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Invited review

An overview of inhibitors of Na^+/H^+ exchanger

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Abstract

The Na^+/H^+ exchanger (NHE) is involved in intracellular pH homeostasis of many mammalian cell types. To date seven NHE isoforms (NHE1–NHE7) have been identified. NHE1 is the most predominant isoform expressed in heart where it contributes to cardiomyocyte pH homeostasis. Although the NHE activation is essential for the restoration of physiological pH, hyperactivation of NHE1 during ischemia–reperfusion episodes disrupts the intracellular ion balance, leading to cardiac dysfunction and damage. Beside its ability to inhibit a conductive Na^+ channel and the Na^+/Ca^{++} exchanger, amiloride was the first drug described as NHE inhibitor. Double substitution of the nitrogen of the 5-amino group of amiloride gave DMA, EIPA, MIBA and HMA. Later, several acylguanidines were prepared to selectively inhibit NHE1. The replacement of the pyrazine ring of amiloride by a pyridine ring or by a phenyl increased the potency and the NHE selectivity. The simultaneous replacement of the pyrazine ring by a phenyl, of the 6-chloro by a sulfomethyl led to drugs such as HOE-694, cariporide, eniporide and BIIB-513 which also selectively inhibited NHE1. In the last decade several bicyclic guanidines were prepared: zoniporide, MS-31038, SM-20220, SM-20550, SMP-300, KB-R9032, BMS-284640, T-162559, TY-12533, S-3226 or SL-591227. Extensive pre-clinical studies indicated that NHE inhibitors afford substantial protection in different animal models of myocardial ischemia (MI) and reperfusion, but the results of clinical trials involving eniporide and cariporide were mixed.

Keywords: Sodium-proton exchanger; NHE; Amiloride; Inhibitor; Ischemia–reperfusion

1. Introduction

The Na^+/H^+ exchanger (NHE) is a protein that is expressed in many mammalian cell types. NHE is responsible for intracellular pH and cell volume regulation by extruding protons from, and taking up sodium ions into cells. To date seven isoforms (NHE1–NHE7) have been identified and cloned. NHE isoforms share ca. 20–60% amino acid identity and a molecular mass from 74 to 99 kDa (Table 1). NHE6 and NHE7 are localized to recycling endosomes and to the *trans*-Golgi network respectively, whereas the other isoforms (NHE1–5) are expressed in the cell membrane [1–3]. NHE isoforms are composed of 12 helical hydrophobic membrane-spanning segments, a N-terminal sequence and a highly hydrophilic C-terminal segment. The segments M3–M12 share a high sequence homology among the various isoforms where M6 and M7 are most highly preserved (95% identity), suggesting that these domains are involved in the transport of Na^+ and H^+ across the membrane [1].

NHE1 is activable by growth factors and expressed in several cell types, mainly in mammalian cardiomyocytes, platelets and on the basolateral membrane of renal tubules [4,5]. NHE2 has been localized in the gastrointestinal system mainly in stomach, colon and small intestine, with lower levels in skeletal muscle and in selected nephron segments [6–8]. Some studies reported basolateral and other apical localization of NHE2 [1]. NHE3 is mainly expressed at high levels in colon, and small intestine, with significant levels also in kidney and stomach [9,10]. It contributes to sodium absorption by the brush-border membrane in intestinal or renal epithelia. NHE4 is highly abundant in stomach and also present at intermediate levels in small intestine and colon [11]. A lower concentration is found on the

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Table 1
Isoforms of the Na^+/H^+ exchanger

Isoform	Species	Structure		Localisation
NHE1	Human	815	91 kDa	Cardiomyocytes, platelets Basolateral membrane of several tissues
NHE2	Rat	813	91 kDa	Stomach, colon, small intestine, adrenal gland
	Rabbit	809	90 kDa	Kidney and intestinal epithelia cell
NHE3	Rat	831	93 kDa	Colon, small intestine, stomach apical membrane of epithelia (proximal tubule, intestine)
NHE4	Rat		81 kDa	Stomach, small intestine, colon, collecting tubule
NHE5	Human	896	99 kDa	Brain (hippocampus), spleen, testis, skeletal muscle
NHE6	Human	669	74 kDa	Brain, skeletal muscle, heart
NHE7	Human	725	80 kDa	Brain (putamen, occipital lobe), skeletal muscle Secretory tissues (stomach, prostate, pancreas, thyroid)

basolateral membrane of collecting tubule. Little is known about the role of NHE5 which is expressed predominantly in nonepithelial tissue such as brain (hippocampus, cortex) [12–14]. NHE5 has been identified at a lower level in spleen, testis and skeletal muscle. In contrary to NHE1–5, NHE6 is the first intracellular NHE. It has been identified on recycling endosomes but not in the inner membrane of mitochondria as primarily assessed [2,15]. NHE6 has been detected with highest abundance in brain and skeletal muscle, followed by heart and other tissues. NHE6 may regulate intravesicular pH and contribute to lysosomal biogenesis. Finally, NHE7 has been localized predominantly to the *trans*-Golgi network [3]. Its expression is ubiquitous but predominant in certain regions of brain (occipital lobe, putamen), in skeletal muscle, in stomach and in glands (pancreas, salivary-, thyroid- and mammary-gland ...).

NHE is working according to the Na^+ and H^+ gradients by exchanging an extracellular Na^+ (Na_o^+) against a intracellular H^+ (H_i^+) with a tightly coupled 1:1 stoichiometry. NHE1-3 and NHE5 exhibited a hyperbolic dependence on Na_o^+ concentration ($[Na^+]_o$) while NHE4 showed a sigmoidal dependence on $[Na^+]_o$. The affinity of these different NHE isoforms for $[Na^+]_o$ is ranging between 5 and 50 mM (Table 2) [1]. Extracellular Li^+ (Li_o^+) and H^+ (H_o^+) competitively inhibit Na_o^+ influx by interacting at a single binding site of NHE1–3 and NHE5. In contrast to NHE2–3 and NHE5, extracellular K^+ (1–100 mM) inhibits NHE1 (Ki = 180 mM) at high and nonphysiological concentrations [14,16]. Only NHE7 and NHE4 are able to mediate the influx of K^+ or Na^+ in exchange for H^+ [3,17]. The decrease of intracellular pH enhanced the $[H_i^+]/[H_o^+]$ gradient and activates NHE isoforms (Table 2) [18]. In absence of Na_o^+, NHE operates in a reverse mode by expelling Na^+.

Table 2
Apparent affinity constants of rat NHE1-3 and human NHE5 isoforms for intra and extracellular monovalent cations

Cation	Apparent affinity constants ($K_{0.5}$) [a]			
	NHE1	NHE2	NHE3	NHE5
Na_o^+ (mM)	10.0	50.0	4.7	18.6
Li_o^+ (mM)	3.4	2.2	2.6	0.32
K_o^+ (mM)	19.5	None	None	Slight inhibition
H_o^+ (pK)	7.00	7.90	7.00	8.13
H_i^+ (pK)	6.75	6.90	6.45	6.43

[a] Values from Refs. [14,16].

NHE activity is regulated by several mechanisms [1]. Regulation of activity can be explained by direct phosphorylation of NHE by PKA and/or PKC [19]. NHE1 has been found to be constitutively phosphorylated in resting cells, and further phosphorylation is induced by phorbol esters, growth factors or phosphatase inhibitors [20]. The phosphorylation sites were detected on the distal part of the cytosolic C-tail. This NHE1 cytosolic tail contains also two calmodulin binding sites. Deletion of this segment constitutively stimulates NHE1 and mimics elevated intracellular $[Ca^{++}]$. The unoccupied domain, able to bind calmodulin with high affinity, exerts an autoinhibitory effect [21]. The binding of a calcineurin homolog protein (CHP) to a NHE1 sequence located on the C-tail inhibits the NHE1 activity. CHP appears to be constitutively phosphorylated [22]. For NHE2, two proline-rich domains that resemble SH3-binding proteins have been identified in the C-tail region [23]. NHE activity is also regulated by GTP-binding proteins. Activated forms of $G\alpha_q$, $G\alpha_{12}$ and $G\alpha_{13}$ activate NHE [24]. Recently, it has been evidenced that nitric oxide inhibited NHE3 activity via activation of soluble guanylate cyclase, resulting in an increase in intracellular cGMP levels and activation of protein kinase G [25].

Following intracellular acidosis, NHE activation is essential to restore physiological pH by H^+ extruding. Nevertheless, an excessive stimulation of NHE results in an increase of intracellular Na^+ concentration and a

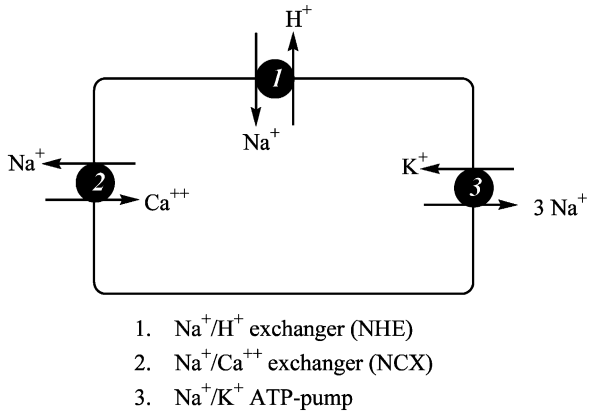

Fig. 1. Activation of NHE in ischemia–reperfusion: ischemia–reperfusion increase the pH_o/pH_i gradient which activate the NHE, enhance the activity of the Na^+/K^+ ATP pump, and of the Na^+/Ca^{++} exchanger leading to intracellular Ca^{++} accumulation.

subsequent activation of Na^+/K^+ ATPase, with a consecutive increase of energy consumption. The high intracellular Na^+ level contributes to activate the sarcolemmal Na^+/Ca^{++} antiporter which lead to raised intracellular Ca^{++} (Fig. 1).

At the cardiac level, this cellular Ca^{++} overload subsequent to NHE-1 activation is involved in ischemic and reperfusion injuries like myocardial infarction activation, stunning and tissue necrosis [26]. It has been demonstrated that the activity of NHE-1 is also increased in red blood cells, platelets, leukocytes, and skeletal muscle cells from patients with essential hypertension [27–31]. In response to chronic or acute hypertension, NHE-3 is redistributed from the apical brush border of proximal tubules to intermicrovillar and endosomal stores [32]. Finally, insulin induced a significantly increased NHE1 activity in normal patients as compared to obese individual were erythrocytes are resistant to insulin [33].

2. NHE inhibitors

With the aim to attenuate the harmful consequences of excessive NHE activation, several inhibitors were developed with the primary goal to provide cardioprotective drugs by inhibiting the NHE1 subtype. Beside its ability to inhibit a conductive Na^+ channel and the Na^+/Ca^{++} exchanger, amiloride, a K^+-sparing diuretic, was the first drug described as NHE inhibitor [34]. NHE1 and NHE2 are the most sensitive isoforms to amiloride inhibition whereas NHE3 and NHE4 are amiloride resistant isoforms [17] (Table 3). NHE5 is inhibited by amiloride at half concentration that was intermediate to those determined for NHE1 and NHE3 isoforms, but closer to the latter [14]. The latest isoform, NHE7 is insensitive to amiloride [3]. Cimetidine, harmaline and clonidine were also reported as weak and non-specific NHE inhibitors [35]. To increase the potency and the selectivity of inhibitors towards the NHE isoforms, and particularly NHE1, several molecules derived from amiloride have been synthesized and investigated. Double substitution of the nitrogen of the 5-amino group gave DMA, EIPA, MIBA and HMA, the most studied pyrazines related to amiloride (Fig. 2). EIPA, HMA and DMA are much more effective than amiloride on each studied isoform and lost the inhibitory potency on Na+ channel and Na^+/Ca^{++} exchanger (Table 3). They are weak selective inhibitors of NHE1. The selectivity of EIPA is ranging as follow: NHE1 > NHE2 > NHE5 > NHE3.

The pyrazine ring of amiloride was then replaced by a pyridine ring or by a phenyl. The phenyl counterpart of amiloride and the pyridine counterpart where the heterocyclic nitrogen was located in *meta* position of the acylguanidine were 54- and 36-times more active than amiloride on human platelet NHE1, respectively [36]. In the same experimental conditions, the pyridine derivative where the heterocyclic nitrogen was in *ortho* position of the acylguanidine was as active as amiloride [36]. Concomitantly to the replacement of the pyrazine ring of amiloride by a phenyl, the 6-chloro has been substituted by a sulfomethyl and the 2-amino has been deleted or replaced by a methyl group. Taken together, these modulations led to benzoylguanidines such as HOE-694 [37], cariporide [38], eniporide [39] and BIIB-513 [40] which completely lost the Na^+/Ca^{++} exchanger inhibitory potency as well as their ability to block Na^+-channels (Fig. 2). For each isoform investigated, HOE-694 is less active than EIPA but more selective towards NHE1 (Table 3). As compared to their inhibitory potency of NHE2, cariporide and eniporide are more NHE1-selective than EIPA. They are inactive on NHE3 and NHE5. As observed for the pyrazine derivatives, the substitution of the distal nitrogen of the acylguanidine moiety strongly decreased the NHE inhibitory potency. Later, several molecules based on a bicyclic template have been designed (Fig. 3). This bicyclic ring was a quinoleine (zoniporide [41], MS-31038 [42]), an indole (SM-20220 [43], SM-20550 [44], SMP-300 [45]), a benzoxazinone (KB-R9032 [46]), a dihydrobenzofurane (BMS-284640 [47]), a tetrahydronaphtalene (T-162559 [48]), or a tetrahydrocycloheptapyridine (TY-12533 [49]). Excepted for T-162559, all these compounds bear an unsubstituted acylguanidine group. Miscellaneous compounds structurally far from amiloride were also prepared (S-3226 [50], SL-591227 [51]) (Fig. 3). Enzymatic studies assessed that zoniporide, BMS-284640, (S)-T-162559 and SL-591227 were selective NHE1 inhibitors as compared to other isoforms (Table 3), whereas SM-20220, SM-20550 and TY-12533 inhibit at least NHE1. Finally, S-3226 was the first NHE3 selective inhibitor.

Table 3
Inhibitory potency of NHE inhibitors towards the different isoforms

Drug	Inhibitory potency (IC_{50} or K_i, in μM) [a]					
	NHE1	NHE2	NHE3	NHE4	NHE5	NHE7
Amiloride	*1–1.6**	*1.0***	*> 100**		*21*	
	5.3*		100–309*	813*		> 2000
EIPA	*0.01*–0.02***	*0.08*–0.5***	*2.4**		*0.42*	
	25.1*		3.3*	> 10*	1.53	
HMA	*0.013**		*2.4**		*0.37*	
DMA	*0.023**	*0.25**	*14**			
HOE-694	*0.085**		*640**		*9.1*	
Cariporide	0.03–3.4	4.3–62	1– > 100		> 30	
Eniporide	0.005–0.38	2–17	100–460		> 30	
Zoniporide	0.059	12	> 500*			
SM 20550	0.010*					
BMS-284640	0.009	1800	> 30		3.36	
T-162559 (S)	0.001	0.43	11			
T-162559 (R)	35	0.31	> 30			
TY-12533	0.017					
SL-591227	0.003	2.3				
S-3226	3.6	80**	0.02			
Harmaline	*140**	330	*1000**		*940*	
Cimetidine	*26**	330	*6200**		*230*	
	51*		> 1000*		> 1000*	
Clonidine	*210**	42	*620**		N.A.	

* = from rat, ** = from rabbit. NA = not active. Values are from references [3,7,14,16,17,41,45,47–51,59].
[a] K_i values are in italic.

The inhibitory potency of amiloride and some derivatives is reduced by high concentrations of Na^+, suggesting that their cationic form bind to the external Na^+ binding site. The potency of NHE inhibitors is not only related to the chemical structure but also to the ionization of the guanidine function. At physiological pH (7.4), the acylguanidine of amiloride ($pK_a = 8.78$) and the aminoguanidine of TY-162559 ($pK_a = 8.4$) are completely protonated, and can interact with NHE under their cationic form [48,49]. During ischemia or reperfusion phase, the pH falls down to 6.2. In these conditions, drugs like cariporide ($pK_a = 6.28$), TY-12533 ($pK_a = 6.93$) or zoniporide ($pK_a = 7.2$) are positively charged and then more efficient [41,49]. Indeed, cariporide and TY-12533 are more active at pH 6.2 than 6.7 (cariporide: $IC_{50} = 22$ nM/120 nM; TY-12533: $IC_{50} = 17$ nM/32 nM). This is also confirmed by the weak activity of the acylguanidine counterpart ($pK_a = 6.2$; $IC_{50} = 210$ nM) of the aminoguanidine TY-162559 ($pK_a = 8.4$; $IC_{50} = 9$ nM) [48].

3. Cardioprotective activity of NHE inhibitors

During myocardial ischemia, mitochondrial ATP synthesis ceases and glycolysis results in the depletion of ATP and in a decrease in intracellular pH which activates the NHE resulting in the extrusion of H^+ and the influx of Na^+. Due to NHE activation and to Na^+/K^+ ATP-pump failure, the overload of intracellular Na^+ activates the Na^+/Ca^{++} exchanger which increases the cytosolic free calcium. The accumulation of intracellular Ca^{++} contributes to cellular damage resulting in arrhythmias and myocardial stunning. With reperfusion, extracellular H^+ rapidly decreases increasing the intracellular to extracellular H^+ gradient. This large H^+ gradient activates NHE which enhances intracellular Na^+ and lead, through the Na^+/Ca^{++} exchanger, to accumulation of Ca^{++} during reperfusion. This contributes to arrhythmia and myocardial contracture during the reperfusion period. As NHE1 is the predominant isoform in mammalian myocardium, the NHE1 inhibitors were investigated in several models of ischemia–reperfusion. Administered 15 min prior to a myocardial ischemia (1 h) induced by occlusion of the left anterior descending coronary artery in dogs, eniporide (0.75 mg kg^{-1}, iv) significantly reduced the infarct size and the area at risk [52]. The reperfusion period was 3 h. In the same model, BIIB-513 (0.75 mg kg^{-1}), administered either prior to ischemia or prior to reperfusion, reduced the infarct size, phase 1b arrhytmias and ventricular fibrillation induced by ischemia and reperfusion respectively [53]. Zoniporide (CP-597396), a highly soluble selective NHE1 inhibitor, was also investigated in a rabbit myocardial ischemia (0.5 h)–reperfusion (2 h) model. Infused 30 min before ischemia until the end of reperfusion phase, zoniporide reduced the infarct size to a greater extent than

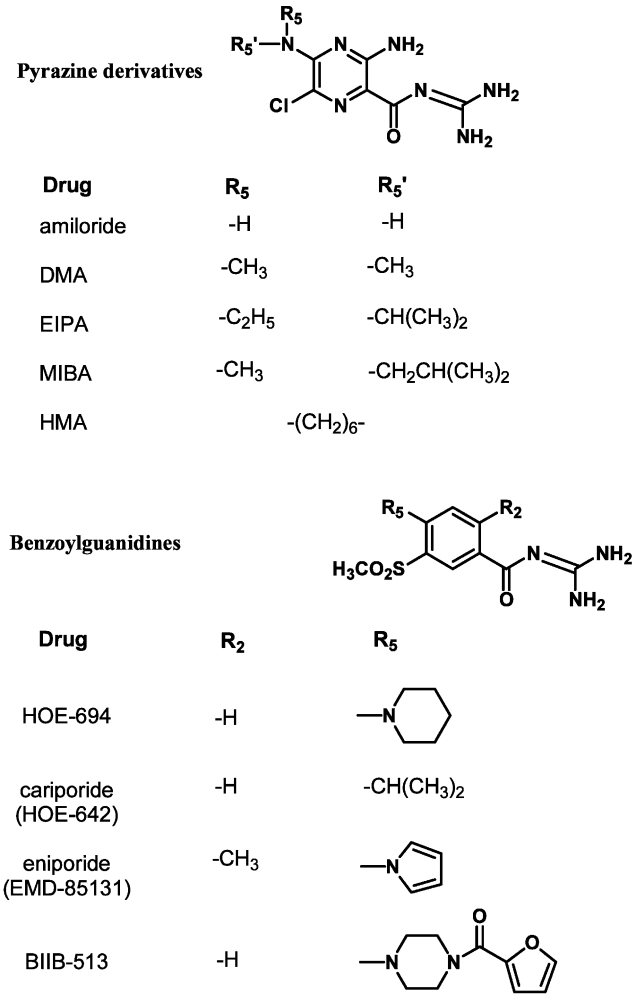

Fig. 2. Chemical structures of amiloride and of its pyrazine and phenyl derivatives.

eniporide or cariporide [54]. Furthermore, zoniporide did not cause any in vivo hemodynamic changes. In rabbit, SM-20550, an indole NHE-1 inhibitor which act on endothelial cells [55], 10 times more potent than EIPA, reduced the infarct size by ca. 30–70% in a dose-dependent manner (iv bolus 1.7–170 μg kg^{-1} followed by iv infusion 2.8–280 μg kg^{-1} h^{-1}) when administered prior to a myocardial ischemia–reperfusion (0.5–5 h) protocol [56]. Infused 10 min prior to the reperfusion period, the reduction was 20–40%. A similar experiment conducted in dogs showed that S-20550 (iv bolus 170 μg kg^{-1} followed by iv infusion 280 μg kg^{-1} h^{-1}) reduced the infarct size of 80 and 41% when administered 15 min prior to the occlusion of the left circumflex coronary artery (2 h) and to the reperfusion period (5 h), respectively [57]. Furthermore, SM-20550 suppressed ventricular fibrillation during both ischemia and reperfusion without affecting the size of the area at risk. SMP-300 inhibits NHE of rat myocytes with an IC_{50} of 6 nM and was therefore 16 times more potent than EIPA. SMP-300 (1 mg kg^{-1}), an orally active specific NHE inhibitor, reduced rat myocardial infarct size after 40 min of coronary artery occlusion followed by 24 h of reperfusion [58]. The cardioprotective effect of (S)-T-162559, a specific NHE1 inhibitor 5 and 31 times more active than eniporide and cariporide, respectively [59], was studied in a rabbit model of ischemia–reperfusion (0.5– 24 h) injury. Intravenously administered 5 min. prior to occlusion, (S)-T-162559 (0.03 and 0.1 mg kg^{-1}) reduced the myocardial area at risk by 36% [60]. The activity of TY-12533 was investigated in a rat model of myocardial ischemia–reperfusion (0.5–24 h), and compared to cariporide. Administered 5 and 10 min before the coronary snare occlusion and reperfusion respectively, TY-12533 and cariporide did not reduce the myocardial area at risk. The pre-occlusion treatment with TY-12533 and cariporide (0.1 mg kg^{-1} iv) reduced the infarct size of 50 and 70%, respectively. After a post-occlusion treatment, only TY-12533 (0.1 mg kg^{-1} iv) reduced the infarct size (44%). In dogs, TY-12533 (3 mg kg^{-1} 10 min^{-1}) injected 10 min before or after a myocardial ischemia–reperfusion (0.25–2 h) did not affect reductions in regional myocardial wall thickening and blood flow during ischemia, but it improved these parameters after reperfusion [61]. SL-591227 is the first potent and NHE1-selective non-guanidine inhibitor. In rat following left coronary artery occlusion (7 min) and reperfusion (10 min), SL-591227 (10–100 μg kg^{-1} min^{-1} iv) inhibited ventricular tachycardia (71–100%) and fibrillation (75–87%) induced by ischemia and reperfusion respectively. In rabbit, SL-591227 (0.6 mg kg^{-1} iv) reduced the myocardial area at risk (−58%) evoked by coronary occlusion and reperfusion (0.5–2 h) [51].

4. Cerebroprotective activity of NHE inhibitors

Three NHE isoforms (NHE 1, 4 and 5) have been found in brain tissues and are expressed in neurons and glial cells. As observed for myocardial infarction, brain ischemia–reperfusion activates NHE which increases intracellular Na^+, cellular swelling and free Ca^{++} accumulation leading to cellular damage. SM-20220 inhibited recovery from acid load in cultured neurons and glial cells with an IC_{50} of 5 and 20 nM, respectively [43]. The effect of SM-20220, a specific NHE inhibitor structurally close to SM-20550 (Fig. 3), has been studied in Mongolian gerbil global cerebral ischemia [62]. Transient ischemia (30 min) was induced by clipping both common carotid arteries, and SM-20220 was intravenously infused (0.3 or 1 mg kg^{-1}) immediately after reperfusion. For each dosage, SM-20220 improved the neurological outcome (McGraw's score) for 24 h, and significantly reduced the mortality rate at 1 mg kg^{-1}. SM-20220 and EIPA reduced free fatty acid from rat cerebral cortex during ischemia–reperfusion injury

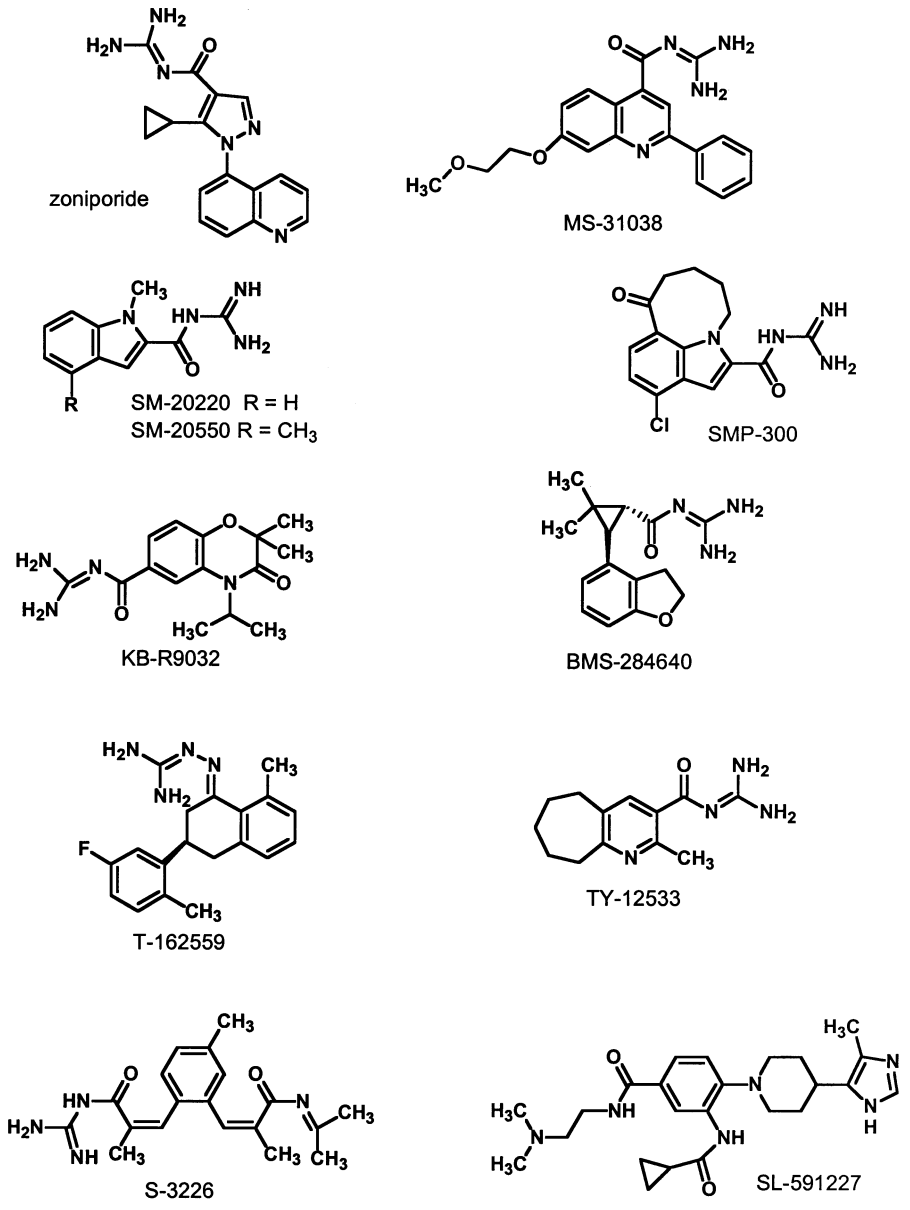

Fig. 3. Chemical structure of bicyclic NHE inhibitors.

[63,64]. These data indicate that these NHE inhibitors prevent the activation of phospholipases that occurs during reperfusion following a cerebral ischemia period. Finally, SM-20220, attenuated cerebral infract volume, water content and neutrophil accumulation at 72 h after permanent occlusion of the rat middle cerebral artery [65].

5. Renal protection of NHE3 inhibitors

Acute renal failure is characterized by sudden loss of the kidney function due to ischemia, trauma, and/or nephrotoxic drugs. As described for heart and brain, renal ischemia activates NHE, and particularly the NHE3 isoform which is expressed at high level in kidney. S-3226 is the first selective NHE3 inhibitor investigated in ischemia-induced acute renal failure in rats [66]. S-3226 (20 mg kg^{-1}, iv) infused before or after global renal ischemia (40 min) enhanced the creatinine clearance and reduced the increase of plasma creatinine as compared to the control. On day 7 following renal ischemia, kidneys revealed pronounced reduction of tubular necrosis, dilatation, protein casts and cellular infiltration when treated with S-3226.

6. Clinical investigations of NHE inhibitors

Extensive pre-clinical studies indicated that NHE inhibitors afford substantial protection in animal models of myocardial ischemia (MI) and reperfusion, with a

high level of conformity between different investigators, species and models. To date, results of clinical investigations with cariporide and eniporide have been reported in patients with evolving myocardial infarction and in those at risk of myocardial infarction [67–70]. The effects of cariporide have been evaluated in patients subject to anterior MI who were expected to receive perfusion therapy by primary coronary angioplasty within 6 h of the onset of symptoms [68]. Patients ($n = 100$) were randomized to receive placebo or cariporide (40 mg bolus iv) 10 min before reperfusion. Administration was completed within 4 h after the onset of symptoms. Cardiac enzymes and their isoforms (CK, CK-MB, LDH) were determined in blood samples taken before and after reperfusion (up to 72 h). Before treatment and at 3-week follow-up, contrast ventriculography was used to evaluate the left ventricular function. This study showed that the area under the curve of CK-MB release was reduced in the cariporide group as compared to the placebo group ($P = 0.047$). The ejection fraction was higher in the cariporide group than in the placebo one, such that the change from baseline to follow-up was greater in the latter group ($P = 0.045$). This study suggested that reperfusion injury could be a target for NHE inhibitors and these results warranted further clinical trials to confirm the therapeutic interest of NHE inhibitors. This led to the large-scale trial ESCAMI (Evaluation of the Safety and Cardioprotective Effects of Eniporide in Myocardial Infarction) [69]. This international, randomized, double-blind, placebo-controlled phase 2 trial enrolled 433 patients undergoing thrombolytic therapy or the percutaneous transluminal coronary angioplasty (PCTA) for acute ST-elevation MI to investigate the efficacy of eniporide on infarct size and clinical outcome. Eniporide was intravenously administered over 10 min. In patients receiving thrombolytic therapy the perfusion had to be completed at least 15 min after the start of treatment, while in patients subject to primary angioplasty, the infusion had to be completed at least 10 min prior to start PCTA. In stage 1 ($n = 430$), four doses of eniporide were considered: 50, 100, 150 and 200 mg. This stage had a triple goal: to evaluated the primary efficacy end point determined by cumulative release of α-hydroxybutyrate dehydrogenase (α-HBDH) and of cardiac markers (CK-MB, troponin T and I), to select a subset of doses to be carried forward in stage 2, and to determine the number of patients to be enrolled for stage 2. Within the first 6 weeks, death, cardiogenic shock, heart failure, arrhythmias, major bleeding were considered as secondary end points. In stage 1, the administration of 100 and 150 mg eniporide resulted in smaller enzymatic infarct sizes, especially in angioplasty group. In contrast, in stage 2 there was no significant difference in the enzymatic infarct size between the three groups (placebo, 100 and 150 mg eniporide). Overall there was no effect of eniporide on clinical outcome of secondary end points. However, a subgroup of patients ($n = 322$, 150 mg eniporide), in whom reperfusion was initiated more than 4 h after symptom onset, showed a significant reduction of heart failure symptoms when compared to the control group.

In the GUARDIAN (*Guar*d *D*uring *I*schemia *A*gainst *N*ecrosis) trial ($n = 11\,590$), the cardioprotective efficacy of cariporide was limited to high-risk patients who underwent coronary artery bypass graft (CABG) [67]. This trial failed to document benefit of cariporide over placebo on the primary end point of death or MI assessed after 36 days. Administered to the subpopulation of patients who underwent CABG, cariporide (120 mg three times a day) reduced of 25% the relative risk in the primary end point of death or MI. Further trials are warranted to confirm the cardioprotective benefit of NHE inhibitors in patients undergoing CABG surgery. The recently initiated EXPEDITION (Na^+/H^+ Exchanger Inhibition to Prevent Coronary Events in Acute Cardiac Conditions) trial will test the hypothesis that NHE inhibition results in a reduction of MI in such patients [70].

These mixed results of clinical investigations with eniporide and cariporide contrast with the encouraging results obtained from preclinical studies, and the potential advantage of NHE inhibitors over other therapies claim further trials. Beside the cardioprotective effects of NHE1 inhibitors, the positive preclinical results obtained in the treatment of brain and renal ischemia reperfusion should be also verified in clinical trials [62–66].

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