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Short communication

Heterogenic contractile response of rat left ventricular myocytes to β_1-adrenoceptor stimulation

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Abstract

We measured the contractile response of left ventricular cardiac myocytes from female rats to selective β_1-adrenoceptor stimulation (isoprenaline, 10^{-8} M and 10^{-7} M in the presence of 10^{-7} M ICI 118,551 a β_2-adrenoceptor inverse agonist). A heterogenic response to stimulation, inversely related to the extent of cell shortening prior to adrenergic stimulation, was observed. Challenge of cardiac myocytes with a selective β_1-antagonist, atenolol (10^{-7} M), suggests the heterogenic response is not caused by basal β_1-adrenoceptor activity. Thus, basal myocyte contractility determines the response to β_1-adrenoceptor stimulation, this should be taken into account when experimental conditions are designed.

Keywords: β-Adrenoceptor; Contractility; Isoprenaline; Cardiac myocyte

1. Introduction

The sympathetic nervous system and circulating catecholamines modulate cardiac excitation–contraction coupling by activating β-adrenoceptors. Several of these receptors have been characterised within the hearts of a variety of mammalian species (Ask et al., 1985; Dukes and Vaughan Williams, 1984; Gauthier et al., 1996; Kaumann, 1997; Barbier et al., 2004). Each receptor type operates via a distinct signalling pathway (Xiao and Lakatta, 1993).

The largest positive inotropic effect is seen in response to the stimulation of β_1-adrenoceptors, caused by the cAMP-dependent protein kinase A activation of several targets (Xiao et al., 1998; Saito et al., 1988; Xiao, 2001). Isoprenaline is routinely applied to cardiac myocytes to stimulate β-adrenoceptors and evoke significant increases in the intracellular Ca^{2+} transient and cell shortening, as well as increasing the rate of relaxation and Ca^{2+} transient decline. The inotropic response to this agonist is typically expressed as a percentage increase in contractility.

However, cardiac myocytes have a finite capacity to increase their shortening and basal cell shortening is variable. In view of this, we wished to test the hypothesis that variability in basal contractility is due to basal β_1-adrenoceptor activity and (possibly as a consequence of this) the inotropic response to β_1-adrenoceptor stimulation in a given myocyte is dependent upon its basal contractility. If this hypothesis was correct it would have important consequences for experimental design and the interpretation of data.

2. Materials and methods

Female, Sprague–Dawley rats (200–230 g) were killed in accordance with U.K. Home Office regulations and single left ventricular myocytes isolated as previously described by Frampton and Orchard (1992). Following isolation, cells were added to the experimental chamber of an inverted microscope and superfused at 37 °C with a physiological salt solution previously used to study the response of rat

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myocytes to isoprenaline (Calaghan et al., 1998) composed of the following (mM): Na^+, 135; K^+, 5; Mg^{2+}, 1; Cl^-, 102; HCO_3^-, 20; SO_4^{2-}, 1; Ca^{2+}, 1; acetate, 20; glucose, 10; insulin, 5 u/l. This solution was equilibrated with 5% CO_2–95% O_2 to give a pH of approximately 7.35. Quiescent rod shaped cells were field stimulated to contract at a rate of 1 Hz with a pulse width of 5 ms. When basal contractile activity was established, cells were superfused with the above solution supplemented first with 10^{-7} M ICI 118,551 (ICI, a β_2-adrenoceptor inverse agonist, O'Donnell and Wanstall, 1980) to block the stimulatory pathway of these receptors and then with ICI in combination with 10^{-8} M or 10^{-7} M isoprenaline to give a selective stimulation of β_1-adrenoceptors, the major stimulatory adrenoceptor sub-type in cardiac muscle. Our index of myocyte contractility was cell shortening, measured using a video-edge detection system sampling at 200 Hz and expressed as a percentage of the cell's resting length.

2.1. Statistics

All data are expressed as mean ± S.E.M. Comparisons between the responses to different concentrations of isoprenaline were determined with Student's unpaired *t*-tests. Statistical significance was assumed at $P<0.05$. If data sets failed a normality test, Mann–Whitney Rank Sum tests were carried out.

3. Results

The mean effect of isoprenaline on the contractility of all cardiac myocytes tested was concentration-dependent, as expected. Application of 10^{-7} M isoprenaline in combination with 10^{-7} M ICI resulted in a significantly increased amplitude of contraction when compared to administration of 10^{-8} M isoprenaline and 10^{-7} M ICI (201% ± 20, $n=74$ vs. 124% ± 12, $n=70$; 10^{-7} M and 10^{-8} M isoprenaline, respectively, $P<0.05$ unpaired *t*-test). However, the contractile response to isoprenaline was heterogenic and dependent upon the initial contractility of the myocytes (Fig. 1A). Cells with low initial shortening (below 5%, Fig. 1B) displayed a large increase in shortening in response to isoprenaline with the response being significantly greater at 10^{-7} M than 10^{-8} M (Mann–Whitney, $P<0.001$). Cells that displayed an initial percentage shortening from 5% to 10% (Fig. 1C) also showed a significantly different contractile response when challenged with 10^{-8} M or 10^{-7} M isoprenaline ($P<0.05$, unpaired *t*-test). Those cells with the highest percentage basal shortening (greater than 10%, Fig. 1D) displayed the smallest response to isoprenaline, with no significant concentration dependent effect ($P>0.05$, unpaired *t*-test).

One explanation for these observations is that the basal shortening of the cell is reflective of the level of basal β_1-adrenoceptor activity. However, although the application of the selective β_1 antagonist atenolol (10^{-7} M) (Lundgren et al., 1979) caused a small negative inotropic effect (−32 ± 3%, $n=69$ myocytes) this effect was not dependent upon the initial contractility of ventricular myocytes as,

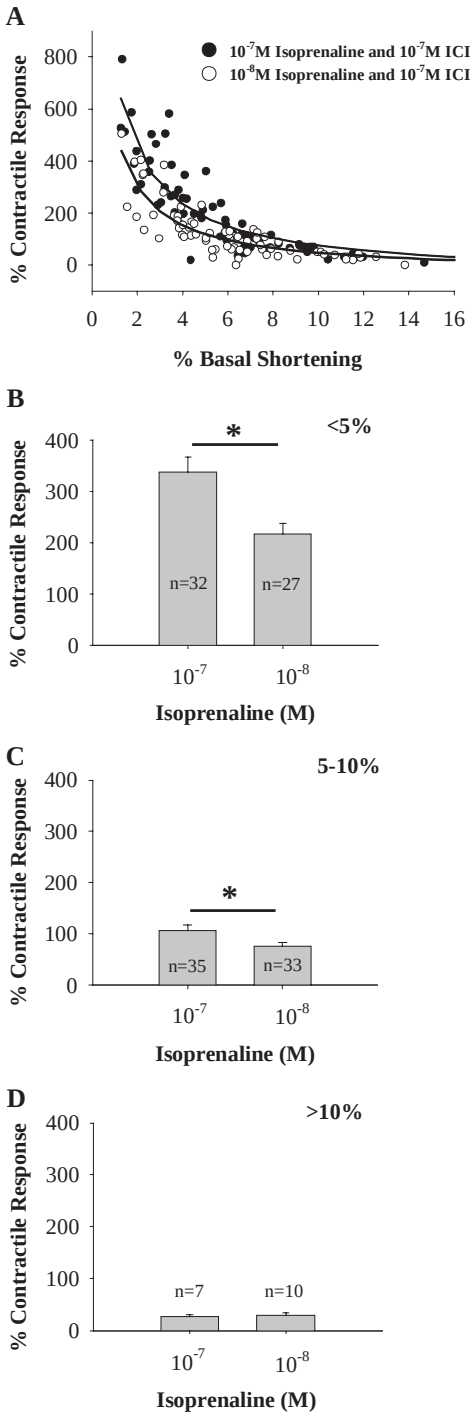

Fig. 1. Effect of isoprenaline (10^{-8} M and 10^{-7} M) with ICI (10^{-7} M) on cell shortening in cardiac myocytes. (A) Response expressed as the % increase in contractility. A hyperbolic decay [$y=y0+(a*b)/(b+x)$] is fitted to each data set ($n=70$–74 myocytes). Mean ± S.E.M. response of cells shown in (A) when divided into those with an initial percentage shortening of up to 5% (B), 5–10% (C) and greater than 10% (D). *$P<0.05$ (unpaired *t*-test or Mann–Whitney for (B).

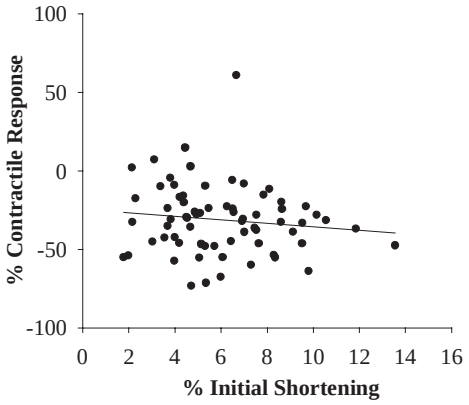

Fig. 2. Effect of 10^{-7} M atenolol on unloaded cell shortening in cardiac myocytes. Response to atenolol is expressed as the % change in contractility for each cell ($n=69$ myocytes). Data were fitted with a linear regression and $r^2=0.016$, demonstrating no relationship between response and basal shortening.

demonstrated by the low r^2 (0.016) of the linear regression in Fig. 2. Neither was the variability in basal contraction dependant upon the presence of insulin in the superfusing solution or the regional (sub-epicardial or sub-endocardial) origin of the myocytes.

4. Discussion

Our observations show that the contractile response to β_1-adrenoceptor stimulation is highly variable. This variability, expressed as a standard error of the mean response, is similar to that previously reported by other studies on the stimulation of β_1-adrenoceptors in rat myocytes (e.g. Xiao and Lakatta, 1993; Xiao et al., 2003) suggesting our cells were not unusually variable in their response. What has not been previously noted is that the variability in response stems largely from an inverse dependence upon the initial level of myocyte contractility. The influence of basal contractility is such that highly contractile cells (shortening greater than 10%) showed a small response to isoprenaline with no significant concentration-dependent effect. The small negative inotropic effect of the β_1-adrenoceptor antagonist atenolol suggests there is some basal activity of these receptors in the absence of agonists, but that this activity does not underlie the variability in the basal contractility of the myocytes. Although the variability in response to isoprenaline was less, it was still apparent, at the lower concentration we used.

Thus the level of basal activation of myocytes and the variability in this parameter needs careful consideration when interpreting the response to isoprenaline. This inherent variability may mask small changes in the inotropic response to β_1-adrenoceptor stimulation, caused by test agents of interest. As an alternative to comparisons of mean response, statistical comparisons of correlations fitted to data may be useful, or transforming data, to account for the effect that basal contractility has on the observed response. For example, using the hyperbolic fits in Fig. 1A to transform observed responses to values at mean basal cell shortening, reduced the S.E.M. of the response data from 10% to 5% of the mean. The response to 10^{-7} M isoprenaline $201\pm20\%$ being transformed to $135\pm6.6\%$ at (the mean) basal shortening of 5.9%.

Basal activation of myocytes is likely to be related to the magnitude of the intracellular Ca^{2+} transient. This will be influenced by experimental factors such as stimulation frequency, temperature and extracellular Ca^{2+} concentration, that are under the control of the experimenter, however variability in basal activation may also be influenced by factors such as action potential duration and signalling factors, possibly downstream of the β_1-adrenoceptor (Bers, 2002).

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