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Forskolin, cyclic nucleotides and positive inotropism in isolated papillary muscles of the rabbit

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1 The effects of forskolin, isoprenaline, sodium nitroprusside and the frequency of stimulation were examined on cyclic nucleotide levels and tension responses in rabbit isolated right ventricular papillary muscles.

2 Increasing the frequency of stimulation from 0.01 Hz to 1.6 Hz induced positive inotropic responses that were not obviously related to alterations in the level of either cyclic AMP or cyclic GMP.

3 Isoprenaline induced rapid, concentration-related positive inotropic responses that were associated with increases in the levels of both cyclic AMP and cyclic GMP. There existed good correlations between the increases in tension and the concentrations of both cyclic nucleotides measured in the tissues.

4 Forskolin induced concentration-related positive inotropic responses that were slow to develop. These responses were accompanied by concentration-related increases in the levels of cyclic AMP but not cyclic GMP. The tension responses correlated well with the levels of cyclic AMP measured. The cyclic AMP levels produced by forskolin were some 8 fold higher than those induced by isoprenaline for similar increases in tension.

5 Sodium nitroprusside was without inotropic effect either positive or negative; it nevertheless elevated cyclic GMP levels whilst slightly reducing cyclic AMP levels.

6 These data show that the ratio of cyclic AMP to cyclic GMP does not correlate well with changes in mammalian cardiac contractility. The data further suggest that whilst the intracellular concentration of cyclic AMP in rabbit ventricular myocardium may be an important determinant of positive inotropism, the relationship between the two parameters is more complex than simple proportionality between the tension generated and the amount of cyclic AMP measured within the cells.

Introduction

It has long been recognised that the strength of cardiac muscle contraction is controlled in large part by interaction of the endogenous catecholamines with cardiac β-adrenoceptors. With the discovery of cyclic 3′,5′-adenosine monophosphate (cyclic AMP) and the general acceptance of its role as a 'second messenger' in many biological systems (Robison *et al.*, 1971) there have been many attempts to try and establish a cause and effect relationship between intracellular cyclic AMP levels and contractile force (for reviews see Tsien, 1977; Entman & Van Winkle, 1979). Despite the amassing of a large volume of circumstantial evidence this relationship has not yet been established conclusively (Tsien, 1977). What is accepted however, is that measurable increases in cyclic AMP ordinarily accompany β-adrenoceptor stimulation and exhibit a time and dose-dependence that is consistent with a hypothetical mediatory role (see for example Schumann *et al.*, 1975; Tsien 1977; Reuter, 1979; Honerjager *et al.*, 1981).

The regulatory role of cyclic nucleotides has been further complicated since the discovery of a second functional nucleotide, cyclic 3′,5′-guanosine monophosphate (cyclic GMP). Levels of this nucleotide have been reported to be increased following cholinergic stimulation to induce negative inotropic responses (George *et al.*, 1970: George *et al.*, 1973). Such observations, and similar ones in other tissues, have led to the *Yin Yang* hypothesis (Goldberg, *et al.*, 1975), this being a concept that symbolises a dualism between opposing natural forces. In its simplest form, therefore, the hypothesis defines cyclic AMP and cyclic GMP as biological effectors involved in regulating cellular functions that are controlled bidirectionally. Thus in cardiac muscle it is envisaged that cyclic AMP acts to facilitate events leading to

contraction whilst cyclic GMP exerts opposing inhibitory effects.

Recently, the results of a series of studies on frog ventricle by Flitney and co-workers demonstrated a remarkably precise correlation between changes in contractility and associated alterations in the levels of both cyclic AMP and cyclic GMP in response to different cardioactive agents (Singh, *et al.*, 1978; Flitney, *et al.*, 1979; Flitney & Singh, 1980; Singh & Flitney, 1981). In their studies Flitney and colleagues showed that the magnitude of the inotropic responses were all paralleled by quantitatively equivalent changes in the ratio cyclic AMP/cyclic GMP. The results of these studies in the frog heart are therefore wholly consistent with the *Yin Yang* concept of cyclic AMP augmenting contractility and cyclic GMP exerting a counter action. Furthermore, Singh & Flitney (1981) have suggested that cyclic GMP may constitute part of a feedback mechanism that serves to regulate cyclic AMP production.

With the exception of the early studies by George *et al.* (1970, 1973) in isolated perfused hearts of the rat there have been few controlled studies in which both cyclic AMP and cyclic GMP concentrations have been measured simultaneously in mammalian ventricular muscle and correlations made with the associated tension responses. The object of the work described here, therefore, was to perform such a study in isolated papillary muscles from rabbit hearts.

Preliminary accounts of these findings have been presented (Rodger & Shahid, 1981: Giembycz *et al.*, 1983).

Methods

Two different methods of inducing positive inotropic responses have been used. In the first series of experiments, the interval-force relationship ('positive staircase' phenomenon) that exists for ventricular muscle (Blinks & Koch-Weser, 1963: Koch-Weser & Blinks, 1963) was used since Endoh *et al.* (1976) have reported a reciprocal relationship between cyclic AMP levels and contractility. In the second series of experiments the β-adrenoceptor agonist isoprenaline, adenylate cyclase activator forskolin and sodium nitroprusside were used.

Male New Zealand white rabbits were stunned with a blow to the back of the neck and exsanguinated. The thorax was opened and the hearts rapidly excised and placed in a dish containing warm oxygenated Krebs-Henseleit solution. Papillary muscles were removed from the right ventricle and suspended in a 60 ml organ bath containing Krebs-Henseleit solution at 32 °C. The solution was bubbled vigorously with a gaseous mixture containing 95% O_2 and 5% CO_2 so as to produce a P_{O_2} of 525–550 mmHg, a P_{CO_2} of 38–40 mmHg and a pH of 7.3–7.4. The Krebs-Henseleit used was of the following composition (mmol l^{-1}): NaCl 118, KCl 4.7, $MgSO_4 7H_2O$ 1.2, KH_2PO_4 1.2, Ca $Cl_2 6H_2O$ 2.5, $NaHCO_3$ 25 and glucose 11.7. The papillary muscles were mounted vertically between two platinum wire electrodes such that the base of each tissue was in contact with the bottom electrode whilst the tendon end, attached to a force displacement transducer (Grass FTO3C), lay just beneath the upper electrode. Throughout the different experiments preparations were stimulated using rectangular pulses of 1 ms duration at a voltage 50–100% above threshold (constant in any one experiment; Grass S88 stimulator attached to stimulus isolation units, SIU5, capacity coupled). Initially the tissues were stretched by adjusting the diastolic tension to 1 g and allowed to stabilize for 60 min (at a driving frequency of 1 Hz) during which time, the bathing solution was changed three times. At the conclusion of this equilibration period any fall off in diastolic tension was corrected by resetting back to 1 g. Isometric contractions of the tissues were recorded on a Grass (model 7) ink writing, curvilinear oscillograph.

Interval force curves were constructed in the following manner. Following the equilibration period the stimulation frequency was reduced from 1.0 Hz to 0.01 Hz. This driving frequency was maintained until a new constant level of developed tension was achieved at which point the frequency of stimulation was increased to 0.05 Hz. This sequence was continued until maximum positive inotropic effects were achieved (usually between 0.8 Hz and 1.6 Hz, see Figure 1a).

Papillary muscle cyclic AMP and cyclic GMP determination

Papillary muscles were rapidly removed from the organ bath, blotted dry on absorbent tissue paper and frozen in liquid nitrogen (time elapsed less than 10 s). The individual papillary muscles were weighed and 2–3 pooled to make 20–30 mg of tissue. Frozen tissue was pulverised under liquid nitrogen and then transferred to a precooled tube and homogenized in 1 ml of ice-cold 6% trichloroacetic acid (TCA) with a 8N Ultra-turrax (TP18/10, 8N shaft) cell disrupter for a period of 90 s (9 × 10 s bursts) at 4 °C. The homogenizer shaft was washed with a further 0.5 ml 6% TCA to recover any residual cell extract. After centrifugation the (8000 *g*) supernatant was extracted 6 times with 5 volumes of water-saturated ether. Residual traces of ether were evaporated by heat (60 °C for 5 min) and the samples stored at − 20 °C. Storing of samples at − 20 °C is sufficient to prevent further breakdown of cyclic nucleotides after deproteinization and ether washing). Before assaying for cyclic nucleotide content, two 500 μl aliquots (one

each for cyclic AMP and cyclic GMP assay) of each extract were freeze-dried. The freeze-dried material was reconstituted in adequate assay buffer to allow duplicate measurements. Preliminary experiments had shown that there was no significant variation in the levels of either cyclic AMP or cyclic GMP in the two 500 μl aliquots. For cyclic nucleotide estimations the commercially available protein binding (cyclic AMP, TRK 432) and radioimmunoassay (cyclic GMP TRK 500) kits were used (Radiochemical International). For each assay a standard curve was constructed and tritium radioactivity was counted in a liquid scintillation spectrometer (tri Carb 460CD Packard) after adding 1 ml (in the case of cyclic AMP) or 4 ml (in the case of cyclic GMP) of the scintillation mixture (Picofluor 30, Packard).

In the text the absolute levels of both cyclic nucleotides are expressed as pmol mg^{-1} wet weight of the tissues. Full recovery of known amounts of unlabelled cyclic AMP (40 pmol) and cyclic GMP (20 pmol) added to 6% TCA before homogenization of the muscles was observed.

Drugs

The following drugs were used: forskolin (Cal Biochem-Behring Corporation), (±)-isoprenaline hydrochloride (Sigma), (±)-propranolol hydrochloride (ICI), sodium nitroprusside (Sigma). Solutions of propranolol and sodium nitroprusside were freshly prepared in 0.9% w/v NaCl solution (saline). Isoprenaline solutions were prepared in acidified (pH 3.5) saline to enhance stability. Forskolin was dissolved in 95% ethanol to provide a stock solution which was, thereafter, diluted in saline.

Results

Effects of stimulation frequency on developed tension and cyclic nucleotide levels

Figure 1a illustrates the typical relationship between stimulation frequency and the force of contraction ('positive staircase' phenomenon) for ventricular cardiac muscle. As the frequency of stimulation is increased from 0.01 Hz to 1.0 Hz there is a stepwise increase in the size of the contractions elicited. These data are summarised graphically in Figure 1b. From this graph it is clear that 0.01 Hz constitutes the pessimal driving frequency for rabbit papillary muscles in that least tension is developed. In contrast, optimal tension development is achieved at a frequency of about 1.0 to 1.6 Hz.

To test for any electrically-induced noradrenaline release from sympathetic nerve terminals present in the tissue that might contribute to and complicate the interval-force relationship, curves were reproduced in the presence of the β-adrenoceptor antagonist (±)-propranolol (1×10^{-7} mol l^{-1}). This concentration of propranolol had been shown in preliminary experiments to produce a 20 fold shift to the right of the concentration-effect curve to exogenously administered noradrenaline. Propranolol was, however, without effect on the interval force curves indicating an absence of neurally-released noradrenaline. This was an important point to establish since cyclic nucleotide levels were to be measured at different driving frequencies. To study the effects of stimulation frequency on developed tension and cyclic nucleotide levels, three different frequencies were selected; the pessimal (0.01 Hz), the nearly optimal (1.0 Hz) and one that lay approximately half-way up the interval-force curve (0.4 Hz) illustrated in Figure 1b. At the end of the equilibration period the frequency of stimulation was reduced from 1.0 Hz to either 0.01 Hz or 0.4 Hz or left at 1.0 Hz. Fifteen minutes later the papillary muscles were rapidly removed from the tissue bath and plunged into liquid nitrogen (see methods) to be assayed later for cyclic AMP and cyclic GMP content. As the inotropic state of the muscle increases there is no parallel increase in the levels of either cyclic nucleotide (see Table 1). Indeed at 0.4 Hz both cyclic AMP and cyclic GMP levels are diminished despite tension having increased about 2.5 fold. There exists a good correla-

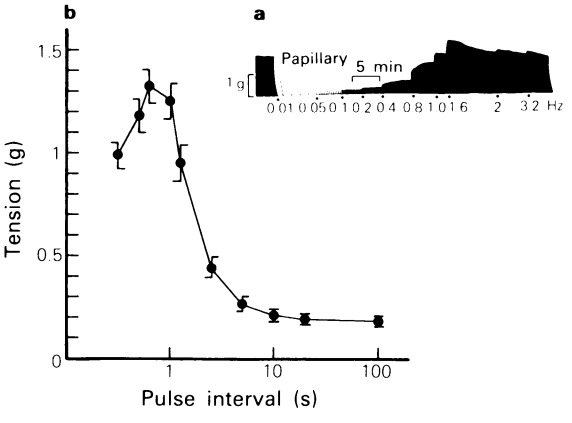


Figure 1 (a) A record of a typical trace illustrating the interval-force relationship for an electrically paced rabbit right ventricular papillary muscle. The left-hand edge of the record shows the tension developed at the conclusion of a 60 min equilibration period of pacing at 1.0 Hz. At the points marked (●) the frequency of stimulation was changed to the values indicated. (b) Graphical representation of the mean interval-force data (vertical lines show s.e. mean) from 10 experiments of the type illustrated in (a).

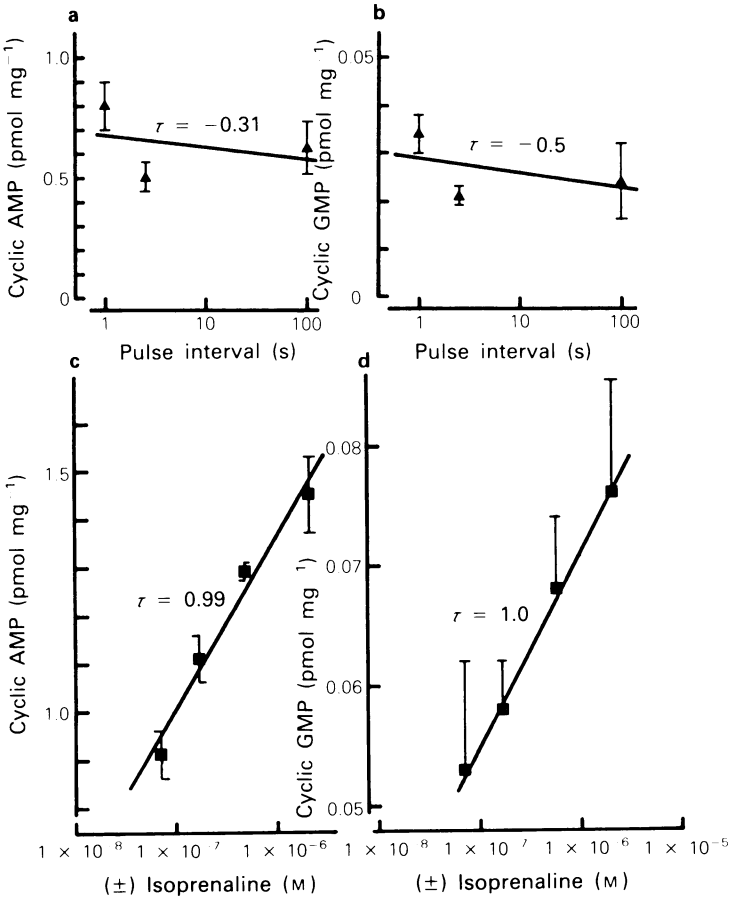


Figure 2 Graphs (a) and (b) illustrate the lack of relationship between pulse interval (stimulation frequency) plotted on the abscissa scale and cyclic AMP (a) and cyclic GMP (b) concentrations plotted in pmol mg^{-1} wet weight of tissue on the ordinate scale. Panels (c) and (d) illustrate a good correlation between the concentrations of isoprenaline (abscissa scale) and the concentrations of cyclic AMP (c) and cyclic GMP (d). Each point represents the mean cyclic nucleotide values (with s.e. indicated by vertical lines) from 4–6 preparations. The regression (r) values are shown for the computer plotted line of best fit through the points.

tion ($r = 0.86$) between the tension developed and the interval between pulses. In contrast, however, the graphs of Figure 2a and 2b clearly demonstrate that there exists no correlation between the levels of either cyclic AMP ($r = 0.31$) or cyclic GMP ($r = 0.5$) and the frequency of stimulation.

Effects of isoprenaline on developed tension and cyclic nucleotide levels

Cyclic nucleotide levels were measured at four different points on the concentration-effect curve to isoprenaline. These points were 25%, 50%, 75% and 100% of the maximum response which were produced by mean concentrations of isoprenaline (± s.e. mean) of 0.065 ± 0.002, 0.17 ± 0.03, 0.55 ± 0.06 and 2.1×10^{-6} mol l^{-1}.

It should be noted that throughout this series of experiments the papillary muscles were driven at 0.4 Hz. To achieve the desired inotropic increase, isoprenaline was added to the tissue bath in a cumulative fashion, each addition of the agonist being made at the peak effect produced by the preceding concentration. Once the desired level of inotropic effect had been reached the tissue was cut down and frozen in liquid nitrogen.

As the concentration of isoprenaline is increased there occurs an increase in developed tension that is associated with increased levels of both cyclic AMP and cyclic GMP. There is good correlation ($r = 0.99$)

Table 1 Effects of stimulation frequency, isoprenaline and forskolin on tension responses and cyclic nucleotide concentrations in rabbit isolated papillary muscles

Treatment	*Concentration* ($mol\,l^{-1}$)	*Tension* (mg)	*Cyclic AMP* ($pmol\,mg^{-1}$)	*Cyclic GMP* ($pmol\,mg^{-1}$)	$\frac{Cyclic\ AMP}{Cyclic\ GMP}$
Stimulation frequency					
0.01 Hz	—	214 ± 42	0.72 ± 0.11	0.024 ± 0.008	30
0.4 Hz	—	537 ± 64	0.60 ± 0.06	0.021 ± 0.002	28.6
1.0 Hz	—	1229 ± 141	0.90 ± 0.10	0.034 ± 0.004	26.5
Isoprenaline	Solvent control	537 ± 64	0.60 ± 0.06	0.021 ± 0.002	28.6
	7×10^{-8}	640 ± 58	0.91 ± 0.05	0.053 ± 0.009	17.1
	1.7×10^{-7}	842 ± 190	1.11 ± 0.05	0.058 ± 0.004	19.1
	5.5×10^{-7}	1319 ± 238	1.29 ± 0.02	0.068 ± 0.006	19.0
	2.1×10^{-6}	1894 ± 176	1.45 ± 0.08	0.076 ± 0.012	19.1
Forskolin	Solvent control	478 ± 60	0.63 ± 0.02	0.051 ± 0.006	12.4
	2.5×10^{-7}	494 ± 65	1.13 ± 0.09	0.06 ± 0.007	18.8
	8.0×10^{-7}	629 ± 94	2.02 ± 0.21	0.07 ± 0.004	28.9
	2.5×10^{-6}	988 ± 123	3.90 ± 0.20	0.056 ± 0.009	69.6
	1.3×10^{-5}	2036 ± 345	11.5 ± 0.90	0.08 ± 0.009	143.8

Each value is the mean ± s.e. mean, $n = 4-6$. Solvent control for isoprenaline was saline and for forskolin the appropriate saline dilution of the 95% ethanol vehicle used to dissolve the forskolin initially. This latter control solution also contained propranolol ($1 \times 10^{-7}\,mol\,l^{-1}$).

between the concentration of isoprenaline and the tension developed by the papillary muscle, as one would expect. There also exists, however, a good correlation between the levels of both cyclic AMP (Figure 2c) and cyclic GMP (Figure 2d) and agonist concentrations.

When the experiments were repeated in the presence of propranolol ($1 \times 10^{-7}\,mol\,l^{-1}$) the tension responses produced by isoprenaline were markedly inhibited (dose-ratio = 20) in a competitive manner. The corresponding levels of both cyclic nucleotides were also reduced in a parallel fashion.

Effects of forskolin on developed tension and cyclic nucleotide levels

Forskolin, a drug that selectively stimulates adenylate cyclase (Seamon & Daly, 1981) produced concentration-dependent increases in developed tension over the range $2 \times 10^{-7}\,mol\,l^{-1}$ to $1.3 \times 10^{-5}\,mol\,l^{-1}$. All experiments using forskolin were performed in the presence of $1 \times 10^{-7}\,mol\,l^{-1}$ propranolol. Compared with isoprenaline, responses produced by forskolin were slow to develop taking approximately 20–30 min to attain peak tension when high concentrations were used. Once established the inotropic effects were only slowly reversed by washing. The mean maximum inotropic response produced by forskolin was 2036 ± 345 mg which is not significantly different ($P > 0.05$) from that produced by isoprenaline (1894 ± 176 mg). Figure 3 illustrates graphically the mean concentration-effect data for forskolin.

Associated with the increased tension responses there occurred marked concentration-related elevations in the levels of cyclic AMP. These increased levels of cyclic AMP were well correlated ($r = 0.94$) with the concentrations of forskolin used (Figure 4a). Compared to the cyclic AMP increases produced by isoprenaline, those induced by forskolin were several times greater for similar inotropic responses (compare the graphs of Figure 2c and Figure 4a). In contrast, although forskolin elevated cyclic GMP levels to a similar level as isoprenaline, these were not concentration-related (Figure 4b).

Comparative data for isoprenaline and forskolin are illustrated in Table 1.

Effects of sodium nitroprusside on developed tension and cyclic nucleotide levels

Sodium nitroprusside ($1 \times 10^{-6}\,mol\,l^{-1}$ to $1 \times 10^{-3}\,mol\,l^{-1}$) was without inotropic effect, either positive or negative, on the papillary muscles driven at either 0.4 Hz or 1 Hz. Figure 5 illustrates that whilst sodium nitroprusside ($1 \times 10^{-3}\,mol\,l^{-1}$) was without inotropic effect it nevertheless caused a 2.5 fold eleva-

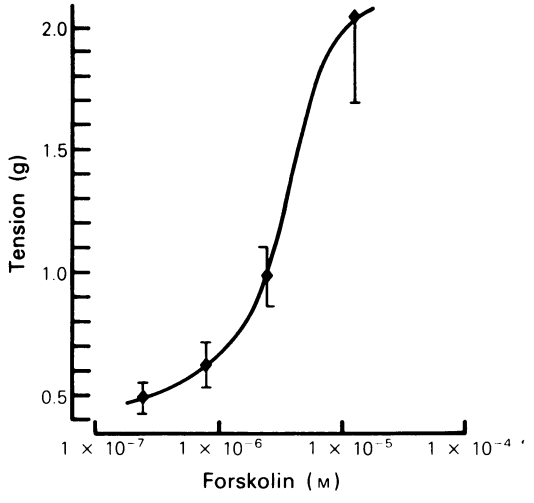
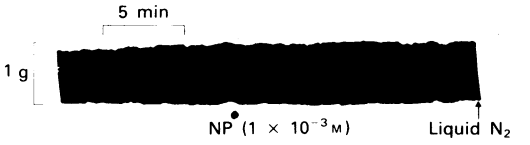


Figure 3 Mean concentration-effect curve for forskolin-induced positive inotropic responses in rabbit right ventricular papillary muscles. Each point represents the mean (s.e. mean shown by vertical lines) of 4–6 preparations.



	Before NP	After NP
Cyclic AMP (pmol mg⁻¹)	0.90 ± 0.10	0.71 ± 0.06
Cyclic GMP (pmol mg⁻¹)	0.034 ± 0.004	0.093 ± 0.003
Cyclic AMP / Cyclic GMP	26.5	7.6

Figure 5 Original tension record from a typical experiment in which the effects of sodium nitroprusside (NP) were examined on cyclic nucleotides. In this experiment the papillary muscle was driven at 1.0 Hz throughout. Fifteen minutes after the addition of sodium nitroprusside, the muscle was removed from the tissue bath and placed in liquid nitrogen (at the point marked Liquid N_2). The table beneath the tension record illustrates the mean cyclic nucleotide data (± s.e. mean) from 5 experiments. The ratio of cyclic AMP to cyclic GMP before and after sodium nitroprusside is also illustrated.

tion in the intracellular levels of cyclic GMP. Only a slight reduction in cyclic AMP levels was observed after sodium nitroprusside. It should be noted that before addition of sodium nitroprusside the cyclic AMP to cyclic GMP ratio was 26.5 whereas afterwards it fell to 7.6 as a consequence of the elevated cyclic GMP value.

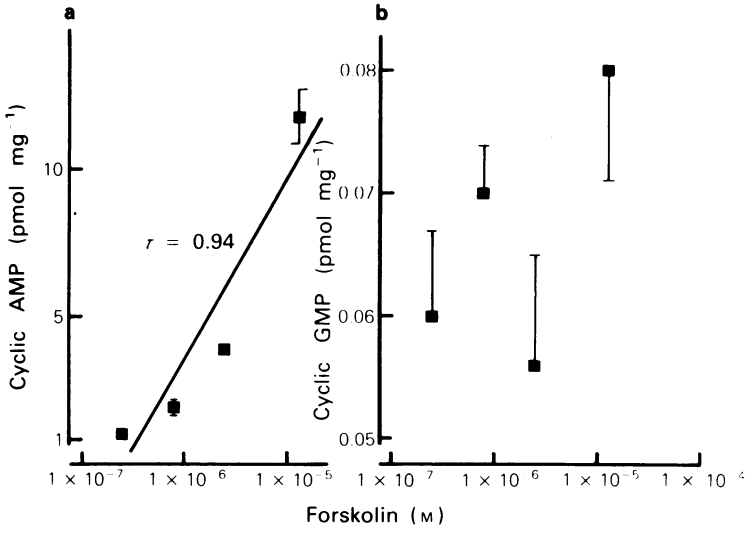


Figure 4 Plots of concentration of forskolin (abscissa scale) against the levels of cyclic AMP (a) and cyclic GMP (b). Each point represents the mean cyclic nucleotide value (s.e. mean shown by vertical lines) for 4–6 preparations. The regression (r) value is shown for the computer plotted line of best fit through the points in graph (a). There is no relationship between cyclic GMP values and concentration of forskolin.

Discussion

In stark contrast to the findings in frog ventricle, (see introduction for details) the results of our studies indicate that the ratio of cyclic AMP to cyclic GMP is unrelated to changes in contractility of mammalian cardiac muscle. Isoprenaline, for example, produced positive inotropic responses via β-adrenoceptor stimulation that were coincident with significant ($P < 0.05$) increases in the levels of both cyclic AMP and (somewhat surprisingly) cyclic GMP. There existed a good correlation between the increases in tension and the levels of both cyclic nucleotides measured in the tissues. The ratio of cyclic AMP to cyclic GMP was little altered during the positive inotropic effects (Table 1).

It has been reported recently that forskolin produces a selective elevation in the intracellular levels of cyclic AMP in a variety of different tissues by directly activating adenylate cyclase (Seamon & Daly, 1981). In the experiments described here forskolin, like isoprenaline, produced increases in developed tension that were well correlated with the levels of cyclic AMP measured in the tissues. Since these experiments were all performed in the presence of propranolol, at a concentration sufficient to produce pronounced β-adrenoceptor blockade, these changes are unlikely to be a consequence of β-adrenoceptor stimulation. Whilst cyclic GMP levels were also raised by forskolin, to levels similar to those produced by isoprenaline, the increases were not related to the concentration of forskolin used. The magnitude of the cyclic AMP increases was such that by comparison the alteration in cyclic GMP was extremely small. The net result was that the inotropic effects of forskolin were accompanied by significantly large increases in the cyclic AMP to cyclic GMP ratio, from about 12 in the absence of forskolin (solvent control data) up to 144 at the maximally effective concentration (see Table 1). Sodium nitroprusside, on the other hand, is known to raise the intracellular levels of cyclic GMP in a variety of tissues (Diamond, 1978) without markedly modifying cyclic AMP levels. Figure 5 illustrates that whilst sodium nitroprusside increased the levels of cyclic GMP approximately 2.5 fold these changes were not accompanied by any alterations in contractility. In these experiments, as the cyclic GMP levels rose, the levels of cyclic AMP fell slightly such that the resultant cyclic AMP to cyclic GMP ratio fell from about 26 in the control period to approximately 8 after addition of sodium nitroprusside.

It is tempting to conclude from these data that in mammalian cardiac muscle it is not so much the ratio of cyclic AMP to cyclic GMP but the absolute level of cyclic AMP within the cells that is the important determinant of β-adrenoceptor-mediated increases in contractility. Such a conclusion must be tempered however, when one considers the cyclic AMP and tension data for forskolin and isoprenaline in greater detail (see Table 1). Both drugs produced tension changes that were well correlated with the levels of cyclic AMP measured within the cells when each drug's effects were plotted separately. It is clear however, that whilst similar tension changes were produced, markedly different cyclic AMP changes were induced within the cardiac cells. For example, the maximum inotropic response to isoprenaline is associated with an intracellular level of cyclic AMP of 1.45 ± 0.08 pmol mg^{-1}. A similar level of cyclic AMP (1.13 ± 0.09 pmol mg^{-1}) is produced by forskolin at a concentration (2.5×10^{-7} mol l^{-1}) that does not produce a significant increase in contractility compared to control levels. Furthermore, the maximum inotropic response to forskolin is associated with a level of cyclic AMP (11.5 ± 0.9 pmol mg^{-1}) that is some 8 fold higher than that produced by isoprenaline for a similar tension increase. Clearly, despite the good correlations that exist between the cyclic AMP levels and developed tensions for forskolin and isoprenaline when considered separately, there is no unifying concentration-effect relationship between the two parameters in the experiments described here.

It is conceivable that the important increases in cyclic AMP, as far as tension is concerned, occur in functional compartments (Brunton *et al.*, 1981) that are readily affected by isoprenaline operating through β-adrenoceptors coupled to certain adenylate cyclase units. Activation via this route could yield marked tension changes that are associated with large cyclic AMP changes in these functional compartments. However, experimental measurements of cyclic AMP are constrained by technical limitations to assessment of the total tissue cyclic AMP which effectively introduces a dilution factor resulting in an underestimate of the actual changes that occur. Additionally, forskolin stimulates all adenylate cyclase present within cells. Consequently many adenylate cyclase units that are not coupled to β-adrenoceptors will be activated. This in turn will lead to a greater production of cyclic AMP within the cardiac cells, much of which may occur in compartments that are divorced from an involvement in tension generation. The net result of such an exaggerated production of cyclic AMP will, in the type of experiments described here, lead to an overestimate of the amount of cyclic AMP required to generate a certain tension change. However, an alternative possibility that must be borne in mind is that the observed changes in contractility may not be causally related to alteration in the levels of cyclic AMP, but merely events occurring in parallel. This being the case, the correlations that can be drawn between the two parameters may be purely fortuitous.

Endoh *et al.* (1976) have shown a reciprocal rela-

tionship between cyclic AMP and contractility in rabbit papillary muscles. In a repeat of their experiments with respect to cyclic AMP we have been unable to corroborate their findings. Furthermore, the results described here indicate that there is no obvious relationship between either cyclic AMP or cyclic GMP levels and the different contractile states induced by changing the frequency of stimulation. Figures 2a and b clearly illustrate that as the frequency of stimulation is increased from 0.01 Hz up to 1 Hz (thus increasing the magnitude of cardiac contraction) there is no associated, parallel increase or decrease in the levels of either cyclic nucleotide. At present we are unable to find a suitable explanation for the discrepancy between our findings and those of Endoh *et al.* (1976). Explanation is further complicated when one considers that not only did we adopt almost identical experimental methods (based upon these authors own descriptions) but also that the changes in cyclic AMP levels measured during our experiments using isoprenaline are remarkably similar to those reported in an earlier paper by Schumann *et al.* (1975).

In conclusion, therefore, whilst these results do not help to establish a cause and effect relationship between cyclic nucleotides and cardiac contractility, they nevertheless highlight the marked differences that exist between amphibian and mammalian cardiac muscle. In contrast to amphibian muscle, the cyclic AMP to cyclic GMP ratio does not correlate well with changes in mammalian cardiac contractility. The data further suggest that whilst the intracellular levels of cyclic AMP in rabbit ventricular myocardium may be an important determinant of positive inotropism, the relationship between the two parameters is more complex than simple proportionality between the tension generated and the amount of cyclic AMP measured within the cells. The data also illustrate that the mechanism underlying frequency-dependent inotropism is unrelated to changes in cyclic nucleotides and remains to be determined.

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