Discussion Letter

Na^+/H^+ exchange and Ca^{2+} influx

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Received 12 September 1989

Cell stimulation raises the cytosolic free Ca^{2+} concentration, $[Ca^{2+}]_i$, and induces activation of Na^+/H^+ exchange which raises the cytosolic pH, pH_i. Recent studies have addressed the question whether Na^+/H^+ exchange plays a role in Ca^{2+} influx and, specifically, whether a rise in pH_i alone suffices to open Ca^{2+} channels in the plasma membrane. Artificial cytosolic alkalinization can induce Ca^{2+} uptake across the plasma membrane of endothelial cells, lymphocytes and smooth muscle cells. Furthermore, inhibition of Na^+/H^+ exchange reduces agonist-induced Ca^{2+} influx in endothelial cells and platelets which supports the concept that pH_i may regulate the opening of Ca^{2+} channels in the plasma membrane. Although these findings argue in favour of a role of Na^+/H^+ exchange in Ca^{2+} influx, the onset of pH_i and Ca^{2+} rises, measured with fluorescent indicators, suggests that the increase in $[Ca^{2+}]_i$ distinctly precedes the increase in pH_i. This challenges the concept that alkalinization per se is a sufficient signal for the opening of Ca^{2+} channels in the plasma membrane.

Platelet; Endothelial cell; Lymphocyte; Smooth muscle cell; Na^+/H^+ exchange

1. INTRODUCTION

All eukaryotic cells possess an ion transport system in their plasma membrane which, driven by the inwardly directed Na^+ gradient, extrudes H^+ from the cytosol to the extracellular space. This ion transport sytem is commonly referred to as the 'Na^+/H^+ exchanger' or 'Na^+/H^+ antiport'.

In resting cells the Na^+/H^+ exchanger is activated by an increase in the intracellular H^+ concentration and replaces intracellular H^+-ions for external Na^+-ions until the original cytoplasmic pH, pH_i, of approximately 7.1 (at pH_0 7.4) is re-established. This recovery of pH_i from cytosolic acidification can be prevented by either removal of Na^+ ions from the extracellular space or by incubation of cells with amiloride and its analogs. Hence, in unstimulated cells the role of the antiport is to keep pH_i constant and to counteract cytosolic acidification [1–4]. In contrast, when cells are activated by specific stimuli such as growth factors or hormones, pH_i increases to ranges close to the extracellular value of 7.4. This increase in pH_i is also abolished in Na^+-free medium and by amiloride analogs which indicates the involvement of Na^+/H^+ exchange in this process. It is generally believed that cell stimulation induces certain modifications in the exchanger that enhance its affinity towards internal H^+ [1–5]. Blocking Na^+/H^+ exchange does not only prevent the agonist-induced increase in pH_i, but also reduces various cell functions (for review see [6–8]). It should be explicitly noted, however, that most of the above-cited studies have been conducted in nominally bicarbonate-free media, and that some cell lines have been reported not to respond with cytosolic alkalinization to stimulation by agonists in the presence of bicarbonate (for review see [3]).

The present review focuses on the potential interrelationship between rises in pH_i and $[Ca^{2+}]_i$ in stimulated cells with special emphasis on the question whether or not a rise in pH_i alone is sufficient to trigger an influx of Ca^{2+} ions.

2. IS A RISE IN pH_i ALONE SUFFICIENT TO INCREASE $[Ca^{2+}]_i$?

One of the earliest events in stimulated cells is a rapid rise in $[Ca^{2+}]_i$. Part of this Ca^{2+} stems from intracellular storage sites and is released through the action of inositol 1,4,5-trisphosphate (Ins 1,4,5P_3) [9–11]. However, the Ins 1,4,5P_3-induced Ca^{2+} mobilization is

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Abbreviations: $[Ca^{2+}]_i$, cytosolic free Ca^{2+} concentration; $Ca^{2+}{}_o$, extracellular Ca^{2+}; EIPA, ethylisopropylamiloride; $[H^+]_i$, cytosolic H^+ concentration; $[Na^+]_i$, cytosolic Na^+ concentration; $Na^+{}_o$, extracellular Na^+; pH_i, cytosolic pH; pH_o, extracellular pH; Ins 1,4,5P_3, inositol 1,4,5 trisphosphate

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

Table 1

Effect of artificial cytosolic alkalinization and agonists on $[Ca^{2+}]_i$ in the presence of extracellular Ca^{2+}, i.e. Ca^{2+} influx plus mobilization from intracellular stores

Cell type	NH_4Cl		Monensin		Agonist		Ref.
	ΔpH_i	$\Delta[Ca^{2+}]_i$ (nM)	ΔpH_i	$\Delta[Ca^{2+}]_i$ (nM)	ΔpH_i	$\Delta[Ca^{2+}]_i$ (nM)	
Endothelial cells	0.34	1410	0.16	1164	0.36	700	15
Lymphocytes	0.32	119	0.26	178	n.d.	n.d.	13
Platelets	0.27	400	0.31	420	0.36	1000	16
Platelets	n.d.	n.d.	0.60	0	0.25	270	18
Platelets	0.27	0	n.d.	n.d.	(0)	1000	17
Smooth muscle cells	0.41	46	n.d.	n.d.	n.d.	n.d.	19

Shown are the rises in pH_i and $[Ca^{2+}]_i$ evoked by natural agonists (where available) in comparison with changes in $[Ca^{2+}]_i$ after artificially elevating pH_i by either NH_4Cl or monensin. Endothelial cells and platelets were activated by thrombin, smooth muscle cells by arginine-vasopressin. In lymphocytes, no data for agonist-induced changes in pH_i and $[Ca^{2+}]_i$ are available in the same study. Since all Ca^{2+} measurements were conducted in the presence of extracellular Ca^{2+}, changes in $[Ca^{2+}]_i$ represent the influx of Ca^{2+} ions from the extracellular space to the cytosol plus mobilization from intracellular stores. All values represent increases of pH_i (units) and $[Ca^{2+}]_i$ (nM) above untreated controls

only transient and, in the absence of extracellular Ca^{2+}, Ca_0^{2+}, $[Ca^{2+}]_i$ rapidly returns to resting levels. Under physiological conditions, i.e. in the presence of Ca_0^{2+}, the elevation of $[Ca^{2+}]_i$ lasts much longer as a result of an influx of Ca^{2+} across the plasma membrane. The exact mechanism by which agonists promote Ca^{2+} influx remains to be defined. Since most non-excitable cells lack voltage-dependent Ca^{2+} channels, one may presume that the entry of Ca^{2+} is mediated via receptor-operated Ca^{2+} channels [12]. An important question is whether cytoplasmic alkalinization alone is a sufficient signal for Ca^{2+} influx. This question has been addressed in different cell types, e.g. lymphocytes, endothelial cells, smooth muscle cells and platelets. In these studies pH_i was artificially elevated by either addition of NH_4Cl or the use of the Na^+/H^+ ionophore monensin. The effect of these manipulations on $[Ca^{2+}]_i$ was assessed from quin2 or fura2 fluorescence, and the translocation of $^{45}Ca^{2+}$. Since the Ca^{2+}-binding properties of quin2 and fura2 are not affected by variations of pH between 7.0 and 7.5 [13,14], the changes in fluorescence seen after artificial cytosolic alkalinization are accurate reflections of $[Ca^{2+}]_i$. Table 1 summarizes the effects of artificially elevating pH_i on $[Ca^{2+}]_i$ in different cell types, and compares these changes with those induced by natural agonists in the same studies (as far as data are available). As these experiments have been conducted in the presence of extracellular Ca^{2+}, both Ca^{2+} mobilization and influx add to the net rise in $[Ca^{2+}]_i$. Ghigo et al. observed that treatment of endothelial cells with either NH_4Cl or monensin evoked a strong influx of Ca^{2+} which even exceeded that induced by thrombin [15]. Similarly, these manipulations induced a significant influx of Ca^{2+} in quin2-loaded lymphocytes [13] which was completely abolished after chelating Ca_0^{2+} by addition of EGTA. The observation that Ca^{2+} influx was not only induced by monensin (which apart from raising pH_i also evelates $[Na^+]_i$), but also by NH_4Cl treatment (which raises pH_i without altering $[Na^+]_i$) points at the rise in pH_i – rather than the change in $[Na^+]_i$ – as the cause of this response. The findings in platelets are controversial. Ghigo et al. [16] reported that artificially elevating pH_i by both monensin and NH_4Cl induced an influx of Ca^{2+} amounting to almost 50% of that evoked by thrombin. This observation was confirmed by measuring monensin-induced uptake of $^{45}Ca^{2+}$ [16]. In contrast, both Simpson and Rink [17] as well as our group [18] failed to detect an influx of Ca^{2+} in response to cytosolic alkalinization along in quin2- or fura2-loaded platelets. Raising pH_i by NH_4Cl in smooth muscle cells induced some minor albeit significant Ca^{2+} influx, and the change in $[Ca^{2+}]_i$ was twice that observed in the absence of Ca_0^{2+} [19]. These observations argue in favour of a role of alkalinization in Ca^{2+} entry in these cells. Unfortunately, the precise mechanisms underlying this process remain obscure. In theory, two different mechanisms may be involved. First, a rise in pH_i might directly open receptor-operated Ca^{2+} channels, e.g. by altering the protonation of the permeation site, thereby allowing Ca^{2+} influx. No data in support of this hypothesis are yet available. Second, alkalinization could induce the intracellular formation of substances which, upon release, combine with specific receptors on the cell surface subsequently promoting agonist-induced Ca^{2+} influx. Possible candidates are certain prostaglandins, products of the phospholipase A_2 pathway, or platelet-activating factor, which are formed via pH-dependent processes [15,20–22].

3. IS INTACT Na^+/H^+ EXCHANGE REQUIRED FOR STIMULUS-INDUCED Ca^{2+} INFLUX?

One means to investigate the contribution of Na^+/H^+ exchange to stimulus-induced influx of Ca^{2+} is to measure changes in fluorescence of quin2- or

Table 2

Effect of inhibition of Na^+/H^+ exchange in different cell types on stimulus-induced $[Ca^{2+}]_i$ rises in the presence of extracellular Ca^{2+}, i.e. Ca^{2+} influx plus mobilization from intracellular stores

Cell type	EIPA $\Delta[Ca^{2+}]_i$ (% of control)	Na_o^+ removal $\Delta[Ca^{2+}]_i$ (% of control)	Stimulus	Ref.
Endothelial cells	0	0	Thr	15
Platelets	n.d.	37	Thr (0.1 U/ml)	16
Platelets	n.d.	36	AA	16
Platelets	40	15	Thr (0.2 U/ml)	25
Platelets	0	n.d.	Thr (0.5 U/ml)	27
Platelets	74	16	Thr (0.1–0.5 U/ml)	26
Platelets	n.d.	100	Thr (0.5 U/ml)	23
Smooth muscle cells	n.d.	100	AII	28

Shown is the effect of inhibiting Na^+/H^+ exchange by either ethylisopropylamiloride (EIPA) or Na^+ substitution on stimulus-induced Ca^{2+} influx. Changes in $[Ca^{2+}]_i$ were deduced from changes in fluorescence of fura2- or quin2-loaded cells. The values represent the rises in $[Ca^{2+}]_i$ (% of control) observed after blockade of Na^+/H^+ exchange. Thr, thrombin; AA, arachidonic acid; AII, angiotensin II. The values in parentheses indicate the agonist concentrations used for cell stimulation

fura2-loaded cells after blocking Na^+/H^+ exchange by either iso-osmotic substitution of Na_o^+ or by direct inhibition of Na^+/H^+ exchange with amiloride analogs. One potential drawback of this method is that this treatment does not only prevent cytosolic alkalinization but also causes dramatic cytosolic acidification in cells stimulated by agonists. Hence, it may be difficult to decide whether an impairment of Ca^{2+} influx by EIPA or removal of Na_o^+ is caused by a fall in pH_i to below the resting value or actually by prevention of cytosolic alkalinization. Most of these studies have been performed in platelets and only a few reports on other cell types have been published. An overview of these experiments is given in table 2. Studies in endothelial cells demonstrated that inhibition of Na^+/H^+ exchange inhibited thrombin-stimulated Ca^{2+} influx as assessed from quin2 fluorescence and the influx of $^{45}Ca^{2+}$ [15]. This observation agrees well with the proposed role of alkalinization as an inducer of Ca^{2+} influx (cf. table 1). Both Na_o^+ removal as well as EIPA also lowered Ca^{2+} influx in platelets stimulated by thrombin or arachidonic acid [16]. Except reports by Sage and Rink [23] and Sanchez et al. [24] most studies demonstrated that intact Na^+/H^+ exchange contributes to agonist-induced Ca^{2+} uptake in human platelets (e.g. [25–27]). Hence, in this respect platelets behave similarly to endothelial cells. In smooth muscle cells, on the other hand, Na_o^+ removal apparently had no effect on angiotensin II-induced Ca^{2+} influx [25], although the finding that artificial cytosolic alkalinization induces Ca^{2+} uptake would predict a role of Na^+/H^+ exchange.

4. CONCLUSIONS

There is increasing evidence that intact Na^+/H^+ exchange is required for the mechanisms that evoke Ca^{2+} influx in stimulated cells. Although an artificially produced rise in pH_i may suffice to trigger Ca^{2+} influx in endothelial cells, lymphocytes, and smooth muscle cells it is doubtful whether a similar mechanism is operative in agonist-stimulated cells. Especially the observation that maximum Ca^{2+} influx precedes the rise in pH_i makes it unlikely that cytosolic alkalinization per se works as a 'second messenger' at receptor-operated Ca^{2+} channels. On the other hand, the reports showing a strong reduction of Ca^{2+} influx after blockade of Na^+/H^+ exchange strongly suggest the involvement of a pH-sensitive step in the opening of Ca^{2+} channels. It remains to be clarified whether the role of Na^+/H^+ exchange is restricted to merely prevent cytosolic acidification in stimulated cells, or whether the observed increases in cytosolic pH also contribute to the mechanisms allowing for Ca^{2+} influx across the plasma membrane.

Acknowledgements: We are indebted to Prof. Dr K.J. Ullrich for helpful comments during the preparation of this manuscript. The authors' work was financially supported by the Deutsche Forschungsgemeinschaft and by the Netherlands Thrombosis Foundation.

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