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ARTICLE

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The voltage-dependent anion channel as a biological transistor: theoretical considerations

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Abstract The voltage-dependent anion channel (VDAC) is a porin of the mitochondrial outer membrane with a bell-shaped permeability-voltage characteristic. This porin restricts the flow of negatively charged metabolites at certain non-zero voltages, and thus might regulate their flux across the mitochondrial outer membrane. Here, we have developed a mathematical model illustrating the possibility of interaction between two steady-state fluxes of negatively charged metabolites circulating across the VDAC in a membrane. The fluxes interact by contributing to generation of the membrane electrical potential with subsequent closure of the VDAC. The model predicts that the VDAC might function as a single-molecule biological transistor and amplifier, because according to the obtained calculations a small change in the flux of one pair of different negatively charged metabolites causes a significant modulation of a more powerful flux of another pair of negatively charged metabolites circulating across the same membrane with the VDAC. Such transistor-like behavior of the VDAC in the mitochondrial outer membrane might be an important principle of the cell energy metabolism regulation under some physiological conditions.

Keywords Mathematical modeling · Membrane potential · Mitochondrial outer membrane · Voltage-dependent anion channel

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Introduction

The voltage-dependent anion channel (VDAC) is the main traffic pore in the mitochondrial outer membrane (MOM), allowing metabolite circulation between mitochondria and the cytoplasm. The known ability of the VDAC to change permeability to negatively charged metabolites in response to changes in the electrical potential across the membrane is of great importance to understanding the molecular mechanisms of regulation of the energy metabolism of cells (Colombini 1979; Hodge and Colombini 1997; Rostovtseva and Colombini 1997; Brdiczka et al. 1998; Lemeshko and Lemeshko 2000; Anflous et al. 2001; Bölter and Soll 2001; Sampson et al. 2001; Vander Heiden et al. 2001; Lemeshko 2002).

In order to close the VDAC incorporated in a lipid bilayer membrane, a transmembrane electrical potential higher than 30 mV has to be applied. The voltage sensitivity of the VDAC is significantly increased by a natural protein localized in the intermembrane space of mitochondria (Liu and Colombini 1992). The voltage dependence of the VDAC is highly conserved, which suggests its great physiological importance. On the other hand, a possibility of energy metabolism regulation by the electrical closure of the VDAC is still being questioned, because the electrical potential across the MOM has not yet been experimentally demonstrated. The Donnan equilibrium potential has been proposed as a possible candidate for modulation of VDAC permeability (Mangan and Colombini 1987; Liu and Colombini 1992). Theoretical consideration of the physical principles and possible mechanisms of generation of the electrical potential across porous membranes such MOM seems to be important for the development of experimental approaches in this area.

We have suggested three steady-state mechanisms by which the electrical potential across the MOM might be generated to modulate the VDAC permeability (Lemeshko and Lemeshko 2000; Lemeshko 2002). According

to one of these mechanisms, the diffusion electrical potential results from the difference in the MOM permeability to various charged metabolites (Lemeshko and Lemeshko 2000). A similar mechanism was suggested recently (Moraru and Loew 2003). The second steady-state mechanism is based on a possibility of the division of the inner membrane voltage between the MOM and the electrogenic contact sites between the inner and outer membranes of mitochondria (Lemeshko 2002). The third mechanism by which the MOM electrical potential might be generated is based on a possibility that the hexokinase reaction catalyzed by the VDAC-hexokinase duplex is vectorially organized in the MOM: the duplex uses ATP^{4-} of the intermembrane space of mitochondria, returns ADP^{3-}, and liberates glucose-6-phosphate(1−) into the cytoplasm. In this case the duplex could function as an active pump, which transfers one negative charge across the MOM using the free energy of the hexokinase reaction (Lemeshko 2002).

The possibility of superposition of the membrane electrical potentials generated by various circulating fluxes of charged metabolites and by different mechanisms allows us to suggest that each of these fluxes could change the common electrical potential across the membrane and thus could modulate the VDAC permeability to other negatively charged metabolites. In this work we have developed a mathematical model for computational analysis of the interaction between fluxes of two pairs of different negatively charged metabolites circulating across the VDAC localized in a membrane. The obtained data predict a transistor-like behavior of the VDAC, because small changes in the flux value of one pair of negatively charged metabolites significantly modulate a more powerful flux of another pair of negatively charged metabolites circulating across the same membrane with the VDAC.

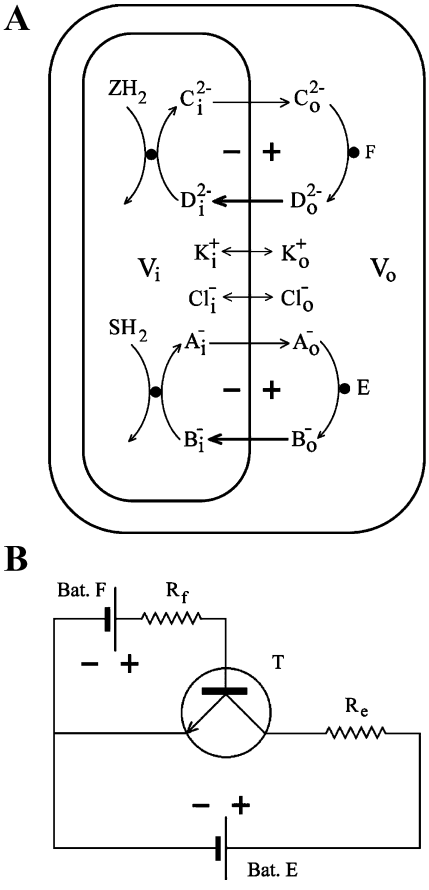

Fig. 1 **A** The biliposomal model explaining a possible interaction between two fluxes of charged metabolites circulating across the same membrane containing the VDAC. According to the model, the steady-state flux of one pare of charged metabolites (C^{2-} and D^{2-}) modulates another flux (A^- and B^-) by influencing the value of the common membrane electrical potential, which modulates the permeability of the VDAC localized in the membrane of the internal liposome. Here, the membrane with the VDAC is more permeable for B^- and D^{2-} than for A^- and C^{2-}, respectively. **B** The common-emitter configuration of a bipolar transistor is shown: a small change in the current through resistance R_f and the base-emitter modulates a more powerful current through resistance R_e and the collector-base

Methods

Description of the model

Let us imagine two different steady-state processes occurring in the system of two liposomes, in which one liposome is enclosed inside another (Fig. 1). One of the processes in the external liposome is the conversion of metabolite A^- to B^- catalyzed by the enzyme E. The maximal activity of the enzyme E is $v_{m,A}$. Another process is the conversion of C^{2-} to D^{2-} catalyzed by the enzyme F with the maximal activity $v_{m,C}$. The rates of both of enzymatic reactions are described by the following equations:

$$v_A = \frac{v_{m,A}\left(\frac{[A^-]_o}{K_{m,A}} - k_A\frac{[B^-]_o}{K_{m,B}}\right)}{1 + \frac{[A^-]_o}{K_{m,A}} + \frac{[B^-]_o}{K_{m,B}}} \quad (1)$$

$$v_C = \frac{v_{m,C}\left(\frac{[C^{2-}]_o}{K_{m,C}} - k_C\frac{[D^{2-}]_o}{K_{m,D}}\right)}{1 + \frac{[C^{2-}]_o}{K_{m,C}} + \frac{[D^{2-}]_o}{K_{m,D}}} \quad (2)$$

The coefficients k_A and k_C are included in Eqs. (1) and (2) to vary the rates of the reverse reactions catalyzed by the enzymes E and F, respectively. The values of $v_{m,A}$ and $v_{m,C}$ were varied to study their influence on the model behavior. The constants $K_{m,A}$ and $K_{m,C}$ were varied, and the constants $K_{m,B}$ and $K_{m,D}$ were set equal to 1 mM. The average concentrations of charged metabolites in the system were set at 5–20 mM for the sum of metabolites A^- and B^-, as well as for the sum of metabolites C^{2-} and D^{2-}.

The volume of the internal liposome (V_i) was set at 0.03 fL and the volume of the external liposome (V_o) was set at 0.36 fL (V_o is the volume between the internal and external membrane of the biliposomal model) (Fig. 1A). These volumes are equal, respectively, to the volume of the intermembrane space of one average mitochondrion (V_i) and to the sum of cytoplasmic and myofibrillar compartment volumes (V_o) relating as 1:12 (V_i:V_o) in cardiomyocytes of rat heart (Smith and Page 1976; Saks and Aliev 1996). These values were used in our model for generation of the metabolically derived potential across the MOM (Lemeshko and Lemeshko 2000).

Inside the inner liposome, the conversion of B^- to A^- is coupled to oxidation of a substrate, SH_2, while the conversion of D^{2-} to C^{2-} is coupled to oxidation of ZH_2. For simplicity, the ratios $[A^-]_i/[B^-]_i = 100$ and $[C^{2-}]_i/[D^{2-}]_i = 100$ are maintained constant in the internal liposome at any $v_{m,A}$ and $v_{m,C}$ in the

external liposome, but the influence of the values of these ratios on the behavior of the model was evaluated. The system also contains K^+ and Cl^- ions. The membrane of the internal liposome contains the VDAC allowing the passage of the ions A^-, B^-, C^{2-}, D^{2-}, K^+, and Cl^-. The VDAC permeability for charged metabolites A^-, B^-, C^{2-}, and D^{2-} may be described by the Goldman equation:

$$J_A = P_A \frac{\Delta\phi F}{RT} \frac{[A^{2-}]_o - [A^-]_i \exp(-\Delta\phi F/RT)}{1 - \exp(-\Delta\phi F/RT)} \quad (3)$$

$$J_B = P_B \frac{\Delta\phi F}{RT} \frac{[B^-]_o - [B^-]_i \exp(-\Delta\phi F/RT)}{1 - \exp(-\Delta\phi F/RT)} \quad (4)$$

$$J_C = P_C \frac{2\Delta\phi F}{RT} \frac{[C^{2-}]_o - [C^{2-}]_i \exp(-2\Delta\phi F/RT)}{1 - \exp(-2\Delta\phi F/RT)} \quad (5)$$

$$J_D = P_D \frac{2\Delta\phi F}{RT} \frac{[D^{2-}]_o - [D^{2-}]_i \exp(-2\Delta\phi F/RT)}{1 - \exp(-2\Delta\phi F/RT)} \quad (6)$$

where $\Delta\phi$ is the membrane electrical potential expressed in volts, F is the Faraday constant, R is the gas constant, $T = 310$ K, and P_A, P_B, P_C, and P_D are the permeability coefficients for A^-, B^-, C^{2-}, and D^{2-}, respectively. These permeability coefficients of the internal liposome membrane with the VDAC may be described by the following equations (Lemeshko and Lemeshko 2000):

$$P_A = 0.72 \times [0.1 + 0.9 \exp\{-(a\Delta\phi)^2\}] \quad (7)$$

$$P_B = 3.6 \times [0.1 + 0.9 \exp\{-(a\Delta\phi)^2\}] \quad (8)$$

$$P_C = 0.36 \times [0.1 + 0.9 \exp\{-(a\Delta\phi)^2\}] \quad (9)$$

$$P_D = 3.6 \times [0.1 + 0.9 \exp\{-(a\Delta\phi)^2\}] \quad (10)$$

according to which the membrane electrical potential $\Delta\phi$ closes the VDAC, thus decreasing its permeability to 10–100% of that in the open state. Here, the maximal permeability of the membrane in the open state of the VDAC was set equal to 3.6 fL s^{-1}, and $a = 300$ V^{-1}, both as in our previous model (Lemeshko and Lemeshko 2000), where a is the voltage sensitivity of the VDAC permeability. The permeability coefficient P_B was arbitrary set five times higher than P_A, and the coefficient P_D was ten times higher than P_C.

The difference in the VDAC permeability to various charged metabolites has been included in the model to allow the generation of the metabolically derived membrane potential, as described earlier (Lemeshko and Lemeshko 2000). The VDAC has been reported to have different permeabilities to various charged metabolites even in the open state, which dramatically diminish in the close state (Hodge and Colombini 1997; Rostovtseva and Colombini 1997). The coefficient a in Eqs. (7)–(10) allows us to set the voltage sensitivity of the VDAC permeability. The relative dependence of the VDAC permeability on the membrane electrical potential, according to this mathematical simulation at $a = 300$ V^{-1}, has been calculated earlier (Lemeshko and Lemeshko 2000; Lemeshko 2002).

The VDAC permeability for K^+ and Cl^- may be considered infinitely high; thus their electrochemical equilibria may be established according to the Nernst equation:

$$\Delta\phi = -\frac{RT}{F} \ln \frac{[K^+]_i}{[K^+]_o} \quad (11)$$

$$\Delta\phi = \frac{RT}{F} \ln \frac{[Cl^-]_i}{[Cl^-]_o} \quad (12)$$

The space-charge neutrality principle requires:

$$[K^+]_i - [Cl^-]_i - [A^-]_i - [B^-]_i - 2[C^{2-}]_i - 2[D^{2-}]_i = 0 \quad (13)$$

If we set the average concentration of the sum of metabolites A^- and B^- at 20 mM and the average concentration of the sum of C^{2-} and D^{2-} at 10 mM, this may be described as:

$$\frac{([A^-]_i + [B^-]_i)V_i + ([A^-]_o + [B^-]_o)V_o}{V_i + V_o} = 0.02 \text{ M} \quad (14)$$

$$\frac{([C^{2-}]_i + [D^{2-}]_i)V_1 + ([C^{2-}]_o + [D^{2-}]_o)V_2}{V_1 + V_2} = 0.01 \text{ M} \quad (15)$$

Setting the average concentration of Cl^- to be constant and equal to 60 mM:

$$\frac{[Cl^-]_i V_i + [Cl^-]_o V_o}{V_i + V_o} = 0.06 \text{ M} \quad (16)$$

requires the average concentration of K^+ to be equal to 100 mM:

$$\frac{[K^+]_i V_i + [K^+]_o V_o}{V_i + V_o} = 0.1 \text{ M} \quad (17)$$

which makes the total charge of the system equal to zero.

At steady state, the equality of the following fluxes has to exist:

$$J_B = -J_A = v_A \quad (18)$$

$$J_D = -J_C = v_C \quad (19)$$

The system of Eqs. (1)–(19) was solved using numerical methods included in the software Mathcad Professional 2001i.

Results

The system composed of two liposomes, one enclosed inside another, and the VDAC localized in the membrane of the internal liposome (Fig. 1A) has been theoretically analyzed with the aim to study a possible interaction between two independent fluxes of charged metabolites circulating across the membrane. In this biliposomal model, the inner liposome represents a mitochondrial intermembrane space (V_i) and the outer liposome the cytoplasm (V_o). It has been assumed that the interaction between two fluxes could be realized owing to the modulation of the VDAC permeability by the common membrane potential generated by both circulating fluxes. Mathematical simulation of the biliposomal model (Fig. 1A) revealed that the VDAC behaves as a transistor (Fig. 1B). Such a phenomenon might be an important principle for regulation in biological systems.

The biliposomal model (Fig. 1A) was studied by varying the maximal activities of the enzyme E ($v_{m,A}$) in the range up to 12 amol s^{-1}, which is comparable with the maximal rate of ATP synthesis (6.7 amol s^{-1}) and with the rate of the reverse creatine kinase reaction (13.3 amol s^{-1}) of an average rat heart mitochondrion (see Lemeshko and Lemeshko 2000 for references). The maximal activity of the enzyme F ($v_{m,C}$) was significantly less than the maximal activity of the enzyme E. Initially, the model was calculated at the following conditions: (1) 20 mM average concentration of the sum of metabolites A^- and B^-; (2) 10 mM average concentration of the sum of metabolites C^{2-} and D^{2-}; (3) $[A^-]_i/[B^-]_i = 100$ and $[C^{2-}]_i/[D^{2-}]_i = 100$; (4) $K_{m,A} = 1$ mM, $K_{m,B} = 1$ mM, $K_{m,C} = 1$ mM, and $K_{m,D} = 1$ mM; (5) $k_A = 0.01$ and $k_C = 0.01$.

When only the enzyme E is active, the calculated steady-state flux of metabolites A^- and B^- across VDAC

in the membrane of the internal liposome (J_A) almost linearly increases with an increase in $v_{m,A}$ up to 12 amol s^{-1} (Fig. 2A, at $v_{m,C}=0$). If we also turn on the cyclic flux J_C of the metabolites C^{2-} and D^{2-}, by setting the maximal activity $v_{m,C}$ of the enzyme F in the range 0–1 amol s^{-1}, a significant restriction of the flux J_A is observed. At $v_{m,A}=8$ amol s^{-1}, the restriction of J_A by the flux J_C takes place at $v_{m,C}=1$ amol s^{-1} (Fig. 2A) or higher (data not shown). At $v_{m,A}=12$ amol s^{-1}, the restriction of J_A is obtained at the values of $v_{m,C}=0.7$ amol s^{-1} or higher. This effect of modulation of one cyclic flux of charged metabolites by another significantly increases with diminishing of $K_{m,A}$ from 1 mM to 0.25 mM (Fig. 1B) or to 0.1 mM (data not shown). A two-fold increase in the permeability coefficients P_C and P_D, from 0.36 to 0.72 fL s^{-1}, and from 3.6 to 7.2 fL s^{-1}, respectively, only slightly diminishes the modulation effect (Fig. 2C).

The observed effect is also revealed at significantly decreased average concentrations of negatively charged metabolites: 5 mM for the sum of metabolites A^- and B^- instead of 20 mM, and 5 mM for the sum of metabolites C^{2-} and D^{2-} instead of 10 mM, at $K_{m,A}=0.25$ mM and $K_{m,C}=0.10$ mM (Fig. 3).

The interaction between two cyclic fluxes of negatively charged metabolites, owing to their influence on the common membrane potential, takes place in the broad range of changes in the ratios of concentrations of these metabolites in the internal liposome of the biliposomal model at the following conditions: 10 mM average concentration of the sum of metabolites A^- and B^-, 10 mM average concentration of the sum of metabolites C^{2-} and D^{2-}, $K_{m,A}=0.1$ mM, and $K_{m,C}=1.0$ mM (Fig. 4). The decrease of the ratio $[C^{2-}]_i/[D^{2-}]_i$ from 100 (Fig. 4A) to 10 only slightly decreases the observed effect (Fig. 4B). At the ratio $[C^{2-}]_i/[D^{2-}]_i=1000$ or higher, the modulation of one flux by another becomes even stronger (data not shown) than at $[C^{2-}]_i/[D^{2-}]_i=100$. The decrease of the ratio $[A^-]_i/[B^-]_i$ from 100 (Fig. 4A) to 10 causes a significant decrease of interaction between two circulating fluxes (Fig. 4C), but no decrease was obtained when this ratio was increased up to 1000 or higher (data not shown). In the considered biliposomal model, the modulation of one circulating flux by another only slightly depends on the rates of the reverse reactions in the external liposome: the modulation effect was well expressed even at $k_A=1.0$ instead of

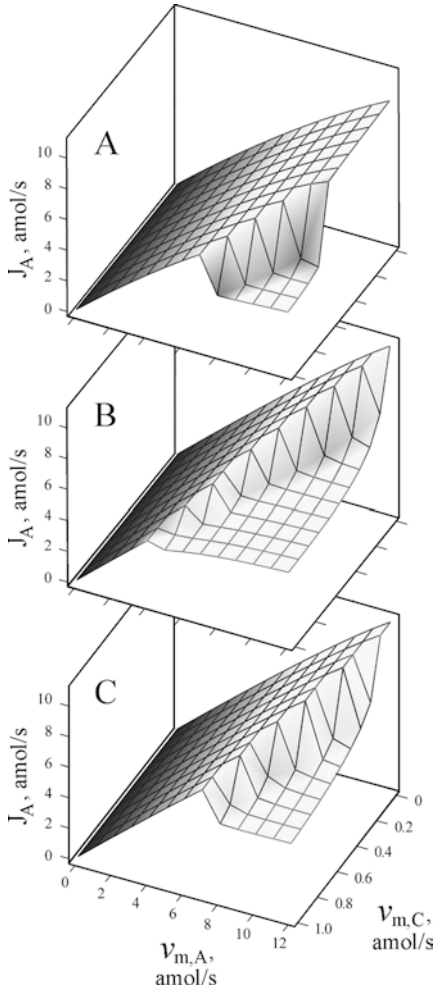

Fig. 2 The dependence of the steady-state flux J_A of metabolites A^- and B^- circulating across the VDAC in the membrane of the internal liposome (Fig. 1A) on the maximal activities of the enzyme F ($v_{m,C}$) and the enzyme E ($v_{m,A}$), according to the system of Eqs. (1)–(19) at the following conditions: **A** the average concentration of the sum of metabolites A^- and B^- is 20 mM, the average concentration of the sum of metabolites C^{2-} and D^{2-} is 10 mM, $[A^-]_i/[B^-]_i=100$, $[C^{2-}]_i/[D^{2-}]_i=100$, $K_{m,A}=1$ mM, $K_{m,B}=1$ mM, $K_{m,C}=1$ mM, $K_{m,D}=1$ mM, $k_A=0.01$, $k_C=0.01$; **B** the values are as in **A** except $K_{m,A}=0.25$ mM; **C** the values are as in **A** except the coefficients of maximal permeability in Eqs. (9) and (10) were increased twice, up to 0.72 and 7.2 fL s^{-1}, respectively

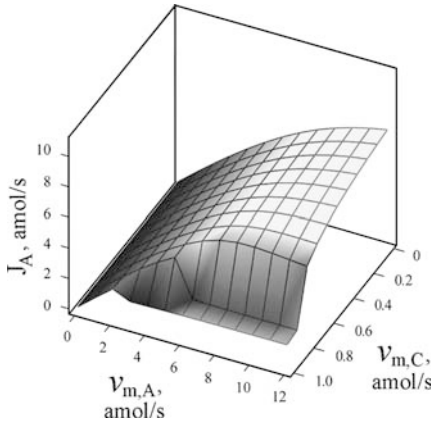

Fig. 3 The dependence of the steady-state flux J_A of metabolites A^- and B^- circulating across the VDAC in the membrane of the internal liposome (Fig. 1A) on the maximal activities of the enzyme F ($v_{m,C}$) and the enzyme E ($v_{m,A}$), according to the system of Eqs. (1)–(19) at the following conditions: the average concentration of the sum of metabolites A^- and B^- is 5 mM, the average concentration of the sum of metabolites C^{2-} and D^{2-} is 5 mM, $[A^-]_i/[B^-]_i=100$, $[C^{2-}]_i/[D^{2-}]_i=100$, $K_{m,A}=0.25$ mM, $K_{m,B}=1$ mM, $K_{m,C}=0.1$ mM, $K_{m,D}=1$ mM, $k_A=0.01$, $k_C=0.01$

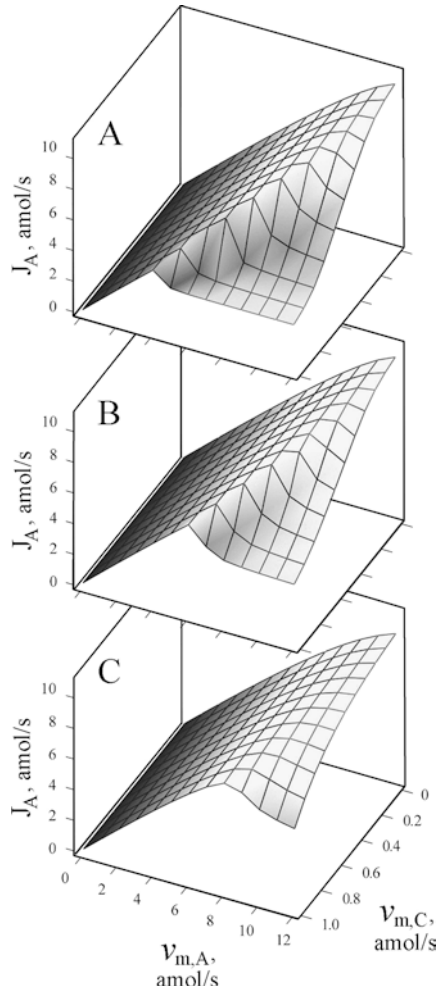

Fig. 4 The influence of the decrease of the ratios $[A^-]_i/[B^-]_i$ and $[C^{2-}]_i/[D^{2-}]_i$ on the effect of modulation of one flux of charged metabolites by another one circulating across the VDAC in the inner membrane of the biliposomal model (Fig. 1A) under the following conditions: **A** the average concentration of the sum of metabolites A^- and B^- is 10 mM, the average concentration of the sum of metabolites C^{2-} and D^{2-} is 10 mM, $[A^-]_i/[B^-]_i = 100$, $[C^{2-}]_i/[D^{2-}]_i = 100$, $K_{m,A} = 0.1$ mM, $K_{m,B} = 1$ mM, $K_{m,C} = 1$ mM, $K_{m,D} = 1$ mM, $k_A = 0.01$, $k_C = 0.01$; **B** as in **A**, except $[C^{2-}]_i/[D^{2-}]_i = 10$; **C** as in **A**, except $[A^-]_i/[B^-]_i = 10$

$k_A = 0.01$ (Fig. 5A), or at $k_C = 1.0$ instead of $k_C = 0.01$ (Fig. 5B), in comparison with the control, $k_A = 0.01$ and $k_C = 0.01$ (Fig. 4A).

The calculations demonstrate that a transistor-like effect of amplification is realized in the considered model. The amplification coefficient for our biliposomal model may be defined as the ratio of the change in the flux J_A (ΔJ_A) in response to the change in the maximal activity of the enzyme F ($\Delta v_{m,C}$), which influences the value of the smaller flux J_C according to Eqs. (2) and (19). The amplification coefficient ($-\Delta J_A/\Delta v_{m,C}$) is equal to 35 in the range of $v_{m,C}$ between 0.7 and 0.8 amol s^{-1} at $v_{m,A} = 8$ amol s^{-1}, or it is equal to 20 in the range of $v_{m,C}$ between 0.5 and 0.7 amol s^{-1} at $v_{m,A} = 10$ amol s^{-1} (Fig. 4A).

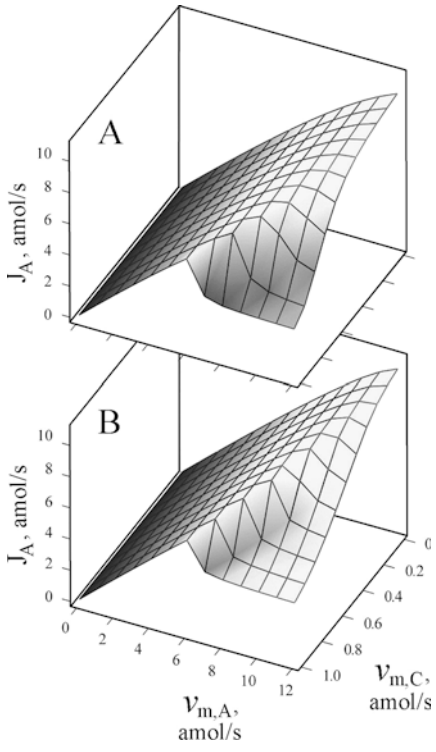

Fig. 5 The influence of the rates of the reverse reactions catalyzed by the enzymes F and E on the effect of modulation of one flux of charged metabolites by another one circulating across the VDAC in the inner membrane of the biliposomal model (Fig. 1A) under the following conditions: **A** as in Fig. 4A, except k_A was changed from 0.01 to 1; **B** as in Fig. 4A, except k_C was changed from 0.01 to 1; control, Fig. 4A, where both $k_A = 0.01$and $k_C = 0.01$

Computational analysis of the biliposomal model (Fig. 1A) demonstrates that two apparently independent fluxes of two different pairs of charged metabolites, circulating across the VDAC localized in the membrane of the internal liposome, interact with one

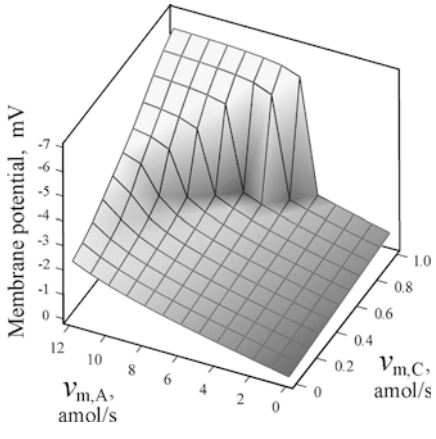

Fig. 6 The dependence of the steady-state value of the electrical potential across the internal membrane of the biliposomal model (Fig. 1A) on the maximal activities of the enzyme F ($v_{m,C}$) and the enzyme E ($v_{m,A}$) at the conditions described for Fig. 4A

another via the common electrical potential across the membrane (Fig. 6). This interaction results from the superposition of the electrical potentials generated by each of these circulating fluxes and it becomes stronger when the common transmembrane electrical potential begins to close the VDAC. As the VDAC closes, it decreases the permeabilities for the metabolites, which in turn allows increasing the generated membrane potential even further. One can see that remarkable restriction of J_A at $v_{m,A} = 10$ amol s^{-1} (Fig. 4A) takes place when $v_{m,C}$ increases from 0.5 to 0.7 amol s^{-1}, leading to a significant increase in the membrane potential (Fig. 6).

At the membrane potential value near the region of the high steepness of the permeability-voltage characteristic of the VDAC, the channel becomes less permeable (Mangan and Colombini 1987; Liu and Colombini 1992). Diminishing permeability of the VDAC allows the generation of higher electrical potential across the membrane (Lemeshko and Lemeshko 2000), thus causing in turn further a decrease of the VDAC permeability, as in a positive feedback loop. Owing to the positive feedback control, the superposition of the potentials generated by two different circulating fluxes is non-additive. According to the data in Fig. 6, the generated membrane potential is equal to only −0.9 mV at $v_{m,A} = 6$ amol s^{-1} and $v_{m,C} = 0$ amol s^{-1}, or to only −0.5 mV at $v_{m,A} = 0$ amol s^{-1} and $v_{m,C} = 0.9$ amol s^{-1}, but it increases up to the value −5.5 mV in a non-additive manner when both of these fluxes are circulating simultaneously at $v_{m,A} = 6$ amol s^{-1} and $v_{m,C} = 0.9$ amol s^{-1} (Fig. 6).

The model was also calculated using four-charge metabolites A^{4-} and B^{4-}, instead of one-charge metabolites A^- and B^-, at the following conditions: (1) 10 mM average concentration of the sum of metabolites A^{4-} and B^{4-} (like the concentration of ATP in the sarcoplasm of cardiomyocytes); (2) 20 mM average concentration of the sum of metabolites C^{2-} and D^{2-} (like the concentration of phosphocreatine in the sarcoplasm of cardiomyocytes); (3) $[A^{4-}]_i/[B^{4-}]_i = 100$ and $[C^{2-}]_i/[D^{2-}]_i = 100$; (4) $K_{m,A} = 0.5$ mM, $K_{m,B} = 1$ mM, $K_{m,C} = 0.5$ mM, and $K_{m,D} = 1$ mM; (5) $k_A = 0.01$ and $k_C = 0.01$. In Eqs. (3) and (4), $4\Delta\phi$ replaced $\Delta\phi$. Equations (7) and (9) were modified to decrease the permeability of the VDAC to A^{4-} in the closed state (Eq. 20) and to increase the permeability to C^{2-} in the closed and open states of the VDAC (Eq. 21):

$$P_A = 0.72 \times [0.05 + 0.95 \exp\{-(a\Delta\phi)^2\}] \quad (20)$$

$$P_C = 0.72 \times [0.1 + 0.9 \exp\{-(a\Delta\phi)^2\}] \quad (21)$$

To adjust the total charge of the system to zero, the average concentration of K^+ in Eq. (17) was set equal to 0.14 M. To satisfy the space-charge neutrality principle, Eq. (13) was modified as:

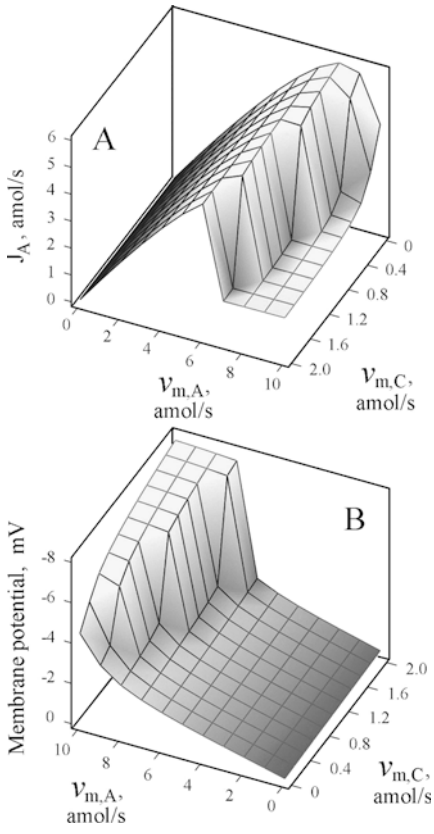

Fig. 7 The dependence of the steady-state flux J_A of metabolites A^{4-} and B^{4-} (**A**) circulating across the VDAC in the membrane of the internal liposome (like A^- and B^- in Fig. 1A) and of the value of generated membrane potential (**B**) on the maximal activities of the enzyme F ($v_{m,C}$) and the enzyme E ($v_{m,A}$) at the following conditions: the average concentration of the sum of metabolites A^{4-} and B^{4-} is 10 mM, the average concentration of the sum of metabolites C^{2-} and D^{2-} is 20 mM, $[A^{4-}]_i/[B^{4-}]_i = 100$, $[C^{2-}]_i/[D^{2-}]_i = 100$, $K_{m,A} = 0.5$ mM, $K_{m,B} = 1$ mM, $K_{m,C} = 0.5$ mM, $K_{m,D} = 1$ mM, $k_A = 0.01$, $k_C = 0.01$

$$[K^+]_i - [Cl^-]_i - 4[A^{4-}]_i - 4[B^{4-}]_i - 2[C^{2-}]_i - 2[D^{2-}]_i = 0 \quad (22)$$

According to calculations at such conditions, a small increase in the maximal activity of the enzyme F ($v_{m,C}$) significantly restricts the flux J_A (Fig. 7A) owing to the generation of a higher membrane potential (Fig. 7B). At higher values of J_A, lower values of $v_{m,C}$ are needed to modulate the flux J_A.

Discussion

Computational analysis of the biliposomal model (Fig. 1A) shows that a small change in a relatively weak flux of one pair of negatively charged metabolites across the VDAC in the membrane of the internal liposome can significantly modulate a more powerful flux of another pair of charged metabolites across the same membrane.

Such behavior is similar to that of a transistor (Fig. 1B), in which a little change in the base-emitter current modulates a significantly higher current in the collector-emitter circuit. In the biliposomal model in Fig. 1A, a small change in the flux J_C causes a significantly higher change in the flux J_A (Figs. 2, 3, 4, 5). The amplification effect depends on the absolute value of J_A (Fig. 4) and on the permeability-voltage sensitivity of the VDAC, i.e. on the parameter a, which was set equal to 300 V^{-1}, as in our previous model (Lemeshko and Lemeshko 2000). No amplification was obtained at $a=0$ or at relatively low values of this parameter in the selected range of changes in the flux J_A (data not shown), i.e. in the range of possible rates of ATP synthesis by one average mitochondrion of rat heart. Interestingly, a peptide found in the intermembrane space of mitochondria, which significantly increases the permeability-voltage sensitivity of the VDAC (Liu and Colombini 1992), could modulate the amplification coefficient discussed here.

The generation of the metabolically dependent electrical potential across the MOM (Lemeshko and Lemeshko 2000) might explain the restriction of ATP and phosphocreatine fluxes between mitochondria and the cytoplasm observed at the cellular level (Vander Heiden et al. 2001). The data for calculation of the biliposomal model for the four-charge and two-charge metabolites, presented in Fig. 7, allow us to assume that the flux of phosphocreatine(2−) across the MOM during the contraction cycle of the heart might temporally restrict the flux of ATP^{4-} across the MOM to the cytoplasm, thus representing one of the possible mechanisms of the energy channelling in the cells suggested by other authors (Saks et al. 1994, 1995 and references therein).

Recently, the generation of the electrical potential across the MOM by the inner membrane of mitochondria was suggested to explain the Crabtree effect (Lemeshko 2002). In this mechanism, one also may expect a transistor-like behavior of the system, because the MOM electrical potential could be generated by a relatively small electrogenic flux of ATP^{4-} in exchange for ADP^{3-} across the contact sites formed by the adenine nucleotide translocator and the VDAC. Because hexokinase II in the tumor cell is also associated with these contact sites, glucose at high concentration might significantly activate the ATP and ADP circulation across the contact sites, causing an increase in the fraction of the inner membrane voltage applied to the MOM, thus closing the VDACs beyond the contacts (Lemeshko 2002). That should in turn inhibit the main flux of ATP beyond the contacts, i.e. the flux of ATP from the mitochondrial matrix to the cytoplasm across the free adenine nucleotide translocator in the inner membrane, across the intermembrane space and the free VDACs in the MOM.

The obtained theoretical data demonstrate the possibility that the VDAC in the MOM might function as a single-molecule biological transistor, allowing mutual interaction between circulating fluxes of charged metabolites due to their influence on the value of the common electric potential across the MOM. Computational analysis of the biliposomal model shows that the probability of generation of a relatively high electrical potential across the membrane with the VDAC significantly increases when two fluxes of charged metabolites circulate simultaneously, instead of only one of them. For mitochondria in the cell, that seems to be even more probable owing to simultaneous circulation of many different fluxes of charged metabolites across the MOM.

It is probable that some physiological phenomena of the energy metabolism regulation in the cells are based on the developed principle of interaction between two fluxes of charged metabolites circulating across the MOM, on the suggested mechanisms of the electrical potential generation across the MOM (Mangan and Colombini 1987; Liu and Colombini 1992; Lemeshko and Lemeshko 2000; Lemeshko 2002), and on the most general concept of the structural and functional channelling of the energy metabolism in the cells (Saks et al. 1994, 1995). Other voltage-gating channels in biological membranes might also function in a transistor-like manner that seems to be an important principle of biological regulation and signaling, similar to the importance of transistor development in electronics.

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