ISOPRENALINE STIMULATION OF CYCLIC AMP PRODUCTION BY ISOLATED CELLS FROM ADULT RAT MYOCARDIUM

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SUMMARY

When 1.25×10^{-6} mol/l isoprenaline is added to suspensions of adult rat myocardial cells in calcium-free bicarbonate buffer, there is a 1.6-fold increase above control of cyclic AMP levels 30 seconds after drug addition. The phosphodiesterase inhibitor methylisobutylxanthine (MIX) at 0.5 mmol/l increases the control level of cAMP and also the level of stimulation to 3-fold. When MIX is present, Ca^{++} (1 mmol/l) has no effect on either control or stimulated cAMP, whereas omission of Mg^{++} results in a lowering of control cAMP, with the levels after stimulation unchanged. Propranolol at 8.45×10^{-6} mol/l abolishes stimulation by isoprenaline.

INTRODUCTION

There is now substantial evidence for the contention that β-adrenergic stimulation of the myocardium promotes increased sarcolemnal adenyl cyclase activity (1-3). Although many studies have been reported of catecholamine-stimulated cyclic AMP production in both isolated perfused hearts (4,5) and plasma membrane preparations (6), data on isolated mature myocardial cells have been precluded until now by the absence of a suitable preparation. We report here on the stimulation by isoprenaline of cAMP production in single-cell suspensions obtained from adult rat myocardium. Also described are the effects of phosphodiesterase inhibition and changes in both calcium and magnesium buffer concentrations on the level of catecholamine stimulation.

MATERIALS AND METHODS

All chemical reagents were 'Analar' grade or equivalent. DL-isoprenaline sulphate was obtained from Ward Blenkinsop, DL-propranolol HCl from ICI and the phosphodiesterase inhibitor 1-methyl-3-isobutylxanthine (MIX) from Aldrich Chemicals.

Purified suspensions of isolated myocardial cells were obtained by timed in vitro perfusions of adult rat hearts with calcium-free Krebs bicarbonate buffer containing crude collagenase, as described in detail previously (7, 8). Cell suspensions (5 ml) were preincubated at 37°C in the appropriate gassed buffer medium containing 2% (w/v) bovine serum albumin (8) for 5 - 7 minutes and then isoprenaline added. Samples (1 ml) were taken at known times, added to 2 ml ethanol, vortex mixed and then stored at -20°C for at least 16 hours. Control flasks were included in each experiment containing an equivalent volume of blank drug vehicle.

After storage, samples were warmed to 4°C and centrifuged at 2250 g for 20 minutes. A portion (2 ml) of each supernatant was withdrawn and evaporated to dryness for 4 hours at 37°C in a vacuum oven. The dried residues were reconstituted in 500 µl of 0.05 mol/l Tris-EDTA buffer (pH 7.5) and cAMP determined in duplicate 50 µl samples by a standard saturation assay (9, 10). Unless stated otherwise, results are given as mean ± standard error of the mean with the number of experiments following in parenthesis. Group means were compared using Student's t-test.

RESULTS

Figure 1 shows typical results from experiments in which myocytes

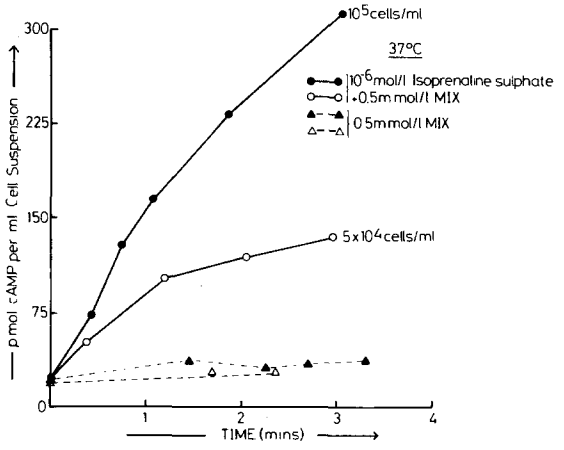

Figure 1 Cyclic AMP production by isolated cardiac myocytes in Ca^{++}- free bicarbonate buffer.

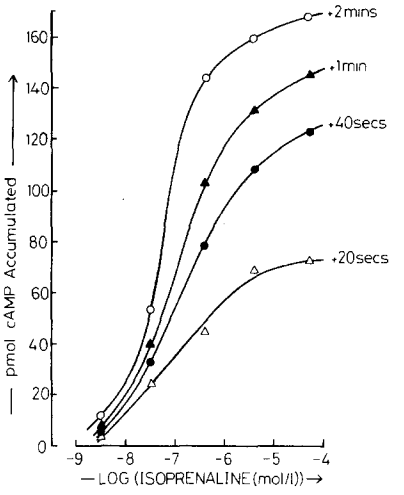

Figure 2 Log-dose response curves for cyclic AMP production. Values obtained from time course responses at each dose of isoprenaline and given as pmol cAMP accumulated per ml. above control. 1 ml. cell suspensions incubated at 37°C, reactions terminated by addition of 2 ml ethanol. Cell count, 8 x 10^4 rod cells per ml. cell suspension.

were incubated in calcium-free bicarbonate buffer containing 0.5 mmol/l MIX with 10^{-6} mol/l isoprenaline added or an equivalent volume (50 µl) of pH 4 saline (control). Two types of responses have been recorded (cf. Figure 1). Of 20 experiments performed with 0.5 mmol/l MIX, 9 have shown a relatively linear cAMP response to isoprenaline, whereas 11 have exhibited saturation after 2 - 3 minutes. In 7 experiments where MIX was omitted from the incubation medium, all responses saturate after 1 - 2 minutes exposure to the drug (1.25 x 10^{-6} mol/l). When the response is alinear, a family of log-dose response curves are obtained (Figure 2) all having half-maximal values on the order of 10^{-7} mol/l isoprenaline, with threshold between 10^{-9}- 10^{-8} mol/l.

Figure 3 summarizes the data from a series of experiments using 1.25 x 10^{-6} mol/l isoprenaline as stimulant, cAMP levels being measured 30 seconds after drug addition. With no phosphodiesterase inhibitor present

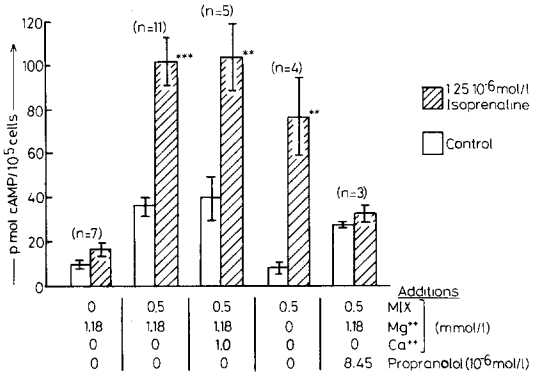

Figure 3 Effects of phosphodiesterase inhibition, Ca^{++}, Mg^{++} and Propranolol on basal and isoprenaline-stimulated cAMP production, measured 30 seconds after drug addition. Ca^{++} and Mg^{++} added as chloride and sulphate salts, respectively. In Mg^{++}- free experiments, magnesium omitted from sedimentation procedure (8) as well as incubation medium. Temperature 37°C.
Each stimulated group compared with corresponding control by Student's t-test.
* = $0.05 < P < 0.01$, ** = $0.01 < P < 0.001$, *** = $P < 0.001$.

in Ca^{++}- free bicarbonate buffer, there are some 10.5 ± 2.2 pmol cAMP per 10^5 rod cells (n = 7) in control flasks and this is not significantly increased statistically to 16.6 ± 9.3 pmol/10^5 cells when isoprenaline is present. On addition of 0.5 mmol/l MIX, control cAMP increased significantly to 36.7 ± 4.0 pmol/10^5 cells (n = 11, $P < 0.001$) and when isoprenaline is present there is a further significant increase to 112 ± 10.6 pmol/10^5 cells (n = 11, $P < 0.001$). The further addition of 1 mmol/l $CaCl_2$ to the medium does not alter either the control or stimulated levels of cAMP. However, the omission of $MgSO_4$ results in a lowering of basal cAMP to a level comparable with that found in the absence of MIX with Mg^{++}, whereas the level of stimulated cAMP is unchanged from that observed with MIX in the presence or absence of Ca^{++}. Propranolol at 8.45×10^{-6} mol/l abolishes stimulation by isoprenaline.

Although the mean level of cAMP in flasks with isoprenaline is not

statistically different from control when all the data are pooled, for each experiment there is a highly significant positive correlation between the level of cAMP in a stimulated flask and the corresponding control (r = 0.951, n = 7, P = 0.001). The data of Figure 3 have therefore been expressed as the ratio (R) of cAMP after 30 seconds in the presence of isoprenaline to the control value at the same time and are presented in Figure 4. Cyclic AMP is increased about 1.6 - fold by isoprenaline when MIX is absent, 3 - fold with 0.5 mmol/l MIX added, irrespective of whether or not calcium is present at 1 mmol/l, and stimulated 10 - fold when Mg^{++} is omitted from the incubation medium. All of these values differ significantly from unity (cf. Figure 4).

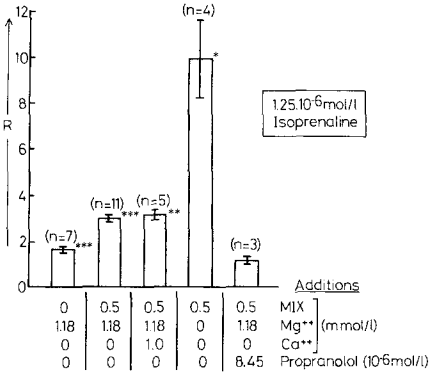

Figure 4 Stimulation of cAMP production by isoprenaline.
$R = \frac{\text{cAMP produced after 30 seconds stimulation.}}{\text{cAMP in control flask after 30 seconds.}}$
t-tests performed assuming that R = 1, i.e. no stimulation.

DISCUSSION

The viable single-cell suspension of cardiac myocytes permits examination of catecholamine-stimulated cyclic AMP production in a preparation where the spatial organisation of the receptor-enzyme complex resembles that found in the intact tissue, without the presence of an extensive extracellular space. The

receptor moiety is exposed to an approximately interstitial fluid environment, whereas the adenyl cyclase unit functions in a cytoplasmic milieu, assuming an intact cell sarcolemma.

In suspensions hypersensitive to calcium, addition of this ion produces cellular contraction and damage (11,12) resulting in a preparation resembling broken-cell or plasma membrane systems. Thus in the only previous report on this subject, Moustafa and co-workers (12), using a calcium-intolerant preparation, reported that Ca^{++} inhibited cAMP production in isolated cardiac myocytes at a concentration (0.5 mmol/l) known to inhibit adenyl cyclase activity in cell-free systems (6). The unchanged levels of basal cAMP found in this study when 1 mmol/l calcium is included (Figure 3) indicate that in the present preparation intracellular free Ca^{++} concentration is being maintained at about 10^{-6} - 10^{-7} mol/l, which is both typical for this type of tissue (13) and also in the range where adenyl cyclase activity is not inhibited by this cation (6). Furthermore, it can be calculated from the data of Moustafa et al (12) that about 10 pmol cAMP/10^5 cells were produced 30 seconds after the addition of 10^{-6} mol/l isoprenaline in calcium-free buffer containing 2 mmol/l theophylline and 0.5 mmol/l EGTA. This is only 12% of the response reported here and could be due to the fact that EGTA might also inhibit adenyl cyclase activity, independent of its metal-ion chelating ability (14).

Adenyl cyclase has an absolute requirement for Mg^{++} (15,16) so that reduction in basal cyclic AMP in myocytes incubated in Mg^{++}- free buffer (Figure 3) is due probably to cell depletion of Mg^{++}. The half-time for magnesium efflux from rat ventricle is on the order of 3 hours at 37°C (17) so that the short incubation times used here would not be expected to reduce intracellular magnesium to a level where adenyl cyclase would be inhibited completely.

Further evidence to support the contention that the sarcolemma is

functioning in a relatively typical manner is obtained from the dose-response curves (Figure 2) where the half-maximal values on the order of 10^{-7} mol/l agree well with those found in other studies (2). The date obtained in the presence of MIX showing both linear and saturable responses to isoprenaline (Figure 1) probably reflect the complex feedback relationship between adenyl cyclase, cyclic AMP and Ca^{++} (6) and might well depend upon the level of trace amounts of calcium which are unavoidable in the nominally calcium-free incubations. This would not only influence the level of residual phosphodiesterase activity, which Ca^{++} is known to stimulate at very low concentrations (18,19), but also transport of trace extracellular calcium might be facilitated by the phosphodiesterase inhibitor itself (20). The consistent finding of cyclic AMP saturating after 1 - 2 minutes where MIX is omitted (7 experiments) agrees well with data from both intact hearts (4) and also other single-cell systems (21). Indeed, studies with these minimally-tensioned isolated myocardial cells might well clarify many of the problems concerning plasma membrane regulation of β-adrenergic activiation of adenyl cyclase.

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