Na⁺/H⁺ exchange in hypertension and in diabetes mellitus – facts and hypotheses

Abstract An enhancement of Na⁺/H⁺ exchange (NHE) in blood cells of selected patients with essential hypertension and with diabetic nephropathy has been described by various investigators. Recent studies have shown that enhanced NHE activity persists in immortalized lymphoblasts from these patients after prolonged cell culture and, thus, appears to be under genetic control. Available evidence strongly argues against a mutation in the encoding gene or an overexpression of the NHE. Immortalized cells from hypertensive patients with enhanced NHE activity display two-fold enhanced agonist-induced rises of the cytosolic free Ca²⁺ concentration and the underlying reason was identified as an increased activation of pertussis toxin (PTX) -sensitive G proteins. The molecular mechanism(s) of this phenomenon have not yet been elucidated. It appears likely that similar changes contribute to the enhanced NHE activity phenotype in diabetic nephropathy, although experimental evidence for this is still lacking. An enhanced activation of PTX-sensitive G proteins could explain many of the hitherto unexplained phenomena in essential hypertension, e.g. inheritance, increased vasoconstriction, hypertrophy or remodeling of arterial blood vessels and the heart, enhanced platelet aggregation etc. In diabetes the same defect could provide the basis for the susceptibility to nephropathy, e.g. by enhancing the deleterious effects of autocrine and paracrine growth factors. Thus, the experimental approach of immortalizing blood cells from patients with essential hypertension and diabetic nephropathy has opened new horizons in the identification of genetically fixed abnormalities in intracellular signal transduction which could contribute to both pathologies and which can now be studied without the confounding influences of the diabetic or hypertensive in vivo milieu.

Key words Hypertension – diabetes – nephropathy – G proteins – hypertrophy

Introduction

Na⁺/H⁺ exchangers constitute a family of membrane proteins which mediate the electroneutral transmembrane exchange of Na⁺ ions against H⁺ ions. Their main physiological function is to mediate cytosolic pH homeostasis, cell volume control, and, in epithelia, Na⁺ reabsorption (33). So far, four different isoforms of Na⁺/H⁺ exchangers have been cloned from mammalian tissues and these isoforms are commonly referred to as NHE-1 –
NHE-4 (for reviews see (8, 16, 17, 59, 67, 92, 99)). They differ with respect to their amino acid composition, tissue distribution, their intracellular regulation by second messengers, and their susceptibility to inhibition by amiloride and its analogs, to name but a few.

During the past decade evidence has been accumulated that the Na⁺/H⁺ exchanger isoform 1 (NHE-1) plays a pivotal role in a variety of cardiovascular pathologies including essential hypertension, diabetic nephropathy, left ventricular hypertrophy, and ischemia/reperfusion injury. The present review summarizes some aspects of the kinetic properties and regulation of the NHE-1 as far as these facts are crucial for the understanding of the alterations that have been observed in hypertension and in diabetic nephropathy. Furthermore, we will give an overview on the most recent findings regarding the potential reason for the enhanced NHE-1 activity in essential hypertension, that is a genetically fixed “hyper-reactivity” of pertussis toxin (PTX)-sensitive G proteins. We will discuss whether similar mechanisms could be operative in diabetic nephropathy and we propose a novel concept in which overactive PTX-sensitive G proteins are the common denominator for both disorders in that such a mechanism could be a major genetic “susceptibility” factor.

**Fig. 1** Kinetic properties of the NHE-1 and their modification by specific stimuli

**A:** Shown is a typical pH_i activation curve of the NHE-1 in human B lymphoblasts and some of the kinetic parameters which describe its course. V_max refers to the maximal transport rate which is usually obtained at pH_i 6.0 or below. The terms “pK” or “K_m” refer to the pH value at which half-maximal transport activity is observed. The Hill-coefficient, n, describes the degree of sigmoidality of the pH_i activation curve. Basal, pH_i, “set-point”, or X_i-value refer to the pH value, at which the antipporter is apparently switched off. (45)

**B:** Effect of direct protein kinase C stimulation (by phorbol ester) or [Ca²⁺]i elevation (by the ionophore ionomycin) on the pH_i activation curve of the NHE-1 in human B lymphoblasts. Both agonists induce an alkaline shift of the pH_i activation curve and increase “pK” as well as the basal pH_i value. In contrast, V_max remains constant. The Hill coefficient, n, is decreased by phorbol ester treatment, but not by ionomycin. Data are compiled from refs. (75) and (93).

**Kinetic properties of the NHE-1, its intracellular and “systemic” regulation, and its relationship to Na⁺/Li⁺ countertransport**

Kinetic properties of the NHE-1

The NHE-1 mediates an electroneutral exchange of intracellular H⁺ against extracellular Na⁺, the inwardly directed Na⁺ gradient being provided by the energy-dependent Na/K-ATPase. The interaction of extracellular Na⁺ ions with the Na⁺/H⁺ exchanger obviously follows simple Michaelis-Menten kinetics: the Hill coefficient (n) of the Na⁺-activation of the NHE-1 equals 1 (4), which suggests the presence of only one binding site for extracellular Na⁺ and neither cooperative activation nor inhibition of the transport by extracellular Na⁺ ions. Thus, K_m for extracellular Na⁺ is one of the relevant parameters of NHE kinetics. The activation by intracellular H⁺ is much more complex. Beside being transported, intracellular H⁺ ions induce an allosteric activation of the antiporter resulting in a sigmoidal activation curve (Fig. 1A). Hence, the dependence of transport activity on cytosolic protons, H⁺_i, does not follow simple Michaelis-Menten kinetics, and Hill coefficient of > 1 are calculated for the NHE-1, the NHE-2, and the NHE-3 (45). Cytosolic protons are not only bound to the transport site of the antiporter but also to a “H⁺_i sensor” or “H⁺_i-modifier site” which directly determines transport activity (94, 95). This “H⁺_i sensor” is obviously located in the transmembrane N-terminal domain of the antiporter, potentially in close vicinity to the transport site, and its “H⁺_i-sensing” properties are largely modified upon phosphorylation of the large cytoplasmic domain of the NHE-1 (94, 95). Thus, the Hill coefficient, which describes the degree of sigmoidality of the NHE activation curve by H⁺_i, and, thereby, partially the state of the antiporter’s H⁺_i sensor, is another important kinetic parameter of the NHE. The maximal