EFFECTS OF ISOPRENALINE ON CONTRACTILE FORCE AND INTRACELLULAR CYCLIC 3′,5′-NUCLEOTIDE LEVELS IN THE HYPODYNAMIC FROG VENTRICLE

J. SINGH, F. W. FLITNEY and J. F. LAMB

Department of Physiology and Pharmacology, University of St Andrews, Bute Medical Buildings, St Andrews, Fife, Scotland

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1. Introduction

The increase in contractility which occurs in response to β-adrenergic stimulation of the heart is thought to be mediated through changes in the levels of intracellular adenosine 3′,5′-cyclic monophosphate (cyclic AMP) [1–5]. The possibility that guanosine 3′,5′-cyclic monophosphate (cyclic GMP) is also involved in regulating the contractile response of the heart has received comparatively little attention. This is somewhat surprising since it has been shown that the negative inotropic response to acetylcholine is associated with a rise in intracellular cyclic GMP [6–8], and that the levels of both cyclic AMP and cyclic GMP undergo oscillatory changes which are synchronised with the cardiac cycle [9]. In addition, we demonstrated that the development of the hypodynamic condition [10] and the inotropic response of the isolated frog ventricle to stimulation with exogenous ATP [11,12] are characterized by changes in the levels of both cyclic 3′,5′-nucleotides. Moreover, in each instance a striking correlation exists between changes in isometric twitch tension and the ratio of (cyclic AMP)/(cyclic GMP).

This paper presents results which show changes in the levels of cyclic AMP and cyclic GMP following stimulation of the hypodynamic frog ventricle with the β-agonist isoprenaline and here too there is a clear correlation between the magnitude of the contractile response and the change in the cyclic nucleotide ratio.

2. Methods

2.1. *Heart perfusion*

Hearts from frogs, *Rana temporaria* and *Rana esculenta*, were isolated and superfused at room temperature (18–20°C) by the method in [13]. The perfusion rate was kept constant at 100 ml. min^{-1} and the preparation was stimulated (Ag wire electrodes) at 30 min^{-1} (square, 5 ms pulses, 10 V). The ventricular strip was allowed to become hypodynamic and subsequently superfused with frog Ringer containing isoprenaline. At a predetermined time during the isoprenaline response it was freeze-clamped in liquid nitrogen. Another strip taken from the same ventricle was allowed to become hypodynamic to the same extent as the first and then freeze-clamped in liquid nitrogen. The latter served as the control strip.

2.2. *Extraction and assay for cyclic nucleotides*

Frozen ventricular strips were pulverized into a powdered extract. Cyclic AMP and cyclic GMP from the latter were extracted and assayed by the techniques in [14] and [15], respectively. The amount of protein present in the extract was estimated by the method depicted by [16]. Cyclic nucleotide results were expressed as pmol cyclic AMP or cyclic GMP/mg protein.

3. Results and discussion

Figure 1 shows the time course of the changes in intracellular cyclic AMP and cyclic GMP following superfusion of isolated frog (*R. temporaria*) ventricular strips with 10^{-6} M isoprenaline. The accompanying contractile response is also shown (dashed line) for comparison. There are two points to emphasise. First, the early increase in contractile force

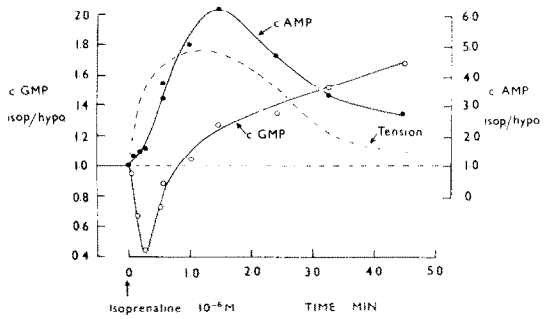

Fig.1. Time courses of changes in contractile force (dashed line) and intracellular levels of cyclic AMP (solid circles) and cyclic GMP (open circles) during exposure of the frog ventricle to 10^{-6} M isoprenaline. Control (hypodynamic) values (mean ± SE) for cyclic AMP and cyclic GMP levels (pmol mg^{-1}. total protein) were 9.39 ± 0.66 and 1.44 ± 0.15, respectively (n = 17).

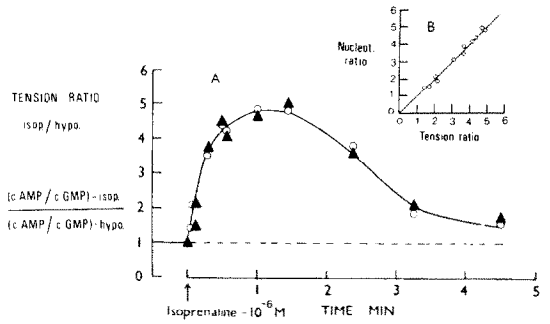

Fig.2. A. Time course of the change in isometric twitch tension (open circles) and cyclic nucleotide ratio (solid triangles) measured at various times during the response to 10^{-6} M isoprenaline. Both parameters expressed as multiples of the control (hypodynamic) values. B. Data taken from fig.1. Cyclic nucleotide ratio plotted against contractile force. Solid line drawn with slope of 1. Correlation coefficient ± SE of estimate: 0.995 ± 0.143, n = 10, $P < 0.001$.

corresponds more closely with an initial decrease in the level of cyclic GMP; the rise in the level of cyclic AMP is delayed and lags somewhat behind the contractile response. This result is of considerable interest since it was demonstrated in mammalian hearts [17–19] that a rise in cyclic AMP preceeds the onset of the inotropic response; indeed, this provides essential corroborative evidence in support of the 'second messenger' role for cyclic AMP [1]. Secondly, cyclic GMP levels reach to the control value (horizontal broken line) at about the time when the contractile response is maximal and further increase is accompanied by a fall in isometric twitch tension and a decrease in the level of cyclic AMP.

It was found earlier that a striking parallelism exists between the time course of the change in ventricular contractility and the ratio of the two cyclic nucleotides during both the development of the hypodynamic condition [10] and during the inotropic response to exogenous ATP [12]. The results of fig.1. yield a similar correlation, as shown in fig.2A. This depicts the change in the ratio of cyclic AMP/cyclic GMP, that is:

$$\left[\frac{\text{cyclic AMP/cyclic GMP (isop)}}{\text{cyclic AMP/cyclic GMP (hypo)}}\right]$$

with time (solid triangles), together with the accompanying tension response (open circles). Statistically, the correlation is highly significant (correlation coefficient ± SE of estimate: 0.995 ± 0.143, n = 10, $P < 0.001$) and reveals a clear correspondence between a change in the cyclic nucleotide ratio and the accompanying change in force (fig.2B, inset).

The effects of altering the concentration of isoprenaline on cyclic nucleotide levels and on contractile force for seven preparations is shown in fig.3,4. In

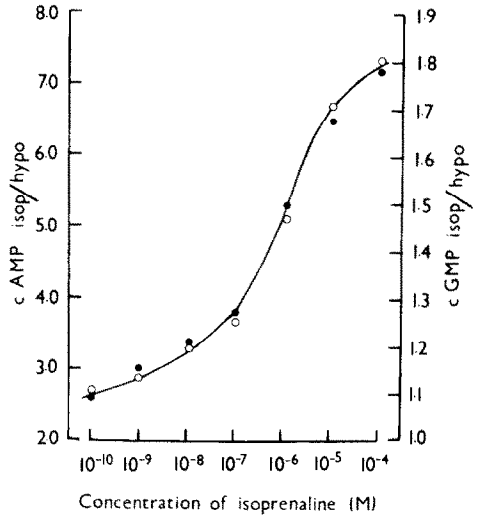

Fig.3. Log dose–response curves for concentrations of isoprenaline ranging from 10^{-10}–10^{-4} M. Solid circles, cyclic AMP; open circles, cyclic GMP. Preparations superfused for approximately 100 s prior to freeze-clamping. Both parameters expressed as multiples of control values.

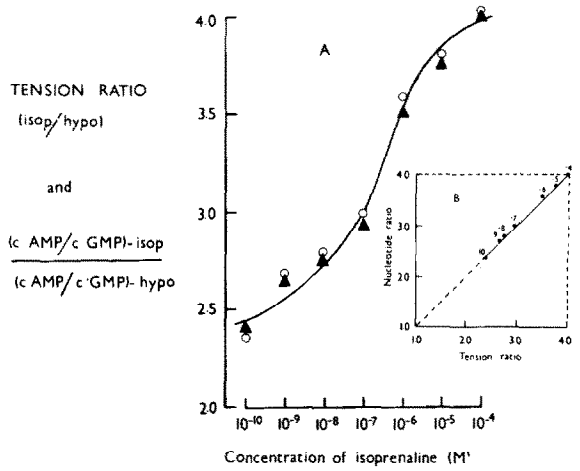

Fig.4. A. Effects of isoprenaline ranging from 10^{-10}–10^{-4} M on contractile force (open circles) and cyclic nucleotide ratio (solid triangles; data from fig.3). B. Relation between isometric force and cyclic nucleotide ratio (multiples of control values). Correlation coefficient ± SE of estimate: 0.996 ± 0.57, $n = 7, P < 0.001$.

these experiments hearts from another species of frogs, *R. esculenta*, were used. Figure 3 shows log dose–response curves for the increase in cyclic AMP (solid circles) and cyclic GMP (open circles) measured ~ 100 s after superfusing with the drug when the response was maximal. The change in the ratio of cyclic AMP/cyclic GMP with varying concentrations of isoprenaline and the corresponding change in isometric twitch tension, measured at the time of freeze-clamping each preparation, are shown in fig.4. The data again reveal a clear correlation (fig.4B. inset: correlation coefficient ± SE of estimate: 0.996 ± 0.057, $n = 7, P < 0.001$) between the cyclic nucleotide ratio and contractile force.

These results, and those referred to earlier [10,12], lead us to postulate that cyclic AMP and cyclic GMP are important components of a control mechanism which regulates the capacity of the ventricle to produce force. The observed correlation between the ratio of the two cyclic nucleotides and isometric force suggests that they play opposing roles in the regulatory process, a feature which is seen in several other biological systems [20]. Both cyclic AMP and cyclic GMP are thought to exert their regulatory effects on cellular metabolism by activating a number of protein kinases, and several substrates for cyclic AMP-dependent protein kinases have been implicated in regulating myocardial contractility, troponin I (TN-I), a subunit of the regulatory protein complex [21]; phospholamban, a 22 000 dalton protein constituent of the sarcoplasmic reticulum [22]; and a surface membrane-bound protein, thought to be a component of the slow inward (calcium) current channel [23]. It has been previously demonstrated that perfusion of the rat hearts with isoprenaline produces a time-dependent increase in the state of phosphorylation of TN-I, which almost exactly parallels the time course of the resulting contractile response [24]. The existence of a similar relationship between the contractile force and the ratio cyclic AMP/cyclic GMP raises the possibility that the state of phosphorylation of TN-I and perhaps of other phosphoproteins involved in regulating force production may be determined by the relative amounts of intracellular cyclic AMP and cyclic GMP. A cyclic GMP-dependent protein kinase has been isolated from the heart [25], although to date there have been no reports of a naturally-occuring substrate for this enzyme in the heart. The phosphatase enzymes responsible for dephosphorylating protein substrates would appear to be plausible candidates in the light of the present results.

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