

SCIENTIFIC LETTER

Increased platelet sodium–hydrogen exchanger activity in patients with variant angina

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The causes of coronary artery spasm in patients with variant angina remain unknown. The segmental location of spasm indicates local hyperreactivity, but a diffuse increased coronary vasoconstriction, suggesting a substrate which could facilitate spasmogenic modifications, has been reported in many patients.¹ The membrane sodium–hydrogen (Na^+/H^+) exchanger (NHE) is a major regulator of intracellular pH (pH_i).² An increased activity of the NHE isoform 1 (NHE-1) in smooth muscle cells has been suggested to favour vasoconstriction by causing intracellular alkalinisation and calcium overload.³ Furthermore, potential triggers of spasm (catecholamines, endothelin-1) have been shown to increase NHE-1 activity.⁴ In this study we investigated NHE-1 activity in platelets of patients with variant angina.

METHODS

The study group included 17 patients (13 men, 58 (9) years) with variant angina (angina attacks at rest, associated with transient ST segment elevation). Patients with hypertension and diabetes were excluded. The control group included 17 healthy subjects (13 men, 55 (6) years) without any history of chest pain, and with normal physical examination, ECG, and laboratory tests.

Study protocol

Because of ethical reasons, calcium antagonist drugs could not be withdrawn in patients, who, however, were invited not to take these drugs on the day of the study. Other drugs were withdrawn for more than one week before the study.

A blood sample of 50 ml was drawn from an antecubital vein. To obtain platelet rich plasma (PRP), blood was anticoagulated with adenosine-citrate-dextrose in a 6:1 ratio and centrifuged at 150 *g* for 15 minute. The upper two thirds of the centrifuged PRP were drawn and incubated at 37°C for 30 minutes with 2'-7'-bis-carboxyethyl-5(6)-carboxyfluorescein acetoxymethylester (BCECF-AM, 7 μM), a fluorescent substance sensitive to pH_i .

To avoid platelet activation, EDTA 1 mM was then added to PRP. Platelets were gel filtered onto Sepharose 2B columns (20×1.6 cm), equilibrated with a buffer HEPES 20 mM, KCl

5 mM, glucose 5 mM, Na Cl 135 mM, albumin 0.2 g%, pH 7.4 (buffer A). The resulting platelet suspension was separated, after adding EDTA 1 mM, into two fractions, and centrifuged at 600 *g* for 15 minutes. To assess NHE activity, one aliquot of platelets was suspended in buffer A, containing a physiologic Na^+ concentration. To obtain calibration curves and conversion of fluorescence values into pH_i values, the second aliquot was suspended in a buffer containing HEPES 20 mM, KCl 140 mM, glucose 5 mM, albumin 0.2 g%, pH 7.4 (buffer B). Ca^{2+} (2 mM) and Mg^{2+} (1 mM) were added to both platelet aliquots.

Fluorescence of platelets labelled with BCECF was recorded by a spectrofluorometer (LS5b Perkin Elmer). Platelet aliquots were put in plastic cuvettes and shaken at 25°C within the cell holder spectrofluorometer. Excitation and emission wavelengths of the fluorometer were fixed at 495 and 530 nm, respectively, with a slit band pass of 5 and 10 nm, respectively. After measuring baseline fluorescence, platelets were exposed to sodium propionate, which enters the cells and induces acidification. This stimulates NHE activation, which restores the initial pH_i by extruding H^+ from the cells. To assess NHE activity in response to increasing grades of acidification, platelets were exposed to solutions with increasing final Na propionate concentrations (8, 17, 26, 33, 40, 50, and 60 mmol/l).

To convert fluorescence into pH_i values, a calibration curve was obtained for each experiment by adding nigericin (2.5 $\mu\text{mol/l}$) to platelet suspended in buffer B. In presence of high extracellular K^+ concentration, nigericin eliminates the intra/extracellular H^+ gradient by changing H^+ with K^+ . Thus, by varying the pH value of the platelet suspension by means of KOH or HCl, the corresponding fluorescence value can be measured. pH_i was measured at baseline and after acidification; the pH_i recovery curve was recorded and the maximal pH_i recovery ($\Delta\text{pH}_i/\text{min}$), taken as a measure of platelet NHE activity, was obtained as the tangent to the curve at the point of maximal slope.

All experiments were repeated while also simultaneously exposing platelets to α thrombin (2 nmol/l), a powerful NHE agonist, together with sodium propionate.

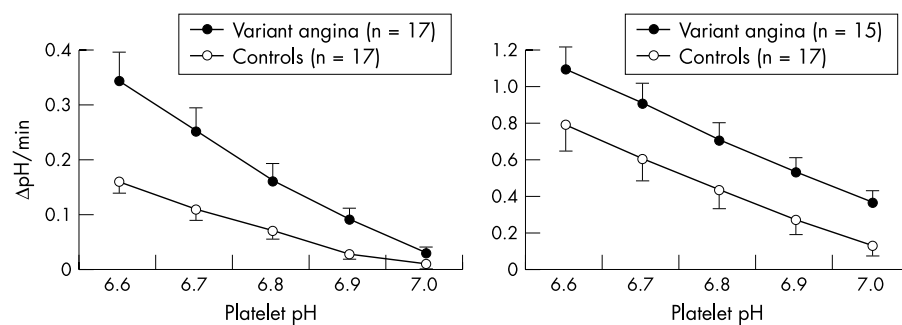


Figure 1 $\Delta\text{pH}_i/\text{min}$ values ($\pm\text{SEM}$), as an expression of the membrane sodium–hydrogen exchanger activity, derived according to individual regression curves for different values of intraplatelet pH_i in the two study groups, obtained after acidification alone (p for trend = 0.01) and after acidification + α thrombin (p for trend = 0.048).

Statistics

Continuous variables were compared by *t* test, and proportions by Fisher exact test. Linear regression analysis between measured $\Delta\text{pH}/\text{min}$ and pH_i achieved at increasing grades of acidification was applied in each subject to derive $\Delta\text{pH}/\text{min}$ values at pH_i 7.0, 6.9, 6.8, 6.7, and 6.6 in response to platelet acidification (and to acidification + α thrombin); two way analysis of variance, with a repeated measure design, was used to compare the values in the two groups. Data are presented as mean (SD). A probability value of $p < 0.05$ was considered significant.

RESULTS

There was a significant inverse linear relation between $\Delta\text{pH}/\text{min}$ and platelet pH_i achieved with increasing Napropionate concentrations, both in patients and controls (data not shown).

After acidification, $\Delta\text{pH}/\text{min}$ values were significantly higher ($p = 0.01$) and the response of $\Delta\text{pH}/\text{min}$ to decreasing pH_i values was significantly steeper in patients than in controls ($p < 0.00001$) (fig 1, left panel).

After acidification + α thrombin, $\Delta\text{pH}/\text{min}$ values were significantly higher in patients than in controls ($p = 0.048$), but there was no difference in the response of $\Delta\text{pH}/\text{min}$ to decreasing pH_i values ($p = 0.92$) (fig 1, right panel).

DISCUSSION

By showing a faster pH_i recovery after acidification (with or without α thrombin), our findings indicate that, in variant angina patients, platelet NHE-1 activity is significantly increased in response to agonistic stimuli. Indeed, NHE is known to be a major regulator of pH_i after acidification.²

As NHE-1 is ubiquitously expressed in body cells, our data may suggest that its activity might also be increased in vascular smooth muscle cells of these patients. Although this needs to be appropriately investigated in future studies, an increased NHE activity in smooth muscle cells in variant angina patients

might represent a substrate contributing to diffuse increased coronary vasoconstriction and facilitate local spasmogenic changes in coronary segments, predisposing to local hyper-reactivity to vasoconstrictor stimuli.

The cause(s) of the increased NHE activity in variant angina patients cannot be derived from our data, but may include an increased NHE molecule expression or increased NHE affinity for agonists. A conformational change in the NHE molecule has, indeed, been suggested to account for functional NHE abnormalities in red blood cells of patients with microvascular angina.³ Also, a dysfunction of the molecular systems which regulate cellular NHE activity could be involved in causing abnormalities in NHE function.

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