is also given in the above publication.

The control coefficient of particular steps \(X_i\) (channels, pumps, exchangers) over different variables \(Y_j\) (amplitude and duration of the action potential, area under the Ca\(^{2+}\) peak, average fluxes through particular channels) were calculated using the following equation:

\[
C_{X_i}^{Y_j} = \frac{\Delta Y_j/Y_j}{\Delta X_i/X_i}
\]

In subsequent computer simulations the original rate constants of particular steps were increased by a relative factor of 0.01 (by 1 %) and the relative changes in the variable \(Y_j\) between the original and the new quasi-steady-state were recorded. In subsequent series of variable estimation \(Y\) represented: A. action potential amplitude (the maximal difference, in mV, between the peak action potential and the resting potential: compare Fig. 2); B. action potential duration (apd50) (width of the action potential peak, in ms, at 50 % of the maximal difference between the action potential and the resting potential: compare Fig. 2) representing the actual time span of electric excitation during each cycle; C. area under the Ca\(^{2+}\) pulse (the integral of Ca\(^{2+}\) concentration over time after subtracting the resting Ca\(^{2+}\) concentration) generally representing the potential magnitude of influence on Ca\(^{2+}\) sensitive subsystems (see Fig. 2); D. average fluxes (the net amounts of translocated charges related to some particular ion divided by the oscillation period, in pA) through selected elements of the system during the whole pulse period (400 ms), which quantitatively represent the contribution of particular elements to the overall ion-cycling activity of the system. The first three of the above mentioned variables are often easily assessed during a typical cell behaviour recording experiment.
Theoretical results

The ion circulation system in cardiac myocytes presented in Fig. 1 is an extremely 'branched' system in the sense that there are many independent ways in which particular ions can be transported across the plasma membrane and sarcoplasmic reticulum membrane. Additionally, apart from two exchangers, namely the ATP-driven Na\(^+\)/K\(^+\) pump (NaK) and Na\(^+\)/Ca\(^{2+}\) exchanger (NaCa), the transport of Na\(^+\), K\(^+\) and Ca\(^{2+}\) ions is independent. Therefore, different channels affect each other only via a common ion transported and, indirectly, via the changes in the membrane potential related to the ion redistribution across the plasma membrane. For this reason it is very important to know the values of the ionic currents through particular channels. Fig. 3 presents the simulated average (in pA) currents (fluxes) during one cycle (400 ms). The ion flow through particular channels is either exclusively or predominantly unidirectional during the cycle (not shown, see also [12]), and therefore the net ion movement is (almost) identical with the total ion movement. It can be seen that the greatest currents are conducted by the sarcoplasmic reticulum pump and channels: SRU, SRL, RyR and SRT. From among the plasma membrane channels, the greatest K\(^+\) currents are conducted by NaK and K1 channels, the greatest Na\(^+\) currents are conducted by NaK and bNSC channels, while the greatest Ca\(^{2+}\) currents are conducted by NaCa and CaL channels. One can expect that these channels play an important role in determining the dynamic properties of the system.

Fig. 4 presents the simulated control coefficients of particular elements of the system over the action potential amplitude. It can be seen that an increase in the activity of the ATP-driven Na\(^+\)/K\(^+\) pump (NaK) increases this amplitude, while an activation of Na channel, bNSC channel and CaL channel moderately decreases the amplitude. The activities of other channels have a much smaller impact on the magnitude of the action potential amplitude. Some channels, namely Ks, Kr, CaT, Cab, to, Kpl, l(Ca) and KATP, have apparently no control.

Fig. 5 shows the control exerted by particular channels on the duration of the action potential (peak width at 50 % of the action potential amplitude). One can see that the pattern of control is significantly different here than in the case of the action potential amplitude. bNSC, K1 and Na channels have a strong negative control, while NaK pump exerts a moderate positive control over the duration of the action potential. Moreover, the absolute values of control coefficients are generally greater here than in the previous case (the action potential duration is more sensitive to the channel activities than the action potential magnitude).

The simulated control coefficients of particular channels over the area under the Ca\(^{2+}\) peak are presented in Fig. 6. Again, the control is distributed among several steps, from among which the NaK pump exerts a strong negative control, the bNSC exchanger and CaL channel exert a moderate positive control, while the control kept by other channels is significantly smaller. And again, several channels, namely Ks, Kr, Kpl, l(Ca), to, KATP, Cab and CaT seem to keep essentially no control.
It also has to be noted that these control coefficient values are much greater than in the previous two cases, meaning that the behaviour of the calcium handling subsystem is much more sensitive to the changes in the activities of the ion transporting elements than the action potential generation subsystem.

Fig. 7 presents the simulated flux control coefficients of particular elements of the system quantifying the control over average ion currents exerted by the ion transporters that have the most significant control over the action potential amplitude and duration, and over the calcium pulse (Na, NaK, K1 and bNSC) and, not surprisingly, also have high values of ion fluxes. It can be seen that the control over the ion fluxes through these channels is distributed mostly among just these channels. The only major exception is the relatively high control of CaL channel over the flux through the NaK channel.
Discussion

The present paper analyses the control exerted by particular components (channels, pumps, exchangers) of the cyclic quasi-steady-state ion transport system in isolated cardiac myocytes over several variables including the amplitude and duration of the action potential, the area under the calcium transient and average fluxes through particular channels during one oscillation period. It is demonstrated that, first, it is possible to determine the pattern of metabolic control not only in a steady-state system, but also in a quasi-steady-state system with periodic changes of fluxes, metabolite concentrations and thermodynamic forces. Second, the control is distributed among many (essentially all) components of the system, like in the majority of the systems analysed using Metabolic Control Analysis. Third, different variables are controlled to different extent by different components of the system. K\(^+\), Na\(^+\) and Ca\(^{2+}\) are transported by several alternative ways: channels, pumps and exchangers. However, as it can be seen in Fig. 3, the average fluxes during one cycle through these ways can be very different for a given ion. In some cases the transport of two ions is coupled and has a fixed stoichiometry (NaK pump, NaCa exchanger), and in some cases an independent (without a fixed stoichiometry) flow of two or three ions through one channel occurs (CaL, bNSC, l(Ca), to, Ks, Na). Of course, one can suppose that the channels with the highest average fluxes play the most important role in the system, although the time variations of the fluxes during one oscillation period are also of a great importance, especially for the shape of the action potential and calcium transient.

The amplitude of the action potential is controlled to the greatest extent (Fig. 4) by NaK pump, the element of the system that conducts the highest K\(^+\) and Na\(^+\) fluxes. Also bNSC and Na channels that conduct the second and third greatest Na\(^+\) currents, respectively, exert a significant control over the action potential amplitude. Interestingly, the action potential amplitude is mainly modified via changing the resting potential value and not the peak value of the action potential. This is why the outward sodium currents (hyperpolarizing currents) have a positive control over the amplitude, while the inward sodium currents have a negative control over it.

The negative control over the action potential duration is mostly exerted (Fig. 5) by bNSC, K1 and Na channels and the positive control by NaK channel. All these channels conduct significant K\(^+\) and/or Na\(^+\) fluxes. A negative control seems to be associated with the Na\(^+\) inward and K\(^+\) outward fluxes, while a positive control with the opposite fluxes.

The area under the Ca\(^{2+}\) peak is significantly increased by an increased activity of bNSC, CaL and Na channels and by a decreased activity of NaK and NaCa exchangers. CaL channel conducts an influx of Ca\(^{2+}\) ions from the extracellular space to the cytosol, while the NaCa exchanger conducts the opposite Ca\(^{2+}\) current, and therefore the effect of their activity on the calcium peak size is straightforward. bNSC and Na channels transport Na\(^+\) ions to the cytosol, while NaK pump transports these ions outside the cell. Because the outward Ca\(^{2+}\) transport by NaCa is coupled with a Na\(^+\) influx to the cytosol, a high external and low internal [Na\(^+\)] promotes the outward Ca\(^{2+}\) transport and therefore diminishes the calcium peak. Interestingly, although the SR-related calcium channels conduct the highest fluxes (see Fig. 4), their
control over the area under the Ca\textsuperscript{2+} peak is very moderate. This is mostly caused by the fact that the release of Ca\textsuperscript{2+} from SR to the cytosol through RyR and SRL and the ATP-driven Ca\textsuperscript{2+} uptake by SRU are significantly controlled by the cytosolic Ca\textsuperscript{2+} itself: as it was discussed above high sensitivities to metabolite concentrations (high elasticity coefficients) are associated with low a control over system variables.

The activities of four channels, namely Na, NaK, K1 and bNSC, control to a significant extent the values of the variables analyzed above: the action potential amplitude, action potential duration and area under the calcium peak. Therefore, it is interesting to test what controls the fluxes just through these channels. The simulated flux control coefficients of all components of the system over the currents through Na, NaK, K1 and bNSC, presented in Fig. 7, demonstrate that the control is distributed mostly among these channels themselves. They are elements of the Na\textsuperscript{+} and K\textsuperscript{+} transport system, but not the Ca\textsuperscript{2+} transport system. This suggests that the Na\textsuperscript{+} and K\textsuperscript{+} transport system is kinetically superior in relation to the Ca\textsuperscript{2+} transport system: the former controls the latter via the membrane potential and Na\textsuperscript{+} gradient across the plasma membrane (driving the NaCa exchanger). In fact, the system composed of only these four channels is able to generate the shape of the membrane potential pulse similar to the original one, shown in Fig. 2, although, of course, it can not generate the original Ca\textsuperscript{2+} pulse (simulations not shown).

The simplistic formulation of the adenosinephophates conversion used in the model makes the concentrations of ATP, ADP and P\textsubscript{i} almost constant during the simulated oscillation cycle. However, in reality, some important changes in these concentrations may take place. Therefore, a more sophisticated ATP production block might also contribute to the distribution of the control over the system variables.

Eight channels of eighteen, namely Ks, Kr, CaT, Cab, to, Kpl, l(Ca) and KATP, seem to have essentially no control over the analyzed system variables: the amplitude and duration of the action potential, area under Ca\textsuperscript{2+} peak and average fluxes through particular channels. This fact seems to be strictly associated with the very low currents conducted by these channels (compare Fig. 3). In fact in the simulation in which these channels were eliminated from the system (their activity was assumed to be zero), the general behaviour of the system remained essentially unchanged (simulations not shown). Therefore a question arises, why these channels did appear during the biological evolution and still exist in cardiac myocytes. It is possible that they play some unknown function and/or are activated by some unknown factors in stressing conditions, for instance during an energetic crisis and/or hypoxia. For example, the current conveyed by KATP channel becomes influential only under a heavy cytosolic ATP depletion to the level of 0.5 mM – in such conditions the channel activity increases 100 times when compared to its basic activity under typical 5 mM ATP. It is also interesting that some of these channels (namely Kr, CaT, Cab, KATP and Kpl) tend to control only their own activities with the values of $C_{X_i}^{Y_i}$ very close to 1. This is contrary to the self control coefficients values for the significant channels, for which the changes in their respective activities are compensated, thus leading to only a minor change in fluxes ($C_{X_i}^{Y_i}$ values equal to or smaller than 0.2).
The ion transport system analyzed in the present study is in many aspects very different from a 'typical' metabolic biochemical pathway (leaving apart the fact that it is a cyclic, quasi-steady-state system). First, there are essentially no (or, at best, very few) linear sequential reaction chains here. Second, the system is extremely 'branched' – many different ways of the flow of a given ion through the plasma membrane or sarcoplasmic reticulum membrane are present. Third, there is no chemical conversion of one metabolite into another (if ATP hydrolysis by NaK and SRU is ignored), but only a purely physical flow of different ions from one compartment into another. This architecture of the system has some important dynamic consequences. First, while the channels conducting a common ion can 'communicate' through this ion concentration and the membrane electrical potential, then the channels not conducting common ions can exert an influence on each other only via this potential (in a sense, the membrane potential is a 'common metabolite' for all channels in a given membrane). Second, the summation property can not be applied to the system – the flux control coefficients of all components over particular (average) fluxes do not sum up to unity. Third, the currents of positively-charged ions in one direction force the currents of positively-charged ions in the opposite direction (this is why the system is cyclic and not unidirectional; however, one must bear in mind that the cyclic ion movement is driven by an unidirectional ATP hydrolysis by NaK and SRU).

The presented coefficients of the control over the action potential amplitude, action potential duration and area under the calcium peak exhibit some resemblance to the concentration control coefficients: for instance, they can be either positive or negative and are related to metabolite concentrations and/or thermodynamic potentials. On the other hand, they refer to quasi-steady-state properties of the system and do not fulfill the connectivity property. Therefore, they are certainly not identical with the concentration control coefficients.

One must also bear in mind that the evaluated coefficients represent only a single point in the parameter (activities) space. Thus, a significant change in some activities will usually redefine the values of different control coefficients. This means, that under non-standard conditions the control over different parameters may be shifted to other components of the system. Such a situation may take place, for example, when some genes coding particular ion channels are knocked out or overexpressed or when a permanent inhibition of particular enzymes occurs.

In the current paper we present only a few system variables selected for control analysis; in our simulations, however, several other variables were considered as well, for instance the area under the action potential, calcium peak amplitude or ATP usage by NaK and SRU, and many more (data not shown). In fact, any conceivable, arbitrary variable describing a particular property of the quasi-steady-state system in mind can be used as a target of the control by the dynamic elements of the entity. Of course, the selected variables should hold a potentially useful biological and/or physical meaning. The purpose of the present study was to analyze only some representative examples. Here, we are showing the plausibility and usefulness of the evaluation of control coefficients over arbitrarily defined parameters of systems oscillating with a constant period, thus enabling a quantitative analysis of interdependencies.
between the elements of the entity. However, our approach does not deal with the changes in the frequencies of oscillations; in fact, the oscillation period is defined as a constant in the employed model.

Of course, in the heart in vivo the cardiac cells are not excited by an artificial electrical stimulation, but by neural signals coming from the sinoatrial (SA) node. The frequency of this excitation may vary depending on conditions, for instance on the presence of different hormones or neural signals coming from the brain. However, the discussed excitation is still external and hierarchically superior in relation to the ion circulation system taken into account within the model (additionally, the model does not involve any feedback signals from cardiac myocytes to the pacemaker cells in the SA node). Therefore, the model can not be used to study the control of different system elements over the frequency of the excitation and contraction events.

The knowledge of the control of particular elements of the system over different system variables gives a better insight into the functioning of the system and therefore is important for the pure science. It has also very important potential practical applications [18], possibly in inventing treatment for heart diseases, for instance those related to the heart beating cycle. The model predictions can be potentially tested by analyzing, in the experimental way, the effect of different mutations leading to small changes in particular channel activities or concentrations on different system variables. They may also be validated by using selective inhibitors of particular ion transporters in experiments recording selected system variables.

Summing up, in the present study the control analysis of chosen variables, namely the action potential amplitude, action potential duration, area under Ca$^{2+}$ peak and average fluxes through particular ion channels in the cyclic ion circulation system in isolated cardiac myocytes was performed. It is shown that: 1. the control is distributed among different components of the system (channels, exchangers, pumps); 2. different variables are controlled by different components to a different extent; 3. the Na$^+$ and K$^+$ transport system exerts a significant control over the Ca$^{2+}$ transport system, but not inversely. Generally, it is practically demonstrated that the approach based on Metabolic Control Analysis can be very useful in analyzing not only steady-state, but also cyclic quasi-steady-state systems.
References


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Figure legends

Fig. 1. Scheme of the ion transport system in cardiac myocytes. Particular channels, exchangers and pumps participating in the Na⁺, K⁺ and Ca²⁺ transport across the plasma membrane and sarcoplasmic reticulum membrane are shown (see Abbreviations for the full names of the transporters). The numbers at the arrows indicate stoichiometries; X₀, Xᵢ, ion X in the extracellular and cytosolic compartments, respectively. Note that several channels/exchangers/pumps conduct more than one ion type.

Fig. 2. Simulated changes in transmembrane potential (Vᵐ) and cytosolic calcium concentration during a single oscillation period of 400 ms. The parameters the control over which is analyzed: action potential amplitude ("amplitude"), action potential duration at 50 % of action potential amplitude ("apd50") and area under Ca²⁺ transient (grey area under Ca²⁺ transient with subtracted resting Ca²⁺ concentration) are graphically defined.

Fig. 3. Average currents (net amounts of translocated charges related to some particular ion divided by the duration of the oscillation period, in pA) through particular channels during one cycle period (400 ms). Iₓ, ion current through system element X (see Abbreviations for full ion transporter names). Na⁺, K⁺, Ca²⁺: currents of Na⁺, K⁺ and Ca²⁺ ions through the cellular membrane; SR Ca²⁺: currents of Ca²⁺ ions through the sarcoplasmic reticulum membrane. For the transporters that conduct different ions particular ion currents are presented separately. Note the alternative scale for the SR (sarcoplasmic reticulum) currents.

Fig. 4. Simulated control coefficients of particular system elements over the action potential amplitude. The control coefficients were calculated using Equ. 7, where X represents a given system element activity and Y represents the action potential amplitude (compare Fig. 2). See Abbreviations for full ion transporter names.

Fig. 5. Simulated control coefficients of particular system elements over the action potential duration at 50% height. The control coefficients were calculated using Equ. 7, where X represents a given system element activity and Y represents the action potential duration (compare Fig. 2). See Abbreviations for full ion transporter names.

Fig. 6. Simulated control coefficients of particular system elements over the area under Ca²⁺ transient. The control coefficients were calculated using Equ. 7, where X represents a given system element activity and Y represents the area under Ca²⁺ transient (compare Fig. 2). See Abbreviations for full ion transporter names.

Fig. 7. Simulated flux control coefficients of particular channels over average currents (net amounts of translocated charges related to some particular ion divided by the oscillation period) through four selected
channels (Na, NaK, K1, bNSC). The control coefficients were calculated using Equ. 2. See Abbreviations for full ion transporter names.
Fig 2.

\[ V_m \text{ [mV]} \]

\[ \text{time [ms]} \]

-100 -- 100
0 -- 2

-100 -- 100
0 -- 1.5

\[ Ca^{2+} \text{ [µM]} \]

amplitude

apd 50

Ca\textsuperscript{2+} peak area
Fig 4.

The figure shows a bar graph with control coefficients for various ions and channels. The y-axis represents the control coefficients ranging from -0.05 to 0.075. The x-axis lists the following ions and channel types:

- Na
- NaK
- K1
- bNSC
- Kr
- Col
- NaCa
- Ks
- CaT
- Cab
- KATP
- l(Ca)
- o
- RyR
- SRU
- SRT
- SRL

The graph highlights the influence of these factors on control coefficients.
Fig 6.
Fig 7.