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Minireview

Molecules and Cells

Modeling the Cardiac Na^+/H^+ Exchanger Based on Major Experimental Findings

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Na^+-H^+ exchanger (NHE) is the main acid extruder in cardiac myocytes. We review the experimental findings of ion-dependency of NHE activity, and the mathematical modeling developed so far. In spite of extensive investigation, many unsolved questions still remain. We consider that the precise description of NHE activity with mathematical models elucidates the roles of NHE in maintaining ionic homeostasis, especially under pathophysiological conditions.

INTRODUCTION

The cellular energy metabolism constantly generates CO_2 which is combined with H_2O and converted into HCO_3^- and H^+. Therefore, removal of H^+ from cytoplasm is a vital process for maintaining the physiological intracellular pH (pH_i ~7.2). Failure in the pH_i homeostasis largely affects overall cellular functions through interaction of H^+ with reactive sites of a majority of cellular proteins. In the cardiac myocytes, the electrical excitation, ion homeostasis including Ca^{2+} and myofilaments sliding are all modified with variation in pH_i (Bountra and Vaughan-Jones, 1989; Choi et al., 2000; Harrison et al., 1992; Orchard and Kentish, 1990). Under the physiological condition, pH_i of cardiac myocyte is regulated by four H^+ equivalent transporters: Na^+-H^+ exchanger (NHE), Na^+-HCO_3^- cotransporter (NBC), Cl^--HCO_3^- exchanger (AE) and Cl^--OH^- exchanger (CHE) (Leem et al., 1999). Among them, the main acid extruder, NHE, has been extensively studied. The H^+ extrusion by NHE is driven by the Na^+ concentration gradient across the membrane. Therefore, the activity is largely dependent on the Na^+ homeostasis, which is determined by a balance between the active transporter Na^+/K^+ pump and all passive movements of Na^+ via ion channels as well as secondary ion exchangers such as the Na^+/Ca^{2+} exchange which is the major pathway for pumping out the intracellular Ca^{2+}. Furthermore, the activity of NHE is dependent on Na^+ and H^+ on both sides of the membrane, especially NHE is activated by the intracellular acidification, but may be inactivated by the extracellular acidification. Thus, to estimate the contribution of NHE to the pH_i homeostasis under various pathophysiological conditions, system biological analysis is required using cell models which incorporate all functional units involved in addition to the NHE model. Up to date, several kinds of mathematical models have been developed. This paper briefly reviews characteristics of NHE described in experimental studies, and then compares the mathematical NHE models.

Ion dependency of NHE disclosed in experimental studies

NHE is an electroneutral antiporter (Demaurex et al., 1995) that exchanges $1H^+/1Na^+$ or $2H^+/2Na^+$ per one cycle, and thereby its activity is independent of the membrane potential. The direction of exchange is determined by the balance between the H^+ and Na^+ concentration gradients across the cell membrane. Properties of NHE in the cardiac myocytes have been investigated mainly in Purkinje fibers (Vaughan-Jones and Wu, 1990; Wu and Vaughan-Jones, 1997) and isolated ventricular myocytes (Ch'en et al., 2003; Hoque et al., 1997; Le Prigent et al., 1997; Leem et al., 1999; van Borren et al., 2004; Wallert and Frohlich, 1989; Yamamoto et al., 2005; Yasutake et al., 1996). The NHE activity was usually measured by the recovery rate of pH_i from the acid loading achieved by superfusing the preparation with extracellular NH_4Cl in the HEPES-buffered solution, and changes of pH was measured with a H^+-sensitive microelectrode (Vaughan-Jones and Wu, 1990; Wu and Vaughan-Jones, 1997) or a pH-sensitive fluorophore (Ch'en et al., 2003; Fuster et al., 2008; Hoque et al., 1997; Le Prigent et al., 1997; Leem et al., 1999; van Borren et al., 2004; Wallert and Frohlich, 1989; Yamamoto et al., 2005; Yasutake et al., 1996). The activity of NHE is measured as the H^+ efflux, J_H (*mM/min*), which is obtained from the measurement of dpH_i/dt using the intracellular pH buffer β (*mM/pH*).

$$J_H = \beta \cdot dpH_i/dt$$

The properties of NHE was also examined under various biophysical conditions by measuring $^{22}Na^+$ influx (J_{Na}) from $^{22}Na^+$-loaded cells that were transfected with NHE1 (Lacroix et al., 2004; Wakabayashi et al., 2003a; 2003b), which is the main subtype in cardiac myocardium.

Intracellular pH dependency

The pH_i-J_H relationship is a standard data in many experimental studies. The NHE activity is negligibly small at the normal pH_i

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(resting flux rate = 0.12 Mm/min (Bers et al., 2003)). However, it is steeply augmented with increasing intracellular acidification, with a Hill coefficient $n_H > 2$ in cardiac myocytes (Gore et al., 1994; Green et al., 1988; Hoffmann et al., 1995; Ng et al., 1994; Wallert and Frohlich, 1989). This high cooperativity in the pH_i-J_H relationship has been attributed to an H^+-binding regulatory site, which has been referred to as "H^+ modifier site" or "H^+ sensor site", and is distinct from the H^+ binding to the transport site. The presence of the H^+ modifier site was also supported by Wakabayashi et al. (2003a), who recorded the reverse mode of J_{Na} by inverting the Na^+ gradient across the cell membrane in NHE1-transfected cell line. They demonstrated a "bell-shaped" pH_i-J_{Na} relationship, namely the H^+ influx through NHE increased with increasing pH_i, providing increasing driving force over a moderate pH_i range, but sharply decreased with stronger alkalinezation because the H^+-mediated activation is removed. Alongside the physiological experiments, the structural analysis of NHE molecule revealed that NHE comprises two domains: an N-terminal transmembrane domain and a C-terminal cytoplasmic domain (Slepkov et al., 2007), but the ion binding sites have not yet been identified. The studies with mutations of several sites in the transmembrane and the C-terminal domain also failed to characterize a unique H^+ modifier site, but suggested multiple sites to be involved (Slepkov et al., 2007).

Recently, an alternative mechanism was suggested by Lacroix et al. (2004) that NHE-1 activation is best described by a Monod-Wyman-Changeux concerted mechanism for a dimeric transporter. During intracellular acidification, a low-affinity form of NHE-1 is converted into a form possessing a higher affinity for intracellular protons, without requirement for an additional proton-sensor site on the protein (Lacroix et al., 2004). However, this model fails to explain the depression of the reverse mode by the intracellular alkalinezation (Aronson et al., 1982; Wakabayashi et al., 2003a).

Extracellular pH (pH_o) dependency

In contrast to the activation by intracellular H^+, the extracellular H^+ seems to inhibit the NHE activity; that is, acidification of the extracellular medium markedly depressed the acid extrusion. Since no obvious cooperativity was observed in the pH_o-inhibition relations (Jean et al., 1985; Vaughan-Jones and Wu, 1990; Wallert and Frohlich, 1989), it has been speculated that the inhibition might be caused by simple competitive interaction between H^+ and Na^+ at the transport binding site on the extracellular side (Green et al., 1988). However, the parallel shifts of pH_i-J_H curves in the acidic direction with decreasing pH_o have been observed in a variety of cell preparations including Purkinje fibers (Vaughan-Jones and Wu, 1990), isolated ventricular myocytes (van Borren et al., 2004), osteoblasts and rat brain synaptosomes (Green et al., 1988; Jean et al., 1985). It is strongly suggested that an additional regulatory mechanism is present for the extracellular H^+. This parallel shift was still detected after verifying the lack of contamination by background acid-base transports (van Borren et al., 2004). Since the parallel shift is hard to be explained by the simple competitive interaction between H^+ and Na^+, further examination will be required to define the mechanisms of extracellular H^+ inhibition.

Na^+ dependency

Comparing to a plenty of data of intracellular H^+ dependency, relatively a few number of references are available for the dependency of NHE on extracellular Na^+ concentrations in the cardiac myocytes. However, it seems to be generally agreed that steady-state NHE flux exhibits simple Michaelis-Menten dependence on extracellular Na^+ with an apparent K_m of 5-50 mM (Gore et al., 1994; Levine et al., 1993; Miyata et al., 2005; Pedersen et al., 2006; Wu and Vaughan-Jones, 1997). In contrast to the parallel shift of pH_i-J_H by extracellular pH, the NHE activity is just scaled down by decreasing the extracellular Na^+ concentration (Wu and Vaughan-Jones, 1997), suggesting that competitive binding of Na^+ to the external transporting site might be involved.

The dependency of NHE on the intracellular Na^+ concentration was recorded in only one study in cardiac myocytes (Wu and Vaughan-Jones, 1997), as far as we know. Although the K_m value or affinity constant for the intracellular Na^+ was hard to determine due to the lack of the data points, the NHE activity seems to be effectively varied at the range from zero to the physiological level of intracellular Na^+ concentration, and the activity nearly saturates over 7 mM. Therefore, the intracellular Na^+ dependency could hardly provide negative feedback even when the NHE activity is largely augmented by intracellular acidification (Wu and Vaughan-Jones, 1997).

Development of NHE models

Monomer models

Based on experimental measurements, a variety of mathematical models of NHE have been developed. These models can be divided into two groups according to the interest of study: 1) Clarifying the role of NHE under various cellular conditions, such as ischemia, acidosis, hypertrophy and so on; 2) Determination of molecular mechanisms of NHE activity by developing a model which can explain the characteristics of NHE, such as the ion dependencies of NHE and the interaction between subunits in the transporter.

As the first category, Leem et al. (1999) derived a pure empirical equation which was a polynomial function of pH_i based on their measurements of pH_i-NHE flux relationship. Their model was then incorporated into two different cardiac cell models and the effects on the contraction and membrane excitation were explored under intracellular acidosis or ischemia/reperfusion (Ch'en et al., 1998; Crampin et al., 2006). Alternatively, NHE activity was described with simple Hill equations for $[H^+]_i$ to investigate the spatial nonuniformity of intracellular pH in the ventricular myocyte, which is induced by the limited intracellular proton mobility and affects the local recovery of pH from acidosis (Swietach and Vaughan-Jones, 2005). Recently, more complicated kinetic NHE models have been proposed, which was described with binding steps of ions to the transporter with simple 6- or 8-state antiport models of ping-pong mechanism (Crampin and Smith, 2006; Niederer and Smith, 2007). They were also included into a ventricular myocyte model (Pandit et al., 2001) and were used for estimating the increase in $[Na^+]_i$, $[Ca^{2+}]_i$ and tension in response to the intracellular acidosis (Crampin and Smith, 2006) or physical stretch (Niederer and Smith, 2007). These simulations gave approximated results because even the latest NHE model (Niederer and Smith, 2007) did not take account of extracellular H^+ or Na^+ dependencies. For more accurate calculations of the temporal change of pH_i or Na^+, the dependencies on the intra- and extracellular concentrations of H^+ and Na^+ should be considered. As for NHE3 in renal proximal tubule, Weinstein (1995) suggested 8-state ping-pong models with or without modifier site. This model described binding and transport of NH_4^+ in addition to H^+ and Na^+ for the calculation of the rate of the ammonia secretion, which is critical in the proximal tubule.

In the second category, the consistency of kinetic models

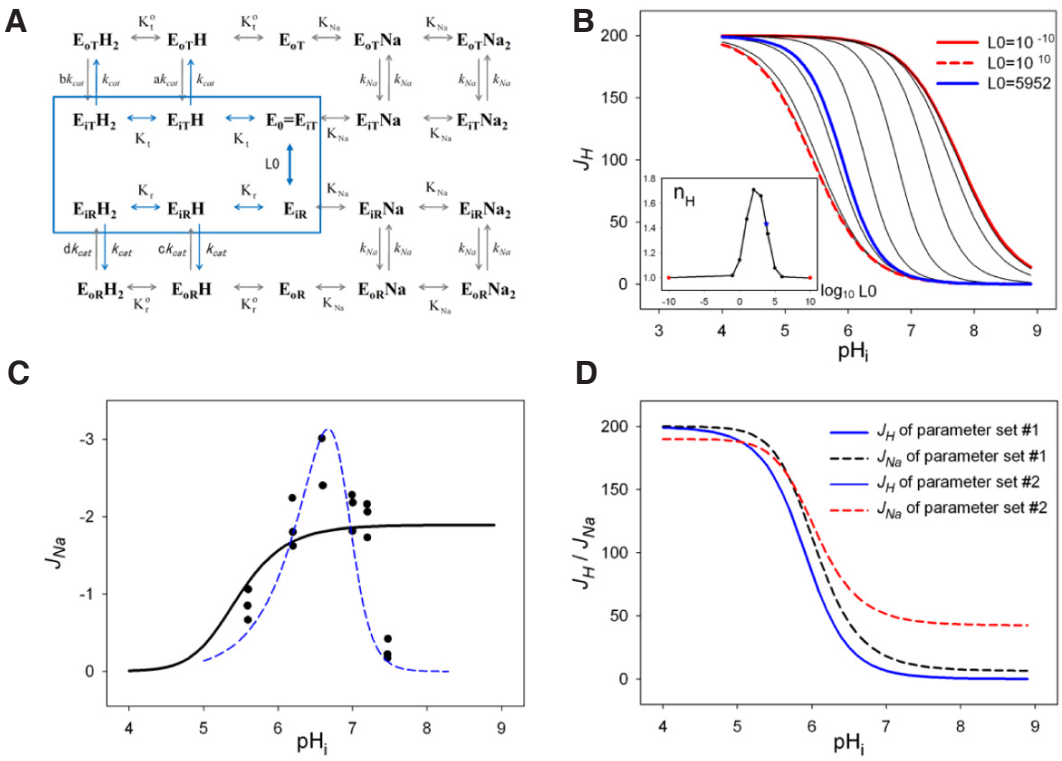

Fig. 1. An NHE dimeric model based on Lacroix et al. (2004). (A) Extended model scheme of NHE model, which includes whole ion-binding and transporting of H^+ and Na^+. The original model of Lacroix et al. (2004) is indicated with blue box and blue arrows. In the extended model, 4 new parameters were added: K_t^o and K_r^o, dissociation constants for external H^+ of low affinity T and high affinity R form, respectively; K_{Na}, dissociation constant for Na^+; k_{Na}, rate constant for transporting Na^+. For simplicity, K_{Na} and k_{Na} are the same for all Na^+-mediated reactions. Constants, a, b, c and d, are uniquely determined by microscopic reversibility for loop-reactions. For determination of the parameters, we used Levenberg-Mar-quardt fitting method. K_t, K_r and L_0, the parameters in the original model of Lacroix et al. were fixed to their original values. Two parameter sets among the best fitting results are presented in Table 1. (B) pH_i-J_H relationship with various L0 (black, L0 is varied by 10 times from 10^{-5} to 10^9; red, L0 = 10^{-10} or 10^{14}; blue, L0 = 5952, the original value of Lacroix et al.). The relationships of both original and extended model were indistinguishable. Inset: Hill coefficients obtained by fitting each curve with Hill equation. (C) The best fitting result for the reverse mode of the extended model (black curve) or of a monomer model with a modifier site (blue dashed curve). Dots were from experimental measurements performed by Wakabayashi et al. (2003a). (D) pH_i-J_H (blue solid lines) or pH_i-J_{Na} relationship (black or red dashed lines) calculated by two different parameter sets among the best fitting results. Note that the pH_i-J_H relationships are identical to the blue line in (B) in both parameter sets.

was examined by comparing with experimental measurements. Wakabayashi et al. (2003a) demonstrated an existence of the modifier site for the intracellular H^+ distinct from transport site by simulating reverse mode of NHE1 with a simplified ping-pong model including independent H^+ modifier. Alexander et al. (2007) suggested a simple model for epithelial NHE3 which kinetically described the activation by pH_i. They compared the different behaviors between NHE1 and NHE3 in hypo-osmotic treatment, and demonstrated the existence of two different inactive states before one active state to explain the activation lag in NHE3, in contrast to the conventional two-state model for NHE1.

Dimer models

Recently, biochemical studies demonstrated that the functional unit of NHE1 is composed of a dimer (Hisamitsu et al., 2006; Moncoq et al., 2008). However, it is not yet clarified how the interactions between monomers influence the transport function. Unlike the above monomer models which concentrated on the interactions between ions and transporters, totally distinct schemes have been proposed for the dimer models by different authors according to their own experimental findings. Three kinds of models are discussed here (Fuster et al., 2008; Lacroix et al., 2004; Otsu et al., 1989; 1993). Since it is impossible to discriminate the dimer models due to insufficiency of experimental data, we separately review individual dimer models.

Otsu et al. (1989; 1993) measured NHE-mediated Na^+ transport after rapid external application of Na^+ at 0°C in renal brush border membrane vesicles, and found a lag phase followed by a monoexponential burst phase of NHE activity in the first turnover. The kinetic analysis of this pre-steady state revealed that activation of the burst phase involves at least two Na^+ transport sites interacting with positive cooperativity. On the other hand, the steady-state Na^+ uptake obeyed simple Michaelis-Menten kinetics. To explain the multiphasic activity, they suggested a flip-flop mechanism for an oligomer in which two ions were transported simultaneously after the two binding sites were occupied. Although it is the first model for dimeric NHE, no mathematical model was proposed for ion transport.

Lacroix et al. (2004) measured pH_i-dependent $^{22}Na^+$ uptake in fibroblasts expressing the human NHE1. They demonstrated that mutations in loops 2, 4 and 5 in the transmembrane domain modified the cooperative behavior of NHE1, and considered that these regions are important for the allosteric regula-

Table 1. Parameters for extended NHE Model of Lacroix et al. (2004)

Parameter set	K_t	K_r	L0	k_{cat}	K_{Na}	k_{Na}	K_t^o	K_r^o
#1	3.63×10^{-3}	1.70×10^{-5}	5952	100	0.075	41300	3.63×10^{-3}	1.70×10^{-5}
#2	3.63×10^{-3}	1.70×10^{-5}	5952	252	6.34	169	3.63×10^{-3}	1.70×10^{-5}

tion of the dimeric exchanger. Especially, R327E mutant exhibited a dramatic loss of cooperativity (Hill coeffeicient changed from 1.69 to 1.19) accompanied with decrease in apparent affinity from K_d (pH unit) (from 6.4 to 5.54). Based on the experimental results, they assumed that the transporter has two distinct conformations with a low- or a high-affinity for H^+ and proposed a Monod-Wyman-Changeux mechanism for a dimeric transporter (blue box and blue arrows in Fig. 1A), in which a low-affinity form (T) of NHE1 is converted into a high-affinity form (R) for intracellular H^+ during intracellular acidification. They successfully reproduced NHE activity with pH_i cooperativity (n_H ~1.4, indicated with blue star in inset of Fig. 1B) without an additional H^+-sensor site. As shown in Fig. 1B, n_H varies with L0; that is, extremely large or small L0 has n_H ~1, and middle value of L0 has high n_H. However, their model fails n_H larger than 2 in cardiac myocytes (Slepkov et al., 2007; Wallert and Frohlich, 1989), as shown in inset of Fig. 1B, and their experimental data were not consistent with those of other group, in which the same mutation R327E had Hill coefficient of 1.68 close to that of wile type (Wakabayashi et al., 2003a). Moreover, due to the absence of the proton-sensor this model scheme cannot explain the bell-shaped reverse mode observed by Wakabayashi et al. (2003a) and Aronson et al. (1982). To examine the reverse mode of their model scheme, we extend their model by including H^+ influx and Na^+ flux pathway (Fig. 1A). Using fitting method, we could obtain multiple parameter sets which had pH_i-J_H relationship almost identical to the original one (Fig. B). We found that all the parameter sets had monotonic reverse mode instead of bell-shape (Fig. 1C). Moreover, the stoichiometry of their model is largely deviated from $1H^+$:$1Na^+$, and NHE activity becomes electrogenic (Fig. 1D).

Fuster et al. (2008) examined the steady-state NHE-mediated H^+ flux in CHO cells. They found that the $[Na^+]_o$-dependency was sigmoidal with a Hill coefficient of 1.8, and suggested a cooperative interaction between the monomers. However, their experimental findings are quite different from previous ones in the following aspects. The measurements of pH_i-dependence were shifted by nearly one pH unit in the alkaline direction (Ch'en et al., 2003; Hoque et al., 1997; Le Prigent et al., 1997; Leem et al., 1999; van Borren et al., 2004; Vaughan-Jones and Wu, 1990; Wakabayashi et al., 2003a; Wallert and Frohlich, 1989; Wu and Vaughan-Jones, 1997; Yamamoto et al., 2005; Yasutake et al., 1996). The findings of biphasic appearance and steep extracellular Na^+-dependence were distinct from those in a variety of cells with the slope of Hill coefficient of ~1 (Goodrich and Suchy, 1990; Gore et al., 1994; Kuwahara et al., 1994; Miyata et al., 2005; Wallert and Frohlich, 1989; Wu and Vaughan-Jones, 1997). In model development, they suggested two coupled-dimer models which had totally different kinetic mechanisms but were broadly consistent with their experimental findings. Thus, obviously the additional experimental findings need to be examined in other cells types such as cardiac myocytes.

CONCLUSION

As the main H^+ transporter to maintain the intracellular pH_i, NHE have been extensively investigated by both experimental studies and mathematical modeling. However, many unsolved questions still remain. Especially, there is no consensus in the dimeric behavior or mechanisms, and experimental data is still insufficient to discriminate possible dimeric models. Besides, determination of adequate model parameters for the interactions between ions and transporters is also important for more precise description of transport flux which is indispensable for studying the ionic homeostasis under various pathophysiological conditions.

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