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Short communication

Cardiac hypertrophy determines digitalis action on intracellular Ca^{2+} in human myocardium

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Abstract

Ventricular hypertrophy alters transporters associated with digitalis action, but the outcome of digitalis treatment on intracellular cations in the hypertrophied human myocardium remains unknown. Using double-barreled Ca^{2+}-selective microelectrodes, we simultaneously measured Ca^{2+} activity and membrane potential in myocardial samples from patients without and with ventricular hypertrophy, prior to and following exposure to strophanthidin, a prototype digitalis. We found that ventricular hypertrophy is associated with greater strophanthidin-induced increase in diastolic Ca^{2+} levels compared to that observed in the absence of hypertrophy. Furthermore, in hypertrophied myocardium the magnitude of the increase in Ca^{2+} induced by strophanthidin was inversely related to increase in myocardial mass. Thus, the extent of ventricular hypertrophy determines digitalis action on intracellular Ca^{2+} within the human myocardium. © 1997 Elsevier Science B.V.

Keywords: Ventricular mass; Ion-selective microelectrode; Ca^{2+} level; Diastolic

1. Introduction

Myocardial hypertrophy occurs early in the progression of heart failure, and has been associated with complex alteration in the expression of Na/K-ATPase, a major regulator of cation homeostasis and the molecular target of digitalis drugs (Swynghedauw et al., 1995; Davies et al., 1996). Specifically, reduction in myocardial Na/K-ATPase concentration, and a shift towards isoform subunits with higher affinity for digitalis have been reported (Charlemagne and Swynghedauw, 1995; Bundgaard and Kjeldsen, 1996). In addition, the activity of the Na/Ca exchanger, as well as other cardiac cation transporters, may also be altered during hypertrophy (Swynghedauw et al., 1995; Schwartz and Mercadier, 1996). However, the effect of digitalis treatment on intracellular cations, such as Ca^{2+}, in the hypertrophied human cardiomyocyte, remains incompletely understood. One major difficulty has been to obtain samples from patients with isolated ventricular hypertrophy in which the contribution of systemic disease, end-stage heart failure or cell damage with membrane depolarization could be excluded (Bundgaard and Kjeldsen, 1996). This is of importance since such factors, alone, could affect intracellular cation homeostasis. Therefore, the purpose of the present study was to determine the effect of a digitalis compound on diastolic Ca^{2+} levels in patients with isolated left ventricular hypertrophy.

2. Methods

2.1. Patients

Biopsy samples of left ventricular myocardium were obtained, with patient consent (and approval of the Institutional Human Ethic Committee), during valve surgery from 15 patients (age from 28 to 67) with left ventricular hypertrophy due to isolated aortic valve stenosis (mean left ventricular mass index: 148 ± 5 g/m^2), and from 16 patients (age from 21 to 42) with isolated mitral stenosis without hypertrophy (mean left ventricular mass index: 88 ± 2 g/m^2). Left ventricular mass was calculated using serial M-mode echocardiography (77020 AC Hewlett Packard), and divided by body surface area to derive left

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ventricular mass index (LVMI), as previously described (Devereux et al., 1986). Other than aortic or mitral stenosis, no patients in either group had significant structural heart disease or coronary atherosclerosis, and were free of congestive heart failure symptoms.

2.2. Ca^{2+}-selective microelectrode measurements

Following removal, biopsy samples were immediately superfused with Tyrode solution (in mM: NaCl 140, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, glucose 10, HEPES 10, pH 7.4), bubbled with a gaseous mixture of O_2 (95%) and CO_2 (5%) at 37°C. Fibrous and connective tissue was removed by fine dissection with the aid of a stereo-microscope. Clean strips of ventricular muscle with clear striation spacing were placed in a temperature-controlled chamber (37°C) positioned on a microscope stage. Diastolic Ca^{2+} levels were determined by double-barreled Ca^{2+}-selective microelectrodes, as previously described (Lopez et al., 1995a). One barrel of the microelectrode contained 3 M KCl to measure membrane potential, while the other barrel contained the ion-selective carrier ETH 1001 (Fluka) to measure Ca^{2+} activity using a high-input impedance electrometer (WPI FD-223). Following impalement of individual cardiomyocytes, diastolic levels of intracellular Ca^{2+} were obtained with reference to a calibration curve constructed for each electrode (Lopez et al., 1995b; Lopez and Terzic, 1996). Only samples in which there was no membrane depolarization were used for analysis. Data are presented as mean ± SEM, where n represents the number of patients (with multiple values for each patient averaged). Statistical significance of differences between two means was determined using the Student's t test, and a value of $P < 0.05$ considered significant.

3. Results

Using a double-barreled Ca^{2+}-selective microelectrode (Fig. 1), resting $[Ca^{2+}]_i$ and membrane potential were recorded simultaneously in ventricular cells from patients without or with ventricular hypertrophy, prior to and following exposure to strophanthidin, a prototype digitalis compound. Diastolic Ca^{2+} levels were three-fold higher in hypertrophied compared to non-hypertrophied cardiomyocytes prior to exposure to strophanthidin (Fig. 2A). Following exposure to strophanthidin (0.01–10 μM), diastolic Ca^{2+} levels increased in ventricular cells from patients without as well as with hypertrophy. Representative recordings from ventricular cells, exposed to 1 μM strophanthidin, show that $[Ca^{2+}]_i$ and membrane potential were 0.21 μM and −82 mV in a patient without (Fig. 1, left panel) versus 0.92 μM and −81 mV in a patient with (Fig. 1, right panel) ventricular hypertrophy. On average, in non-hypertrophied cardiomyocytes, diastolic Ca^{2+} increased from 107 ± 3 nM ($n = 16$); to 131 ± 4 nM ($n =$

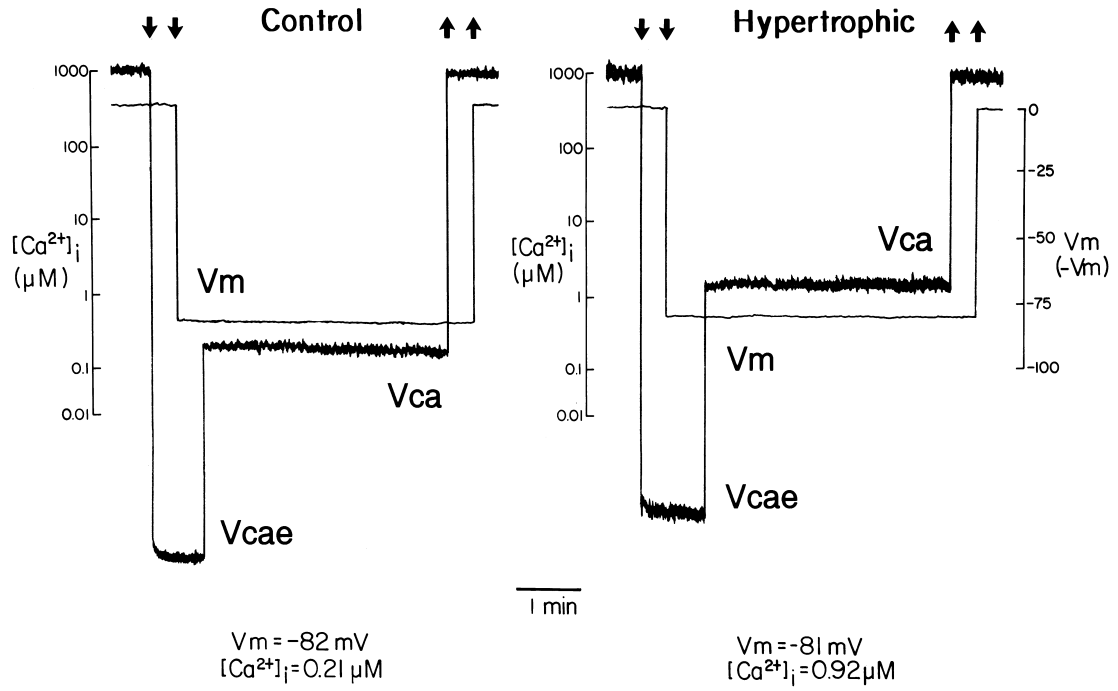

Fig. 1. Strophanthidin induces a larger increase in diastolic Ca^{2+} in human hypertrophied versus non-hypertrophied myocardium. Simultaneous recording with double-barreled microelectrodes of membrane potential, V_m and $[Ca^{2+}]_i$ ($V_{ca} = V_{cae} - V_m$ where V_{cae} is the potential recorded by the Ca^{2+}-selective barrel), in a cardiac biopsy sample from a patient without (left panel) and with myocardial hypertrophy (right panel) exposed to 1 μM strophanthidin. V_m and V_{ca} traces are not superimposed. Downward arrows indicate cell impalement. Upward arrows indicate withdrawal of microelectrode. Calibration bar for V_m applies to both panels.

12), 182 ± 4 nM ($n = 10$), 218 ± 4 nM ($n = 8$) and 252 ± 6 nM ($n = 8$) after treatment with 0.01, 0.1, 1, and 10 μM strophanthidin, respectively (Fig. 2A). In hypertrophied cardiac cells, diastolic Ca^{2+} increased from 383 ± 25 nM ($n = 15$) to 552 ± 17 nM ($n = 14$), 754 ± 12 nM ($n = 11$), 934 ± 15 nM ($n = 10$) and 985 ± 13 nM ($n = 10$) after treatment with the same concentrations of strophanthidin (Fig. 2A). Thus, the increase in diastolic Ca^{2+} levels induced by the digitalis compound was significantly greater in cells from patients with hypertrophy.

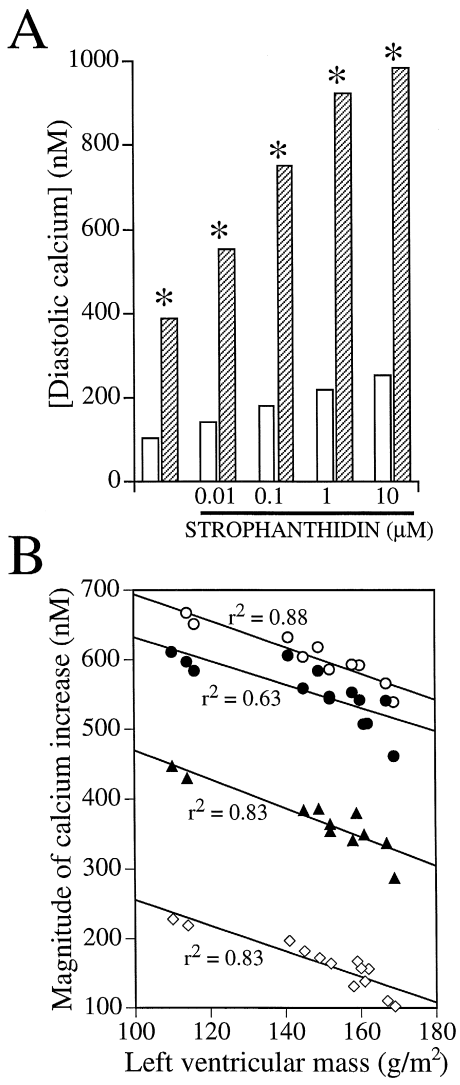

Fig. 2. The presence (A) and extent (B) of ventricular hypertrophy determine the action of digitalis on diastolic Ca^{2+} levels in human myocardium. (A) Effect of strophanthidin (0.01–10 μM) on diastolic Ca^{2+} levels in non-hypertrophied (open bars) and hypertrophied (hatched bars) myocardium. Note that strophanthidin induced a concentration-dependent increase in diastolic Ca^{2+} levels in both groups. Stars indicate significant difference between the mean values of Ca^{2+} levels obtained in non-hypertrophied versus hypertrophied cardiomyocytes. SEM varied between 3 and 25 nM. Number of measurement is provided in the text for each concentration. (B) Relationship between the magnitude of digitalis-induced increase in diastolic Ca^{2+} levels and ventricular mass in patients with ventricular hypertrophy. Values of Ca^{2+} levels for each patient were averaged from 2–4 measurements and plotted against corresponding ventricular mass at each concentration of strophanthidin (open diamonds: 0.01 μM; closed triangles: 0.1 μM; closed circles: 1 μM; open circles: 10 μM). Solid lines were obtained by linear regression, and r^2 values are provided for each concentration of strophanthidin.

To determine the relationship between digitalis effect and increasing ventricular mass, the magnitude of the increase in Ca^{2+} levels, induced by strophanthidin, was plotted against ventricular mass for each patient with ventricular hypertrophy (Fig. 2B). At each concentration of strophanthidin (0.01–10 μM), the magnitude of the increase in Ca^{2+} was less with increasing ventricular mass (Fig. 2B). Thus, the response of hypertrophied myocardium to the digitalis compound was inversely related to the degree of ventricular hypertrophy.

4. Discussion

The present study demonstrates that in biopsies from hypertrophied versus non-hypertrophied human hearts, diastolic Ca^{2+} is elevated under control conditions, and is increased to a greater extent in response to strophanthidin. The magnitude of the effect of the digitalis compound was found to correlate inversely with the degree of myocardial hypertrophy.

It is conceivable that Ca^{2+} loading, and an enhanced responsiveness to digitalis could have been secondary to the presence of myocardial ischemia and heart failure or cell damage resulting from the process of obtaining biopsy samples and/or the hypertrophic process itself (Bundgaard and Kjeldsen, 1996). However, in this study the effects of ischemia and heart failure were largely excluded since no patient had significant coronary disease or heart failure at the time of biopsy collection. In addition, the contribution of cell damage or membrane depolarization was excluded by measurement of membrane potential simultaneously with intracellular Ca^{2+}, and by including measurements only from non-depolarized cardiomyocytes. To our knowledge the present study is the first to determine diastolic Ca^{2+} levels in the presence and absence of digitalis in myocardial samples from patients with isolated ventricular hypertrophy due to aortic stenosis in which the contribution of systemic disease, end-stage heart failure or cell damage could be excluded.

It is also conceivable that levels of Ca^{2+} obtained from patients with hypertrophic myocardium were only apparently elevated because of abnormally low levels in control patients without hypertrophy associated with lower left ventricular pre-load in the setting of isolated mitral stenosis. However, against this is the finding that left ventricular mass index obtained from control patients with mitral stenosis were similar to values found in patients without cardiac disease (Shub et al., 1994). Thus, the values of intracellular Ca^{2+} obtained, herein, are likely to reflect changes due to myocardial hypertrophy rather than loading conditions of the heart.

The finding that human hypertrophic myocardium exhibits an enhanced responsiveness to digitalis is consistent with recent findings which have demonstrated alteration in the level and affinity of Na/K-ATPase, the major sarcolemmal target of digitalis action (Charlemagne and Swynghedauw, 1995; Bundgaard and Kjeldsen, 1996). In addition, abnormal activity of other cation transporters involved in the pharmacological action of digitalis, such as the Na/Ca exchanger, have also been demonstrated in hypertrophied myocardium (Swynghedauw et al., 1995; Schwartz and Mercadier, 1996). To date, however, the consequences of these changes on Ca^{2+} loading in hypertrophic human myocardium exposed to a digitalis compound have remained controversial. In this regard, the present study indicates that the outcome of digitalis action on diastolic Ca^{2+} loading in hypertrophied myocardium is related to the extent of hypertrophy. The biological relevance of the inverse relationship found between the magnitude of increase in diastolic Ca^{2+} induced by digitalis and the degree of hypertrophy is unclear, but could, in principle, be due to several factors. On one hand, progression of hypertrophy has been associated with a progressive decrease in the concentration of Na/K-ATPase molecules, the main target of digitalis action (Charlemagne and Swynghedauw, 1995). On the other hand, increased intracellular calcium concentration per se has been associated with altered responsiveness of cardiac cells to pharmacological agents (Jovanovic et al., 1996), including a decreased response to digitalis (Ahlemeyer et al., 1992). Taken together, this could explain the apparently paradoxical finding that, although Ca^{2+} increases with hypertrophy, the degree of Ca^{2+} increase induced by digitalis actually decreased with the progression of hypertrophy.

The significance of the present study is that it provides evidence, at the cellular level, that the presence and extent of ventricular hypertrophy could determine the action of digitalis within the human myocardium. However, the relationship between the effects observed herein in quiescent myocardial samples to the working human heart remains to be determined. Although digitalis is not indicated in the treatment of patients with isolated myocardial hypertrophy, our findings could have relevance for patients receiving digitalis since recent evidence suggest that myocardial hypertrophy is an early component of clinical heart failure. Thus, our findings could provide a basis for the variable efficacy of digitalis observed in clinical practice (The Digitalis Investigation Group, 1997).

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