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Cyclic AMP levels in ventricular myocytes from noradrenaline-treated guinea-pigs

Dylan G. Wynne, Federica Del Monte, Sian E. Harding *

Cardiac Medicine, National Heart and Lung Institute, Imperial College, Dovehouse St., London SW3 6LY, UK

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Abstract

Chronic activation of the sympathetic nervous system in human heart failure is believed to cause cardiac β-adrenoceptor desensitisation. We have investigated the relationship between β-adrenoceptor desensitisation and cyclic AMP levels in cardiac myocytes isolated from the ventricle of guinea-pigs chronically infused with noradrenaline hydrochloride for 7 days. Functional β-adrenoceptor desensitisation was confirmed by a significant decrease in the maximum isoprenaline-stimulated contraction amplitude and an increased EC_{50} for isoprenaline. In the absence of β-adrenoceptor stimulation, basal cyclic AMP levels were significantly depressed in populations of myocytes from noradrenaline-treated animals compared to sham-operated controls, and this was not accounted for by myocyte hypertrophy or necrosis. Similarly, there was a significant decrease in cyclic AMP levels at maximally inotropic isoprenaline concentrations. Threshold and maximum inotropic concentrations of the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX), restored isoprenaline-stimulated cyclic AMP levels in noradrenaline-treated guinea-pig cardiac myocytes, although we have previously reported no increase in maximum inotropic effect of isoprenaline with these compounds.

Keywords: Cardiac myocyte; cAMP; β-Adrenoceptor desensitization; Contractility

1. Introduction

β-Adrenoceptor desensitisation plays a pivotal role in the progression of human heart failure (Bristow et al., 1990; Harding et al., 1994). The lack of response of failing human myocardium to β-adrenoceptor agonists is paralleled by a decrease in production of the second messenger, cyclic AMP. It is likely that this represents an agonist-induced desensitisation produced by the high levels of noradrenaline release in these patients, since the infusion of noradrenaline or isoprenaline into animals reproduces the syndrome well (Eschenhagen et al., 1991a; Brown and Harding, 1992; Harding et al., 1993). However, basal cyclic AMP levels in the absence of β-adrenoceptor stimulation are also reduced in myocardium taken from the failing human heart (Danielsen et al., 1989; Von der Leyen et al., 1991; Bohm et al., 1994). This may be part of the desensitisation process, secondary to the rise in the activity of the inhibitory guanine-nucleotide binding protein. It could also represent an increase in non-viable myocardium in the samples from the failing heart compared to those from normal subjects. The presence of endogenous catecholamines within intact myocardium may also influence the result: the one study where biopsy material was used, rather than the washed papillary muscle, did not show a decrease in basal cyclic AMP (Regitz-Zagrosek et al., 1994). This has been ascribed to residual stimulation by noradrenaline in the sample.

Apart from some preliminary reports, few data from animal models are available to address the question of whether a decrease in basal cyclic AMP is a consequence of β-adrenoceptor desensitisation. A study on dogs infused with noradrenaline shows no significant change in basal cyclic AMP levels within the myocardium but, as with the human biopsy samples, the tissue was not washed free of endogenous noradrenaline (Raum et al., 1983). Cultures of neonatal rat cells exposed to noradrenaline for 1–5 days showed reduced accumulation of cyclic AMP (in the presence of a phosphodiesterase inhibitor) over a 15 min incubation period (Reithmann and Werdan, 1989).

The question is important because of the suggestion that a reduction in tonic stimulation by cyclic AMP contributes

* Corresponding author. Tel.: (+44-171) 352 8121, ext. 3311; fax: (+44-171) 823 3392; e-mail: sian.harding@ic.ac.uk.

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to the poor contractility of the failing heart in the *absence* of β-adrenoceptor stimulation. The failing human heart relaxes slowly (Souffer et al., 1985), and does not increase contraction force with increasing heart rate (Feldman et al., 1988b; Hasenfuss et al., 1994). These changes are reflected in isolated myocytes and muscle strips from failing human ventricle, which also contract and relax more slowly than those from non-failing (Del Monte et al., 1995), and show a marked reduction of the increase in contraction amplitude with frequency (Mulieri et al., 1992; Schwinger et al., 1993; Davies et al., 1995). Raising (or mimicking) intracellular cyclic AMP with threshold concentrations of forskolin or cyclic AMP analogues can reverse both these changes (Schwinger et al., 1993; Harding et al., 1996).

The first aim of this study was to investigate whether β-adrenoceptor desensitisation in the absence of heart failure causes a decrease in basal cyclic AMP levels. We have shown that myocytes from the hearts of guinea-pigs infused with noradrenaline for 7 days exhibit a functional β-adrenoceptor desensitisation that is quantitatively similar to that seen in failing human heart (Harding et al., 1993), although the animals exhibit no signs of cardiac failure. In the present study we correlate functional responses to isoprenaline in isolated ventricular myocytes with biochemical assay of myocardial cyclic AMP. The isolated myocyte preparation provides numerous advantages over intact myocardium for this study, particularly for estimating the contribution of non-viable or non-myocyte tissue to the measurement of nucleotide levels. Importantly, the system is free from endogenous catecholamines and consequently permits a more accurate assessment of basal over isoprenaline-stimulated cyclic AMP levels.

In addition, we have compared the effect of phosphodiesterase inhibitors to restore isoprenaline-stimulated cyclic AMP with their ability to reverse the functional β-adrenoceptor desensitisation in contraction experiments. In a previous study (Wynne et al., 1993) we had shown that threshold inotropic concentrations of 3-isobutyl-1-methylxanthine (IBMX) (or other phosphodiesterase inhibitors) increased the sensitivity of myocyte contraction to isoprenaline, but did not fully reverse the depression in the maximum β-adrenoceptor-mediated response in noradrenaline-treated guinea-pigs. Here we match cyclic AMP measurements to those functional studies, to investigate the hypothesis that restoring maximum cyclic AMP production does not completely overcome β-adrenoceptor desensitisation.

2. Materials and methods

2.1. Noradrenaline treatment of guinea-pigs

Male Dunkin-Hartley guinea-pigs of weight 300–500 g were anaesthetized using 2% Hypnorm (0.5 ml · kg^{-1}) and a local injection of lignocaine hydrochloride (2 ml · kg^{-1}). Osmotic minipumps (model 2002, Alzet, USA) containing (−)-noradrenaline hydrochloride (Sigma) dissolved in saline and 1 mM ascorbate were incubated in saline for 4 h at 37°C and implanted subcutaneously in the neck. The mean pumping rate of the pumps was 0.97 ml · h^{-1} with a concentration of noradrenaline such that the guinea-pigs received, over a period of 7 days, 900 μg · kg^{-1} · h^{-1}. Weight-matched, sham-operated animals were given the same anaesthesia and incision, but no minipump was implanted. The guinea-pigs were fed standard laboratory diet and water ad libitum.

2.2. Isolation of guinea-pig ventricular myocytes and contractile studies

Guinea-pigs were heparinised (3000 U · kg^{-1}) and killed by cervical dislocation. Left ventricular myocytes were enzymatically isolated from the excised hearts by a retrograde Langendorff perfusion method as previously described (Brown and Harding, 1992). The contractility of electrically stimulated (0.5 Hz), superfused myocytes at 32°C in Krebs-Henseleit solution (KH) (composition in mM: NaCl 119, KCl 4.2, $MgSO_4$ 0.94, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11.5, $CaCl_2$ 1), bubbled with 95% O_2/5% CO_2 was monitored as previously described using a video-microscopy edge-detection system (Harding et al., 1988).

Concentration–response curves were constructed cumulatively in increments of 2 mM (Ca^{2+}) or half log-units (isoprenaline) increased until no further increase in contraction amplitude was shown, or until arrhythmic contractions were obtained. The EC_{50} values for isoprenaline and the isoprenaline/Ca^{2+} ratio (the maximum contraction amplitude with isoprenaline relative to that with maximally stimulating concentrations of Ca^{2+} in the same myocyte) were calculated for each cell. For noradrenaline-treated guinea-pig ventricular myocytes these two measures indicate the degree of functional β-adrenoceptor desensitisation (Brown and Harding, 1992; Wynne et al., 1993).

2.3. Measurement of cell size

From a separate series of animals, video-hardcopies of > 30 myocytes from each preparation were taken to measure cell dimensions (area and perimeter) using a digitising tablet with associated software (VIDS III, Analytical Measuring Systems, Cambridge).

2.4. Extraction and assay of myocyte cyclic AMP

Myocyte preparations were separated on a continuous Percoll gradient to remove non-myocyte cells and enrich the proportion of viable myocytes; the procedure was modified from that of Maisch (1981). In order to create the gradient, 12 ml of 42.5% Percoll in KH buffer, pre-equilibrated to pH 7.4 with 95% O_2/5% CO_2, was ultracen-

Table 1
Average concentrations of inotropes to produce effects on guinea-pig myocytes

	Concentration of inotrope	
	Control	Noradrenaline- treated
Maximum inotropic isoprenaline	30 nM	300 nM
Threshold inotropic IBMX	3 μM	10 μM
Maximum inotropic IBMX	30 μM	300 μM
Maximum inotropic forskolin	3 μM	3 μM

trifuged at $20000 \times g$ for 50 min; 1 ml of the cardiac myocyte suspension (approx. 10^6 cells) was layered on this and separation achieved by centrifugation at $400 \times g$ for 45 min. Enriched rod-shaped myocytes were found in a narrow band towards the lower part of the gradient, and round cells in a broader band above. After separation, cell populations were washed twice by centrifugation in KH at $400 \times g$. Following purification and again after cell incubations at 32°C in KH containing 1 mM Ca, myocyte viability was estimated, both morphologically and in the extent of trypan blue exclusion.

Following 5 min incubation at 32°C under the conditions detailed below, cyclic AMP was extracted from cardiac myocyte suspensions using Amprep ion-exchange chromatography SAX minicolumns. For each experimental day the percent recovery of cyclic AMP was estimated with ^{3}H-labelled cyclic AMP and corrections calculated. Cyclic AMP was assayed using a standard ^{125}I radioimmunoassay Kit supplied by Amersham. Cell protein was determined using the method of Bradford (1976), and bovine serum albumin used as standard. Cyclic AMP values were expressed as pmol · mg^{-1} protein.

For each intervention, concentrations of inotropes were matched with the modal concentrations giving specific degrees of inotropy in control and noradrenaline-treated guinea-pig myocytes (Wynne et al., 1993). These are shown in Table 1.

Since the analysis is on a heterogeneous population of both viable (rod-shaped) and non-viable (rounded) cells, it was necessary to establish the contribution of the non-viable cells alone towards the measured levels of basal cyclic AMP. Thus a further set of incubations was performed under basal conditions for a sample of myocytes consisting of 100% non-viable cells. Such a homogenous population was obtained from the uppermost layer of cells following centrifugation through the Percoll gradient.

2.5. Statistical analysis

Statistical significance was assessed by Student's *t*-test (for paired data where possible). Values quoted throughout as mean ± standard error of the mean (S.E.M.). *N* represents the numbers of animals. Results obtained from several cells for one heart preparation were pooled. For the comparison of the EC_{50} values, *t*-tests were performed on the log-transformed variable (the pD_2).

3. Results

3.1. Morphological changes following noradrenaline treatment of guinea-pigs

Osmotic mini-pump infusions of noradrenaline induced a significant increase in the heart to body weight ratio compared to sham-operated animals (Table 2, $P < 0.05$). However, this was due to a decrease in relative body mass in noradrenaline-treated animals during treatment, rather than an increase in heart weight.

Enzymatic digestion of excised hearts resulted in morphologically intact, Ca^{2+} tolerant ventricular myocytes. Following centrifugation through a continuous Percoll gradient, the final percent yield of viable rod-shaped myocytes (trypan blue exclusion) from the hearts of noradrenaline-treated guinea-pigs remained significantly lower than the yield obtained from the hearts of sham-operated animals (Table 2). In a separate series of animals, average cell length, width and 2-dimensional area was measured from video-hardcopies of myocytes (> 30 per animal) to determine whether the increase in heart to body weight ratio represented a true hypertrophy (Table 3). There was no significant change in cell dimensions following noradrenaline treatment. This is in contrast to our previous work with the renal hypertensive guinea-pig, where cell area was increased by 50% in the 3 weeks after renal banding (Naqvi et al., 1994). Consequently it is unlikely that chronic catecholamine infusion had elicited true cardiac hypertrophy in the present guinea-pig model.

3.2. Contractile responses of single myocytes

The maximum contraction amplitude in high extracellular Ca^{2+}, indicated as the percent cell shortening, was not impaired in the noradrenaline-treated guinea-pig myocytes (Table 4). β-Adrenoceptor desensitisation was confirmed in the noradrenaline-treated guinea-pig ventricular myocytes by the attenuated contractile response to the β-adrenoceptor agonist isoprenaline. Concentration–response curves were right-shifted compared to control, with the isoprenaline EC_{50} increased 13-fold. This was in addition to a significant reduction in the maximum isoprenaline/Ca^{2+} ratio (see Table 4).

Table 2
Animal, heart and myocyte preparation characteristics: control and noradrenaline-treated guinea-pigs

	Control (8)	Noradrenaline-treated (8)
Initial body weight (g)	441 ± 28	425 ± 18
Change in body weight (g)	65 ± 10	11 ± 1 [b]
Heart weight (g)	1.90 ± 0.13	1.84 ± 0.07
Heart weight/body weight (%)	0.37 ± 0.01	0.42 ± 0.01 [a]
Viable myocytes (%)	46.6 ± 2.8	36.6 ± 0.6 [b]

Mean ± S.E.M., (*n*) = animals, significantly different from control. [a] $P < 0.05$; [b] $P < 0.01$.

Table 3
Morphological parameters of left ventricular myocytes from control and noradrenaline-treated guinea-pigs

	Control (7)	Noradrenaline-treated (10)
Length (μm)	117 ± 9	122 ± 14
Width (μm)	32.0 ± 2.1	32.6 ± 3.2
Area (μm²)	2459 ± 299	2575 ± 426

Mean ± S.E.M., (*n*) = animals.

3.3. Basal cyclic AMP measurements

Cyclic AMP measurements for myocyte suspensions were corrected for percent recovery and expressed as pmol cyclic AMP/mg protein. Fig. 1 summarizes the total amount of cyclic AMP for myocytes isolated from sham-operated and noradrenaline-treated guinea-pigs under basal and inotropically-stimulated conditions. Noradrenaline treatment resulted in a significant decrease in basal cyclic AMP levels ($P < 0.02$), to values 37% of those measured for myocytes from the hearts of sham operated animals.

Through extrapolation of data obtained from cell suspensions consisting of 100% round-shaped cells, it was calculated that under basal conditions an average rod-shaped myocyte contributed over 10 times the amount of cyclic AMP as a round (Table 5); 204 ± 65 pmol $\times 10^{-6}$ cyclic AMP per rod vs. 17 ± 2 pmol $\times 10^{-6}$ cyclic AMP per round ($P < 0.05$) for the sham-operated group ($n = 7$), and 100 ± 30 pmol $\times 10^{-6}$ cyclic AMP per rod vs. 10 ± 2 pmol $\times 10^{-6}$ cyclic AMP per round ($P < 0.05$) for the noradrenaline-treated group, $n = 8$.

The mean percentage of viable cells in preparations from the noradrenaline-treated animals was lower than preparations from sham-operated animals. However, the magnitude of the difference (21%) was not sufficient to explain the large decreases in basal levels of cyclic AMP (63%) in noradrenaline-treated guinea-pig ventricular myocytes. Omitting one myocyte preparation resulted in a more even matching of yields between treated and untreated groups ($44 \pm 6\%$ for sham-operated ($n = 7$) vs. $40 \pm 6\%$ for noradrenaline-treated ($n = 7$), P = NS) but did not decrease the significance of the difference in basal cyclic AMP between control and noradrenaline-treated animals (see Table 5b).

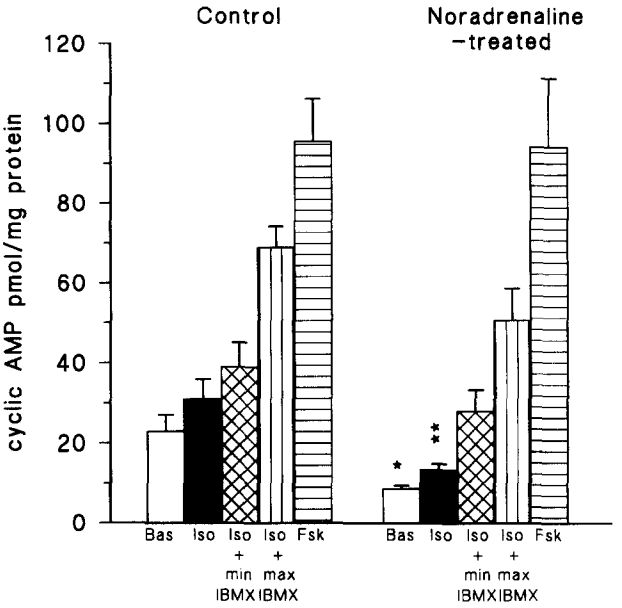

Fig. 1. Cyclic AMP levels in ventricular myocytes of sham-operated ($n = 8$) and noradrenaline-treated guinea-pigs ($n = 8$) for: drug-free basal (open columns); maximum inotropic concentrations of isoprenaline (solid columns); maximum isoprenaline plus threshold inotropic IBMX (cross-hatched columns); maximum isoprenaline plus maximum inotropic IBMX (vertical lined columns); maximum IBMX plus maximum forskolin (horizontal lined columns). For concentrations of agents, see Table 1. Values are mean ± s.e. mean. * $P < 0.02$, ** $P < 0.01$, significantly different from corresponding control.

3.4. Isoprenaline-stimulated cyclic AMP levels

The concentration of isoprenaline used in each case was the mode of the concentrations that had given maximum inotropic stimulation in the contractile experiments (see Methods). Isoprenaline induced increases in cyclic AMP levels (see Fig. 1). The increase over basal was 35% for the sham-operated group and 56% for the noradrenaline-treated group. β-Adrenoceptor stimulation by isoprenaline in noradrenaline-treated guinea-pig ventricular myocytes gave total values of cyclic AMP which remained significantly depressed compared to control ($P < 0.01$)—only 43% of the level in sham-operated animals. As with the basal values, matching preparations for cell yield did not remove the difference between control and noradrenaline-treated groups. It should be noted that, since full concen-

Table 4
Contractile responses of isolated ventricular myocytes from control and noradrenaline-treated guinea-pigs

	Control (6)	Noradrenaline-treated (7)
Contraction amplitude (% shortening) in maximum Ca^{2+}	13.3 ± 1.7	9.7 ± 0.9
Contraction amplitude (% shortening) in maximum isoprenaline	11.6 ± 1.6	3.5 ± 0.7 [c]
Isoprenaline/Ca^{2+} ratio	87.7 ± 2.9	35.8 ± 7.2 [c]
pD2	8.68 ± 0.06	7.63 ± 0.08 [c]

Mean ± S.E.M., (*n*) = animals, [c] $P < 0.001$ compared to control.

tration–response curves to isoprenaline were not performed for cyclic AMP levels, it cannot be stated from the present study whether increasing isoprenaline beyond the maximum inotropic concentration would have produced a further cyclic AMP increase.

3.5. Potentiation of isoprenaline-stimulated cyclic AMP levels by PDE inhibition

The degree of β-adrenoceptor desensitisation reported in this study is consistent with that previously obtained in the same model (Wynne et al., 1993). In this latter study, cardiac ventricular myocytes from noradrenaline-treated guinea-pigs showed a 20-fold increase in the isoprenaline EC_{50} and an isoprenaline/Ca^{2+} ratio of only 60%. The presence of phosphodiesterase inhibitor caused a significant leftward shift in the isoprenaline concentration–response curve in noradrenaline-treated guinea-pig myocytes. However, potentiation of the maximum inotropic response to isoprenaline was incomplete, with levels remaining significantly lower than control. Our aim was to identify the level of myocyte cyclic AMP during such inotropic interventions. Average threshold and maximum inotropic concentrations of the non-specific phosphodiesterase inhibitor IBMX were taken from this prior study (Table 1)

IBMX increased isoprenaline-stimulated cyclic AMP levels in ventricular myocytes (Fig. 1). In noradrenaline-treated guinea-pig ventricular myocytes, threshold inotropic concentrations of IBMX (10 μM) were sufficient to restore isoprenaline-stimulated levels of cyclic AMP to control (31 ± 5 pmol · mg^{-1} protein for isoprenaline alone in sham-operated, versus 28 ± 5 pmol · mg^{-1} protein for isoprenaline plus IBMX in the treated group). Despite restoration of cyclic AMP levels, we have previously demonstrated incomplete reversal of positive inotropy under identical conditions (Wynne et al., 1993). The maximally inotropic concentration of IBMX raised cyclic AMP in cells from noradrenaline-treated animals to 50.6 ± 8.0 pmol · mg^{-1} protein, while there was little additional effect on the maximum contractile response to isoprenaline (Wynne et al., 1993). Further increases in cyclic AMP were caused by the addition of 3 μm forskolin in the presence of maximum inotropic concentrations of IBMX, with final values being similar in both preparations (Fig. 1).

4. Discussion

4.1. Changes in basal cyclic AMP levels

In this study we show that prolonged in vivo exposure to noradrenaline results in a decrease in resting, unstimulated cyclic AMP production. The use of a density gradient-purified myocyte preparation precludes residual contamination by endogenous catecholamines or the presence of non-myocyte cells as possible explanations of the effect. Additionally, we have shown that myocyte hypertrophy does not account for the difference between control and noradrenaline-treated animals.

Preparations of myocytes from noradrenaline-treated animals did contain a higher proportion of non-viable myocytes than control. This could be due either to some catecholamine-induced necrosis in vivo, a phenomenon which has been reported before (Wheatley et al., 1985; Mann and Cooper, 1989), or to a greater susceptibility of cells to damage during the isolation procedure. A non-viable myocyte contained on average around 10% of the cyclic AMP of a viable cell. However, matching of preparations by yield showed that the decrease in basal cyclic AMP was not due to an excess of dead myocytes in the noradrenaline-treated group. It should, however, be noted that the presence of necrotic myocytes within an intact myocardial sample will presumably have the same effect to decrease overall cyclic AMP levels. This is of particular importance in studies on failing human heart, where there may be considerable scarring of the ventricle.

Despite this proviso, the present study shows that it is possible for the high local catecholamine levels to produce

Table 5
Cyclic AMP levels in control and noradrenaline-treated guinea-pig myocytes

a. Basal cyclic AMP levels (1 mM Ca^{2+}), pmol · mg^{-1} protein, rod- and round-shaped myocytes

	Control (8)	Noradrenaline-treated (8)
Whole myocyte preparation (rods + rounds)	22.8 ± 4.7	8.0 ± 0.8 [a]
100% round-shaped myocytes	0.9 ± 0.1	0.6 ± 0.1

Mean ± S.E.M., (n) = animals, [a] $P < 0.05$ from control

b. Cyclic AMP levels, pmol · mg^{-1} protein, in yield-matched preparations

	Control (7)	Noradrenaline-treated (7)
Basal (1 mM Ca^{2+})	20.1 ± 3.6	8.8 ± 0.9 [d]
Maximum isoprenaline	27.7 ± 4.3	14.0 ± 1.6 [d]

Mean ± S.E.M., (n) = animals, [d] $P < 0.02$ from control.

the decrease in unstimulated cyclic AMP that has been observed in failing human myocardium (Danielsen et al., 1989; Von der Leyen et al., 1991; Bohm et al., 1994). Our results are in agreement with those of Reithmann and Werdan (1989) who obtained similar effects by exposing neonatal rat cell cultures to noradrenaline in vitro. The magnitude of the change, approximately 50–60% reduction in basal cyclic AMP, was the same in the present study, in that of Reithmann and Werdan (1989), and in the samples from the failing human heart (Danielsen et al., 1989).

Low basal cyclic AMP may have consequences for contraction and relaxation of the heart: it has been shown that the poor frequency response of failing myocardium can be partially reversed by threshold inotropic concentrations of isoprenaline. We have shown that the speed of shortening and relaxation of myocytes from failing human heart is slow compared to non-failing (Del Monte et al., 1995). β-Adrenoceptor stimulation has a greater effect to accelerate the beat in failing myocytes than in non-failing, despite the poor inotropic effect of β-adrenoceptor agonists in failing heart. Isoprenaline thus effectively normalises the speed of contraction and relaxation of myocytes from the failing human heart (Harding et al., 1996).

It might be argued that in vivo the failing human heart does not operate under basal conditions, but is always exposed to a stimulating level of noradrenaline. However, in advanced heart failure the loss of endogenous noradrenaline stores, together with the reduction in β-adrenoceptor numbers (Bristow et al., 1992), may reduce activation of the β-adrenoceptor pathways to a low level. Treatment with β-blockers could allow recovery of basal cyclic AMP production, and so reverse some of the deleterious changes described in the preceding paragraph. This would explain the paradoxical clinical improvement obtained by blocking the β-adrenoceptor (Waagstein et al., 1993), which might have been expected to withdraw vital inotropic support from the failing heart.

The subcellular mechanism of the reduction in cyclic AMP following catecholamine exposure is not addressed in the present study, but several possibilities exist. It is now thought that the β-adrenoceptor itself, even when unoccupied by agonist, may exert a tonic stimulatory effect on cyclase activity (Mewes et al., 1993; Adie and Milligan, 1994; Milano et al., 1995). A decrease in β-adrenoceptor numbers might therefore reduce this tonic effect. However, infusion of isoprenaline (Molenaar et al., 1990) or noradrenaline (Kompa et al., 1992) did not reduce β_1-adrenoceptor number assessed autoradiographically in guinea-pig ventricle, and we have shown that it is this subtype which primarily mediates contractile increases in the guinea-pig myocyte (Del Monte et al., 1993).

A more likely alternative is that decreased basal (and β-adrenoceptor-mediated) activation of adenylate cyclase following catecholamine exposure arises from increased tonic inhibition by inhibitory G-proteins (G_i). Rat cardiac myocytes cultured in the presence of noradrenaline demonstrate increased immune-detectable levels of $G_{i\alpha}$ (Reithmann et al., 1989). It has been well documented that G_i is also increased in failing human heart, while G_S is unaffected (see Harding et al., 1994). Inactivation of G_i with pertussis toxin reversed the depression of basal adenylate cyclase activity in sarcolemmal membranes from failing human heart (Feldman et al., 1988a). Interestingly, short-term (3 h) exposure of human trabeculae to noradrenaline did not significantly decrease basal adenylate cyclase activity (Kaumann et al., 1989). This would suggest that some induction of protein synthesis may be required to produce the change in basal cyclase. In support of this hypothesis, it has been shown that infusion of isoprenaline in rats led to an increase in $G_{i\alpha}$ mRNA (Eschenhagen et al., 1991b), with specifically enhanced transcriptional activity of the $G_{i\alpha\text{-}2}$ gene (Muller et al., 1993).

4.2. Stimulated cyclic AMP levels: correspondence with contractile effects

Cyclic AMP levels generated by maximally inotropic effects of isoprenaline were decreased in myocytes from the NA-treated guinea-pigs. However, detailed conclusions concerning coupling of β-adrenoceptors should not be drawn from the present study, since full concentration–response curves for β-adrenoceptor stimulation of cyclic AMP generation were not constructed. It has been shown in several species, including cat, that maximum cyclase activity may be attained at concentrations of isoprenaline several orders of magnitude higher than those giving maximum effects on contraction (Kaumann et al., 1989). It is therefore likely that we did not elicit the maximum increase in cyclic AMP with the isoprenaline concentrations used in the present study in myocytes from either control or treated animals.

The concentrations of isoprenaline were chosen to parallel contractile experiments where we had attempted to overcome desensitisation at the contractile level by the combined use of β-agonists and phosphodiesterase inhibitors (Wynne et al., 1993). It is clear from the present study that either IBMX (in the presence of isoprenaline) or forskolin is able to overcome the defect in cyclic AMP production in the noradrenaline-treated animals. This is in contrast to the results on neonatal cultures, where the effect of forskolin was also decreased after noradrenaline exposure (Reithmann and Werdan, 1989), but close to those in failing human heart (Bohm et al., 1989).

Does reversal of the deficit in cyclic AMP production lead to reversal of the decreased maximum contractile response to β-adrenoceptor stimulation? The lower concentration of IBMX used here restored isoprenaline-stimulated cyclic AMP levels of myocytes from noradrenaline-treated animals to those seen in untreated animals. The functional effect of IBMX was to increase the inotropic effect of submaximal concentrations of isoprenaline, shift-

ing the concentration–response curve to the left. However, the maximum effect of isoprenaline was not restored in the presence of IBMX (Wynne et al., 1993). Even the high concentration of IBMX used here, which increased isoprenaline-stimulated cyclic AMP 4-fold, did not increase maximum isoprenaline-stimulated contraction in myocytes from noradrenaline-treated guinea-pigs (Wynne et al., 1993). These results support our previous contention that raising cyclic AMP levels is not sufficient to overcome the depression of maximum contraction. In human myocytes, forskolin or cyclic AMP analogues do not produce the same maximum contraction amplitude in myocytes from failing human heart as in those from non-failing (Harding et al., 1992). We have speculated that, in β-adrenoceptor-desensitised human or guinea-pig myocytes, the arrhythmias which limit the maximum effect of β-adrenoceptor stimulation occur before the full inotropic effect of cyclic AMP can be expressed (Harding et al., 1992; Wynne et al., 1993).

4.3. Summary

Noradrenaline infusions in guinea-pigs induced cardiac β-adrenoceptor desensitisation, detectable both with reduced isoprenaline-stimulated inotropy and decreases in isoprenaline-stimulated cyclic AMP. In addition, basal cyclic AMP levels in the absence of β-adrenoceptor stimulation were markedly decreased. Both functional and cyclic AMP changes were quantitatively similar to those seen in human heart failure. IBMX restored isoprenaline-stimulated cyclic AMP levels in noradrenaline-treated guinea-pig cardiac myocytes, but did not reverse the functional depression of the maximum contractile response to β-adrenoceptor agonists. This suggests that additional events occur either independently of, or downstream to, cyclic AMP production which limit positive inotropy. The inability of phosphodiesterase inhibitors to fully restore the maximum β-adrenoceptor response has also been observed in myocytes from failing human hearts (Wynne et al., 1993). We conclude that exposure to noradrenaline can reproduce in guinea-pig myocytes the main post-receptor defects seen in cells from failing human heart, confirming the role of the sympathetic nervous system in the progression of heart failure.

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