Minireview

Regulation of mitogen-activated protein kinase pathways by the plasma membrane Na\(^+\)/H\(^+\) exchanger, NHE1

Stine Falsig Pedersen *, Barbara Vasek Darborg, Maria Louise Rentsch, Maria Rasmussen

Department of Molecular Biology, University of Copenhagen, DK-2100 Copenhagen, Denmark

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Abstract

The mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK, play a major role in the regulation of pivotal cellular processes such as cell death/survival balance, cell cycle progression, and cell migration. MAPK activity is regulated by a three-tiered phosphorelay system, which is in turn regulated by a complex network of signaling events and scaffolding proteins. The ubiquitous plasma membrane Na\(^+\)/H\(^+\) exchanger NHE1 is activated by, and implicated in, the physiological/pathophysiological responses to many of the same stimuli that modulate MAPK activity. While under some conditions, NHE1 is regulated by MAPKs, a number of studies have, conversely, implicated NHE1 in the regulation of MAPK activity. Here, we discuss the current evidence indicating the involvement of NHE1 in MAPK regulation, the mechanisms by which this may occur, and the possible physiological and pathophysiological relevance of this phenomenon.

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The family of mitogen-activated protein kinases (MAPKs)\(^1\) are central regulators of pivotal cellular processes including cell death/survival balance, cell cycle progression, cell migration, and differentiation [1,2]. It is not surprising, therefore, that the activity and subcellular localization of MAPKs are tightly controlled by a complex network of signaling events and direct protein–protein interactions [see 3–5].

Many of the stimuli regulating MAPK activity, such as exposure to mitogens or physiological or pathophysiological stress conditions, are also associated with marked changes in the activity of specific ion transport proteins [see, e.g., 6,7]. An important such transporter is the ubiquitous plasma membrane Na\(^+\)/H\(^+\) exchanger, NHE1 [see 8–10]. It is well established that NHE1 is, under some conditions, regulated by MAPKs [11,12]. However, a

\(^*\) Corresponding author. Fax: +45 35321567.

E-mail address: sfpedersen@aki.ku.dk (S.F. Pedersen).

\(^1\) Abbreviations used: Ang II, angiotensin II; BMK1, big MAPK1; [Ca\(^{2+}\)], the free, intracellular Ca\(^{2+}\) concentration; CHP, calcineurin homolog protein; EIPA, 5-N-ethyl-N-isopropyl-amiloride (NHE1 inhibitor); EMD87500, N-[2-methyl-4,5-bis(methylsulphonyl)-benzoyl]-guanidine (NHE1 inhibitor); ERK, extracellular signal-regulated kinase; ERM, ezrin/radixin/moesin; ET-1, endothelin 1; 5-HT, 5-hydroxytryptamine; HOE642, 4-isopropyl-3-ethylsulphonylbenzoyl-guanidine methanesulphonate (cariporide); HOE694, 3-methylsulphonyl-4-piperidino-benzoyl guanidine mesylate; Hsp70, heat shock protein 70; IKAP, IκB kinase complex-associated protein; iNOS, inducible nitric oxide synthase; JIP, JNK-interacting protein; JNK, c-Jun N-terminal kinase; KSR, Kinase Suppressor of Ras; LPA, lysophosphatidic acid; MAPK, mitogen-activated protein kinase; MEKK, MAPK/ERK Kinase Kinase; MIA, 5-N-methyl-N-isobutyl-amiloride (NHE1 inhibitor); MKK, MAPK kinase; MKKK, MAPK kinase kinase; MKP, MAPK phosphatase; MPT, mitochondrial permeability transition; NHE, Na\(^+\)/H\(^+\) exchanger; NIK, Nck-interacting kinase; OSM, Osmosensing Scaffold for MEKK3; p90RSK, p90 ribosomal S6 kinase; PP1, protein phosphatase 1; PtdIns(4,5)P\(_2\), phospholipid phosphatidylinositol(4,5)P\(_2\); ROS, reactive oxygen species.

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number of studies have, conversely, pointed to a central role for NHE1 upstream of MAPKs after a variety of stimuli. The aim of this contribution is to discuss current evidence regarding the involvement of NHE1 in regulation of the various MAPK subfamilies, the possible mechanisms involved, and the physiological and pathophysiological implications of this regulatory interaction.

Mitogen-activated protein kinases (MAPKs)

Mitogen-activated protein kinases (MAPKs) are a family of highly conserved proline-directed protein kinases found in all organisms from yeast to human [13]. MAPKs are regulated by a plethora of signaling events, including both hormone- and growth-factor receptor activation and mechanical/physical stimuli. In turn, MAPKs convert these signals into the coordinated regulation of pivotal physiological processes including cell death/survival balance, cell cycle progression, cell migration, and differentiation [1,2]. Four subfamilies of MAPKs have been extensively characterized: the extracellular signal-regulated kinases (ERK1/2), the c-Jun N-terminal kinases (JNK1/2/3), the p38 MAPKs (p38α/β/γ/δ), and ERK5 (also known as big MAPK1, or BMK1), while relatively little is known about the more recently identified family members ERK7 and ERK8 [see 1,14,15]. Most commonly, ERK1/2 and ERK5 are activated by mitogenic stimuli, and tend to favor proliferation and cell survival, while the JNK and p38 MAPK pathways are activated by stress stimuli such as hyperosmotic stress, ischemia/anoxia, or UV irradiation, and tend to promote cell cycle arrest and cell death [1,14]. However, there are multiple exceptions to this rule, and the pattern of MAPK regulation in response to a given stimulus, and the effects of a given MAPK on cell proliferation and death, are highly context-, isoform-, and cell type dependent [see, e.g., 1,4,16].

Consistent with the important physiological roles of these kinases, the regulation of MAPKs is highly complex. MAPK activation involves a highly conserved, three-tiered phosphorelay “core module”, in which a MAPK kinase (MKK) phosphorylates a MAPK kinase (MKK), which in turn activates the MAPK by dual phosphorylation of Thr and Tyr in a characteristic Thr-x-Tyr motif. Although there is significant cross-talk between the MAPK core modules, it is generally such that MKK1 and MKK2 activate ERK1 and ERK2, MKK3 and MKK6 activate p38 MAPK, MKK4 and MKK7 activate JNK, and MKK5 activates ERK5 [1,14,17]. Upstream of the MKKs, multiple MKKs, themselves controlled by a diverse range of protein kinases, small GTPases, and other proteins, regulate each MAPK subfamily in response to specific stimuli [see 1,18]. It has been established that Raf proteins (Raf-1, A-Raf, and B-Raf) act as MKKKs for ERK1/2, MAPK/ERK Kinase Kinase 1 (MEKK1) for ERK1/2 and JNKs, MEKK2 for JNKs and ERK5, and MEKK3 for p38 MAPKs and ERK5 [18,19]. However, the pattern of MKKKs regulating each MAPK subfamily is complex and yet incompletely understood, with for instance 13 MKKKs shown to regulate JNKs [19]. For further details, see also the Science’s STKE Connection maps for ERK1/2, JNK, and p38 MAPK: http://stke.sciencemag.org/cgi/cm/stkecm; CMP_10958 (ERK), CMP_10827 (JNK), and CMP_10958 (p38 MAPK).

Also central to the function of MAPKs is their spatial regulation by specific scaffolding proteins. For the ERKs, important examples include the Kinase Suppressor of Ras (KSR), β-arrestins1/2, paxillin, and apparently also Raf-1 itself. For JNK and/or p38 MAPK, important scaffolds are the JNK-interacting proteins (JIPs), filamin, and the recently described Osmosensing Scaffold for MEKK3 (OSM) [20; see 3,21]. MAPK scaffolding proteins generally interact with several components of the core three-kinase module, and control the physiological functions of MAPKs by regulating the subcellular localization and proximity of these components, as well as having direct effects on their activity [see 3,21].

In addition, the MAPKs can also regulate each other, an example being the regulation of ERK by p38 MAPK [22,23], which has been proposed to reflect the formation of a direct complex between these kinases [22]. Finally, dephosphorylation of MAPKs is mediated by a family of dual specificity protein phosphatases, the MAPK phosphatases (MKPs), which dephosphorylate MAPKs at Thr and Tyr. The MKPs exhibit distinct, but overlapping specificities for the MAPK subfamilies, and are themselves regulated by induction and stabilization in response to many of the stimuli regulating MAPKs, including exposure to mitogens or hyperosmotic stress [25,26; see 27].

The plasma membrane Na+/H+ exchanger, NHE1

Many of the stimuli regulating MAPK activity are associated with marked changes in the activity of specific ion transport proteins. An important such transporter is the ubiquitous plasma membrane Na+/H+ exchanger, NHE1. NHE1, belongs to the SLC9A family of Na+/H+ exchangers, of which nine mammalian members have been identified [see 28]. NHE1, which is ubiquitously expressed and highly conserved across vertebrate species, plays a major role in the regulation of intracellular pH (pH_i) and cell volume in most cell types studied, and has more recently been implicated in the regulation of cell morphology and cytoskeletal organization via its physical interactions with the actin-based cytoskeleton [see 8–10]. NHE1 has been assigned important roles in the regulation of cell proliferation, cell death/survival balance, and migration/invasion, although it is a point of some controversy whether the role of NHE1 in these processes is a regulatory or a permissive one [see 8–10]. NHE1 is activated by a wide range of stimuli including many growth factors and hormones, cell adhesion, or stress stimuli such as hyperosmotic cell shrinkage or ischemia/anoxia [see 7,8,10]. The NHE1 protein has an amphipathic N-terminal region with a proposed topology of 12 transmembrane domains, and a long (about 300
MAPKs and the regulation of membrane transport proteins

MAPKs are, via their downstream effectors, important regulators of many membrane transport proteins at the level of gene transcription [40]. MAPKs and/or their downstream effectors also regulate the activity of multiple transporters posttranscriptionally, either by direct phosphorylation of the transporter, or indirectly by phosphorylation of regulatory enzymes [e.g., 12, 41–43]. ERK, p38 MAPK, and JNK have all been implicated in the acute regulation of NHE1 activity after various stimuli [12, 41–43; see 7]. In some cases, regulation of NHE1 activity by MAPKs appears to involve direct phosphorylation. Thus, the above-mentioned Ser703 residue is directly phosphorylated by the MAPK effector p90 ribosomal S6 kinase (p90RSK) [11,12], and NHE1 has also been proposed to be directly phosphorylated by p38 MAPK [48].

NHE1 and the regulation of MAPK activity

Interestingly, a number of recent reports have, conversely, suggested that NHE1 plays a central role in the regulation of MAPK activity after certain stimuli. In this section, we discuss findings from studies directly addressing the role of NHE1 in MAPK regulation in a variety of tissues and after various stimuli (summarized in Table 1). To our knowledge, the first study directly proposing a role for NHE1 in the regulation of MAPK activity was published in 1995 by Takewaki et al. [47], who investigated the role of NHE1 in cardiac hypertrophy and proliferation of vascular smooth muscle cells. Takewaki et al. found that NHE1 mRNA expression in rabbit heart and arteries was increased in vivo with stimuli inducing hypertrophy (balloon injury, ventricular pressure overload), and that the specific NHE1 inhibitor HOE694 partially inhibited stretch-induced activation of ERK as well as of NFκB in cultured cardiomyocytes. In a later study in cultured neonatal rat cardiomyocytes [48] it was reported that NHE1 inhibition by HOE694 (cariporide) inhibited the stretch-induced activation of Raf-1 and ERK, while angiotensin II (Ang II) and endothelin 1 (ET-1)-induced ERK activation was unaffected. The mechanism of NHE1-mediated Raf-1–ERK activation was not elucidated, although roles for increased free, intracellular Ca2+ concentration ([Ca2+]i) or pH, were tentatively suggested [48]. In apparent contrast to the lack of role of NHE1 in Ang II–induced ERK activation reported in neonatal cardiomyocytes [48], Mukhin et al. in a study in rat aortic smooth muscle cells (RASM), showed that both NHE1 and ERK were activated by Ang II, and that activation of ERK by Ang II was strongly attenuated by inhibition of NHE1 or removal of extracellular Na+ [49]. Similarly, exposure to 5-hydroxytryptamine (5-HT) or intracellular acidification induced by an ammonium chloride prepulse stimulated ERK activity in an NHE1-dependent manner in RASM [49]. Ras- and MEK1/2 activation by Ang II or ET-1 were also attenuated by NHE1 inhibition, indicating that the effect of NHE1 was at or above the Ras level [49].

ERK1/2 is not the only MAPK regulated by NHE1. Thus, a study in intact rabbit hearts showed that the specific NHE1 inhibitor BIB722 prevented p38 MAPK phosphorylation, and strongly attenuated apoptosis, fibrosis, myocyte swelling, and inducible nitric oxide synthase (iNOS) protein expression after pacing-induced, non-ischemic heart failure, pointing to a major role for NHE1 in these events [50]. The mechanism by which NHE1-regulated p38 MAPK activity was not elucidated, yet a role for [Ca2+]i was tentatively suggested [50]. A recent study substantiated these findings by demonstrating that the NHE1 inhibitor EMD87580 attenuated the activation of both ERK1/2 and p38 MAPK after phenylephrine (PE) treatment (a model of hypertrophy) in neonatal rat cardiomyocytes [51]. NHE1 inhibition also prevented the PE-induced opening of the mitochondrial permeability transition (MPT) pore, loss of mitochondrial membrane potential, and reactive oxygen species (ROS) production. Finally, antioxidants and inhibitors of mitochondrial dysfunction also inhibited MAPK activation. Hence, it was proposed that NHE1-mediated activation of ERK and p38 MAPK was secondary to NHE1-dependent ROS production [51]. Very recently, Chen et al. demonstrated that HOE642 prevented glucose-induced ERK1/2 activation and hypertrophy in a high glucose model of cardiomyocyte hypertrophy, further supporting the notion that an NHE1–ERK1/2 pathway plays an important role in hypertrophy development [52].

All of the studies discussed above were performed in cardiac or vascular smooth muscle cells. However, the effects of NHE1 on MAPK activity are not limited to the cardiovascular system, or indeed to contractile cell types (although this may be the case for the Ang II and 5-HT induced, NHE1-dependent ERK activation, see [49]). Studies in human colon cancer epithelial cells indicated a major role for NHE1 in interleukin-8 (IL-8) production in...
response to a variety of inflammatory signals [53]. NHE1-dependent regulation of IL-8 production appeared to be mediated via ERK and NFκB, both of which were activated in an amiloride-sensitive manner [53]. Unfortunately, amiloride is not a specific inhibitor of NHE1, hence, the interesting potential role of NHE1 in MAPK regulation in the gut inflammatory response should be confirmed using more specific means of NHE1 inhibition.

We recently employed two systems to further explore the possible roles of NHE1 in regulation of MAPK activity. In many tissues, ischemia/anoxia is associated with increased activity of both NHE1 and MAPKs [7]. Both have been shown to contribute to ischemia/anoxia-induced cell damage, however, beyond the proposed roles of the ERK1/2–p90RSK (stimulatory) and p38 MAPK (inhibitory) pathways in regulation of NHE1 after ischemia/anoxia [54; see 7], the relationship between NHE1 and MAPKs under these conditions has received little attention. In NIH3T3 mouse fibroblasts, chemical anoxia rapidly activated NHE1 and, at a slower time scale, also p38 MAPK, while ERK1/2 activity was unaffected [24]. The anoxia-induced activation of p38 MAPK was completely abolished in the presence of either EIPA or HOE642 to inhibit NHE1, while ERK1/2 activity was unaffected by EIPA. Interestingly, anoxia-induced cell death was exacerbated by inhibition of p38 MAPK, and modestly attenuated by inhibition of NHE1, indicating that the regulation of p38 MAPK is not the only way in which NHE1 modulates anoxia-induced cell death [24]. Anoxia-induced activation of the tumor suppressor protein p53, as well as protein kinase B (PKB) activity and ERM protein phosphorylation were NHE1-independent, arguing against their involvement in p38 MAPK regulation [24]. Since NHE1 is known to play an important role in ischemia/anoxia-induced elevation of [Ca2+]i, in some tissues [see 7], a role for [Ca2+]i, is possible by analogy with the findings of [51], however, this remains to be addressed.

The second system employed was osmotic cell shrinkage, which potently activates NHE1 and regulates MAPK activity in many cell types [see 6,55]. In Ehrlich Lette Ascites (ELA) cells, cell shrinkage rapidly and transiently inhibited ERK1/2, and this effect was abolished by EIPA, HOE642, or by replacement of extracellular Na+ with NMDG+ [56]. MEK1/2 activity exhibited a similar pattern
of shrinkage- and EIPA sensitivity. Moreover, ERK1/2 activity under basal and hyperosmotic conditions was strongly suppressed by the expression of human (h)NHE1 in NHE1-deficient AP1 cells [56]; B.V.D., M.R. and S.F.P, unpublished. JNK activity was transiently increased by osmotic shrinkage in a partially EIPA-inhibitable manner in ELA cells, and was increased by hNHE1 expression in API cells. Finally, p38 MAPK was activated by shrinkage in a manner independent of NHE1 [56]; B.V.D., M.R. and S.F.P, unpublished. Thus, it appears that in osmotically shrunken ELA cells, NHE1 negatively regulates ERK1/2 activity, acting at or above the level of MEK1/2, and contributes to JNK activation, possibly in part due to the strongly alkaline pH, optimum of this kinase [56]; B.V.D., M.R. and S.F.P, unpublished.

From the examples presented above, it is clear that NHE1-mediated MAPK regulation is cell type- and context-specific, and indeed, evidence has also been presented against a role for NHE1 in MAPK regulation after some stimuli. In U937 cells (a human monocyte-like cell line), JNK activity also exhibited an alkaline pH optimum, yet shrinkage-induced JNK activation was unaffected by inhibition of NHE1 by amiloride or Na+ removal [57]. Moreover, in C6 glioma cells, lysophosphatidic acid (LPA) increased proliferation in an EIPA-sensitive manner, yet the LPA-induced ERK1/2 activation was unaffected by EIPA and HOE642 [58].

Possible mechanisms of NHE1-mediated MAPK regulation

By what mechanisms may NHE1 regulate MAPK activity? Two major categories of mechanisms—both of which may play a role—may be envisaged, namely, those dependent and -independent of the transported ions. In the failing heart, a role was proposed for the following series of events: NHE1 activation—increased [Na+]i—increased [Ca2+]i—mitochondrial dysfunction—ROS production—activation of ERK and p38 MAPK [51] (Fig. 1A). Similarly, NHE1 was reported to be activated by elevation of [Na+]i, after palytoxin-induced perturbation of Na+,K+,2Cl- cotransporter NKCC1, which associates which pHi (and presumably also [Na+]i) differ dramatically from the global cellular values [61]. Similar to NHE1, ERK1/2, JNK, and p38 MAPK have all been reported to be enriched in caveolae [63], hence a role for such local effects in MAPK regulation can by no means be excluded (Fig. 1B).

The other category of events that might conceivably be responsible for NHE1-mediated MAPK regulation involve conformational coupling via the C-terminal cytosolic tail region (Fig. 1B). A number of candidate mechanisms of this kind, all of which have yet to be experimentally tested, may be envisaged. First, the Ste20 related kinase NIK, which interacts directly with NHE1 [64], has been shown to interact with MEKK1, and act upstream of MEKK1 to activate JNK [65]. It is interesting in this regard that an apparently parallel scenario has been reported for the Na+,K+2Cl cotransporter NKCC1, which associates directly with the Ste20 related kinase and alanine rich kinase (SPAK), forming a complex which was proposed...
to interact in a regulated manner with p38 MAPK [66]. Second, ezrin, which also interacts directly with NHE1 [33], has been proposed to positively regulate ERK activity [67]. Third, a role for the actin cytoskeleton is also possible, as the ezrin-mediated coupling of NHE1 to the actin-based cytoskeleton has been reported to regulate cytoskeletal organization [33, and ERK, JNK, and p38 MAPK have all been found to be regulated by cytoskeletal reorganization after various stimuli [68,69]. Finally, 14–3–3 proteins, which interact with the Ser703-phosphorylated NHE1 [39], have been shown to function as scaffolds for MEKK1, -2, and -3 [70], and hence both a scaffolding role of NHE1, and competition between NHE1 and MEKKs for 14–3–3 may be envisaged.

Conclusions and perspectives

As discussed above, a substantial body of evidence indicates that NHE1 can regulate the activity of ERK1/2, JNK, and p38 MAPK, yet that the effects of NHE1 on MAPK activity are highly context- and cell type-specific. The precise pathways leading from the transporter to MAPK regulation remain to be determined, including the roles of the transported ions versus events involving conformational coupling of NHE1 to enzymes or cytoskeletal elements. Clearly, the concept of a transporter as part of a macromolecular signaling complex is in no way restricted to NHE1 [see 71–74]. Notably, regulation of signaling events by such complexes in a manner independent of ion transport has been proposed for several types of transporters [75; see 73,76]. Moreover, other transporters than NHE1 have been proposed to modulate, or act as a scaffold for, MAPK activity, including the Na+,K+,2Cl- cotransporter, NKCC1 [66,77], the Na+,-K+ ATPase [76], and voltage-dependent ether-a-go-go K+ channels [75]. Regardless of the mechanisms, the findings discussed in the present contribution indicate that at least under some conditions, regulation of MAPKs is an important consequence of NHE1 activation. Given the central roles of both NHE1 and MAPKs in control of, e.g., cell migration, proliferation, survival, and cell death, and in clinically important conditions such as cancer and ischemic cell death [7,16,78–80], NHE1-mediated MAPK regulation could be of considerable physiological and pathophysiological relevance, and may be an important link to understanding the role(s) of NHE1 in these processes. Future studies should aim at elucidating the mechanisms by which NHE1 regulates MAPK pathways, and at further exploring the physiological and pathophysiological consequences of NHE1 activation upstream of MAPKs.

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References
