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INOTROPIC RESPONSES OF THE FROG VENTRICLE TO DIBUTYRYL CYCLIC AMP AND 8-BROMO CYCLIC GMP AND RELATED CHANGES IN ENDOGENOUS CYCLIC NUCLEOTIDE LEVELS

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Abstract—Isolated frog ventricles were superfused with solutions containing either N^6, O^2-dibutyryl adenosine 3′,5′-cyclic monophosphate (dibutyryl 3′,5′-cyclic AMP) or 8-bromo guanosine 3′,5′-cyclic monophosphate (8-br 3′,5′-cyclic GMP) and changes in isometric twitch tension and in the levels of endogenous 3′,5′-cyclic nucleotides were measured. The results show that dibutyryl 3′,5′-cyclic AMP potentiates the twitch, whereas 8-br 3′,5′-cyclic GMP depresses it. Both effects are dose-related. The magnitude of the decrease in contractile force produced by 8-br 3′,5′-cyclic GMP, and of the increase caused by dibutyryl 3′,5′-cyclic AMP, are each paralleled by quantitatively-equivalent changes in the ratio 3′,5′-cyclic AMP: 3′,5′-cyclic GMP. The effect of 8-br 3′,5′-cyclic GMP on the twitch is associated with a marked reduction in endogenous 3′,5′-cyclic AMP levels, which is linearly related to the increment in intracellular 3′,5′-cyclic GMP (slope of regression line ± S.E. : -10.4 ± 0.6 pmoles 3′,5′-cyclic AMP·pmole^{-1} increase in 3′,5′-cyclic GMP). The entry of dibutyryl 3′,5′-cyclic AMP into the fibres is accompanied by a relatively small change in intracellular 3′,5′-cyclic GMP, in this case, an increase (slope of regression line ± S.E. : $+0.018 \pm 0.002$ pmoles 3′,5′-cyclic GMP·pmole^{-1} increase in 3′,5′-cyclic AMP). These results suggest that both 3′,5′-cyclic nucleotides are normally involved in regulating contractility, 3′,5′-cyclic AMP potentiating the twitch and 3′,5′-cyclic GMP exerting a counter-action; and that 3′,5′-cyclic GMP may constitute part of a feedback mechanism which serves to regulate 3′,5′-cyclic AMP levels.

The physiological responses produced by many hormones, drugs and neurotransmitters are mediated through changes in intracellular adenosine 3′,5′-cyclic monophosphate (3′,5′-cyclic AMP) [1]. The ensuing cellular changes are thought to be initiated by the activation of enzymes (3′,5′-cyclic AMP-dependent protein kinases) which catalyse the phosphorylation (by adenosine 5′-triphosphate) of regulatory proteins, whose physiological function depends critically upon their state of phosphorylation [2] and is altered as a result. The discovery of a cyclical from of a close-related nucleotide, guanosine 3′,5′-cyclic monophosphate (3′,5′-cyclic GMP [3]), and of a corresponding 3′,5′-cyclic GMP-dependent protein kinase [4], has led to the suggestion that this substance may also function as a 'second messenger' [5] and, more recently, to the view that many cellular processes may be regulated in a bidirectional fashion, by 3′,5′-cyclic AMP and 3′,5′-cyclic GMP acting antagonistically [6]. This idea arose initially from observations concerning the effects of acetylcholine on the heart; it was found that whereas many positive inotropic responses are accompanied by elevated levels of 3′,5′-cyclic AMP [7], the negative inotropic effects evoked by acetylcholine are characterised instead by increases in intracellular 3′,5′-cyclic GMP [8–10]. Later studies by Brooker [11] and by Diamond, Ten Eick and Trapani [12] have cast some doubt on the interpretation of these earlier experiments and opinion is now more divided over the question of the involvement of 3′,5′-cyclic GMP in regulating myocardial contractility [13].

The results of a recent series of studies, concerned with the effects of several cardioactive agents on the frog ventricle, have revealed a remarkably precise correlation between changes in contractility and associated changes in endogenous 3′,5′-cyclic nucleotide levels; specifically, the magnitude of the inotropic responses produced by adenosine 5′ triphosphate [14], isoprenaline [15], uridine 5′ triphosphate [16], adenosine [17] and sodium nitroprusside [18] are all paralleled by quantitatively-equivalent changes in the ratio 3′,5′-cyclic AMP : 3′,5′-cyclic GMP. These findings then, appear to offer some support for the idea that the contractile performance of the ventricle is regulated, at any given moment, by the relative amounts of 3′,5′-cyclic AMP and 3′,5′-cyclic GMP in the fibres. However, they can equally well be explained by postulating that the observed changes in 3′,5′-cyclic nucleotide levels and in twitch tension arise simultaneously, as a result of an underlying common cause, and are only incidentally (and not causally) related.

The experiments now to be described were undertaken in an attempt to resolve this question. The

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approach has been to investigate the effects of manipulating the levels of intracellular 3′,5′-cyclic AMP and 3′,5′-cyclic GMP directly, by superfusing preparations with their more permeant analogues, dibutyryl 3′,5′-cyclic AMP and 8-br 3′,5′-cyclic GMP, respectively. Previous studies have established that dibutyryl 3′,5′-cyclic AMP augments the contractile response of the heart [19, 7] whereas 8-br 3′,5′-cyclic GMP depresses it [20, 21], but the precise nature of the relationship between the altered levels of 3′,5′-cyclic AMP and 3′,5′-cyclic GMP, consequent upon such treatment, and of the associated inotropic effects, has not yet been established. This has now been investigated and the results obtained are broadly consistent with the idea that 3′,5′-cyclic AMP and 3′,5′-cyclic GMP do indeed act in a reciprocal manner to regulate ventricular contractility. They also offer some evidence for the existence of a mechanism whereby these antagoniic effects might be expressed, since it transpires that 8-br 3′,5′-cyclic GMP can markedly depress the levels of 3′,5′-cyclic AMP. This observation suggests that 3′,5′-cyclic GMP may constitute part of a feedback system which serves to regulate the synthesis and/or degradation of 3′,5′-cyclic AMP.

MATERIALS AND METHODS

Dissection, superfusion and recording procedures. Isolated ventricles from adult frogs (males and females, *Rana temporaria*) were used thoughout this study. Animals were killed by stunning and pithing and the heart excised rapidly. The atria were removed and the ventricle divided into posterior and anterior halves by a single incision. The methods used for recording isometric contractions and for superfusing preparations are described elsewhere [21, 23]. Preparations were superfused with Ringer's solution (composition, mM: NaCl, 115; KCl, 2.5; $CaCl_2$, 1.0; Na_2HPO_4, 2.15; NaH_2PO_4, 0.85; glucose, 5.6; pH 7.2) containing either N^6,O^2 dibutyryl adenosine 3′,5′-cyclic monophosphate (Sigma Ltd) or 8-bromo guanosine 3′,5′-cyclic monophosphate (Uniscience Ltd) at a flow rate of 100 ml·min^{-1}. Ventricles were stimulated electrically (square pulses, 5 msec duration; amplitude 10 V; frequency, 0.5 Hz) through silver wire electrodes located on either side of the preparation. All experiments were conducted at room temperature (18–19°).

Experimental procedure. The contractile response of the superfused ventricle declines over a period of time, leading to a relatively stable but depressed state, termed the hypodynamic condition. This process is itself accompanied by changes in intracellular 3′,5′-cyclic AMP and 3′,5′-cyclic GMP [24], and so in all experiments care was taken to ensure that the degree of hypodynamic depression in each 'test' half-ventricle was exactly the same as that for its control partner.

The experiments were performed as follows. First, each control and 'test' (partner) half-ventricle was stabilised by superfusing with Ringer's solution for a period of 80 min; typically, contractile force fell to around 30 per cent of its initial level during this period. The control half-ventricle was further superfused with Ringer's solution for an additional 25 min. It was then crush-frozen between forceps which had been cooled previously by immersion in liquid nitrogen. The resulting frozen tissue was stored in liquid N_2 until required for assay purposes. The 'test' half-ventricle was exposed to a solution containing either dibutyryl 3′,5′-cyclic AMP (range 10^{-5} to 10^{-3} M) or 8-br 3′,5′-cyclic GMP (range 10^{-9} to 10^{-4} M). The resulting inotropic responses developed relatively slowly, approaching a steady-state after approximately 25 min. The perfusing solution was then exchanged for Ringer's solution alone, in order to remove traces of extracellular dibutyryl 3′,5′-cyclic AMP or 8-br 3′,5′-cyclic GMP, prior to being crush-frozen. (A 'wash-out' period of 30 sec was allowed, Lamb and McGuigan [21], using similar perfusing conditions, found that changes in the twitch tension produced by lowering extracellular $[Ca]_0$ from 2.0 to 1.0 mM required only 1 sec for completion; a 'wash-out' period of 30 sec was therefore considered adequate to remove 3′,5′-cyclic nucleotide derivatives from the inter-fibre spaces.)

3′,5′-cyclic nucleotide and total protein assays. 3′,5′-cyclic AMP and 3′,5′-cyclic GMP were extracted from frozen tissue and assayed using Radiochemical Assay Kits, TRK 432 [25] and TRK 500 [26], respectively. Total protein was estimated by the Biuret method [27]. Details of the procedures are given elsewhere [23]. 3′,5′-cyclic nucleotide levels are expressed throughout in either pmoles·mg^{-1} protein, or alternatively, test values are given as multiples of the control levels. Since the aim of these experiments was to increase either 3′,5′-cyclic AMP or 3′,5′-cyclic GMP levels inside the fibres by use of their more permeant analogues, it was necessary to investigate the following two points: first, to determine whether there is any detectable difference in the sensitivities of the assay methods to parent compounds, on the one hand, and to their derivatives, on the other; and second, to ascertain whether the assay for one 3′,5′-cyclic nucleotide is influenced by the presence of the other.

Control experiments showed that both assay kits are equally sensitive to the analogues as to the parent substances (to within ± 2%; in the sample range 0.5 to 10 pmoles). The presence of a large excess (up to 10^8 times more) of dibutyryl 3′,5′-cyclic AMP (or 3′,5′-cyclic AMP) had no detectable effect (<0.5%) on the sensitivity of the 3′,5′-cyclic GMP assay; and likewise, a large excess (10^7 times more) of 8-br 3′,5′-cyclic GMP (or 3′,5′-cyclic GMP) did not interfere with the assay for 3′,5′-cyclic AMP.

Since neither assay method is able to distinguish between naturally-occurring (endogenous) 3′,5′-cyclic nucleotides and their corresponding analogues, and because the extent to which the synthetic derivatives are converted to the parent compounds on entering the fibres is not known, the proportion of each present in the fibres after superfusion cannot be readily ascertained. Hence, 3′,5′-cyclic AMP levels in preparations which were superfused with solutions containing dibutyryl 3′,5′-cyclic AMP actually represent endogenous 3′,5′-cyclic AMP *plus* dibutyryl 3′,5′-cyclic AMP; and similarly, the levels of 3′,5′-cyclic GMP in fibres superfused with 8-br 3′,5′-cyclic GMP represent endogenous 3′,5′-cyclic GMP *plus* 8-br 3′,5′-cyclic GMP.

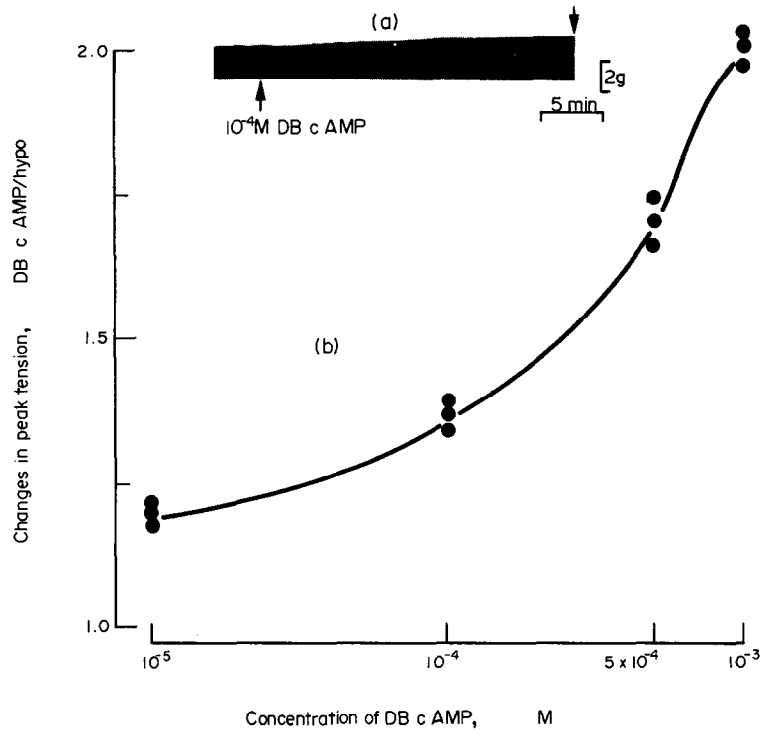

Fig. 1. (a) Effects of 10^{-4} M dibutyryl 3',5'-cyclic AMP on contractile force. The upward and downward pointing arrows indicate the times of application of dibutyryl 3',5'-cyclic AMP and of freezing – clamping the preparation, respectively. (b) Log-dose–response curve showing the effects of different concentrations of dibutyryl 3',5'-cyclic AMP on isometric twitch tension measured at the peak of the response. Contractile force expressed as multiples of control values.

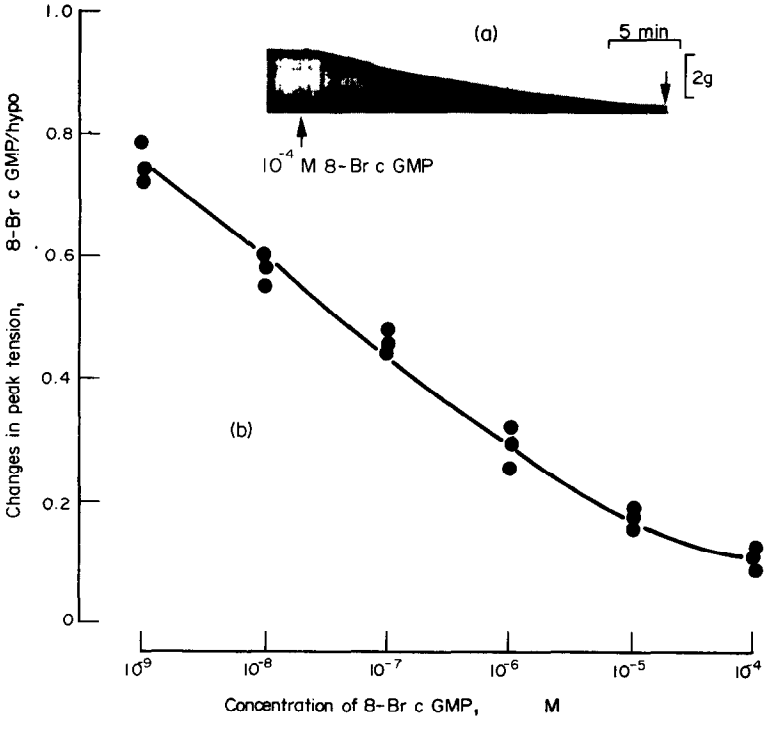

Fig. 2. (a) An original chart recording of a response produced by superfusing a half-ventricle with 10^{-4} M 8-br 3',5'-cyclic GMP. (b) Dose–response curve showing the effects of 8-br 3',5'-cyclic GMP (10^{-9} to 10^{-4} M) on isometric twitch tension. Contractile force expressed as multiples of control values.

RESULTS

Inotropic responses of the frog ventricle to dibutyryl 3',5'-cyclic AMP and 8-br 3',5'-cyclic GMP

Figure 1(a) is an original chart recording showing the time course of the increase in isometric twitch tension during superfusion of a hypodynamic ventricle with 10^{-4} M dibutyryl 3',5'-cyclic AMP. The effects of different concentrations of dibutyryl 3',5'-cyclic AMP (range 10^{-5} to 10^{-3} M) on the 'steady state' twitch tension (measured after 25 min) are shown in Fig. 1(b).

Figure 2(a) shows the response of a preparation to 10^{-4} M 8-br 3',5'-cyclic GMP. After an initial, rapid decline, the twitch amplitude decayed relatively slowly, in this case, to around 10 per cent of its initial value. The log dose–response curve, for concentrations of 8-br 3',5'-cyclic GMP in the range 10^{-9} to 10^{-4} M, is shown in Fig. 2(b). Here again, the steady-state values shown were measured after 25 min.

Effects of dibutyryl 3',5'-cyclic AMP and 8-br 3',5'-cyclic GMP on intracellular 3',5'-cyclic nucleotide levels

The inotropic responses produced by superfusing preparations with 3',5'-cyclic nucleotide analogues are accompanied by changes in intracellular 3',5'-cyclic nucleotide levels. The extent to which they were altered was measured by assaying frozen tissue and, somewhat surprisingly, the results obtained showed that each of the two derivatives influenced the levels of both 3',5'-cyclic AMP and 3',5'-cyclic GMP, and not merely those of the corresponding 'parent' nucleotide.

The effects of superfusing ventricles with dibutyryl 3',5'-cyclic AMP (10^{-5} to 10^{-3} M) are shown in Fig. 3. Dibutyryl 3',5'-cyclic AMP produced dose-related increases in both 3',5'-cyclic AMP (open circles) and 3',5'-cyclic GMP (closed circles).

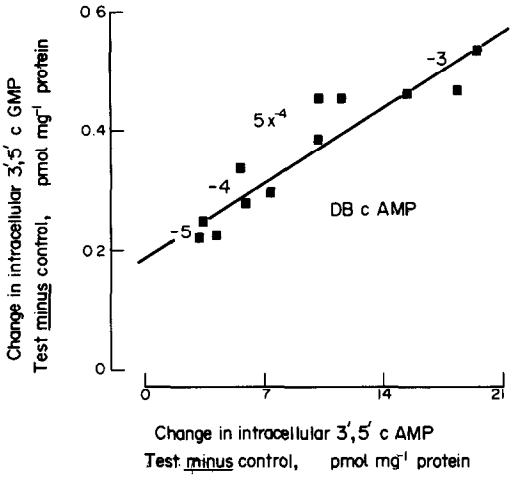

Fig. 4. Relationship between changes in endogenous 3',5'-cyclic AMP and 3',5'-cyclic GMP levels (treated ventricle – control ventricle) following treatment with dibutyryl 3',5'-cyclic AMP (10^{-5} to 10^{-3} M). The slope of the line relating the increases in intracellular 3',5'-cyclic AMP and 3',5'-cyclic GMP is fitted using regression analysis (slope of regression line ± S.E.: ±0.018 ± 0.002 pmole 3',5'-cyclic GMP·pmole^{-1} increase in 3',5'-cyclic AMP; $P < 0.001$, $n=12$). Control 3',5'-cyclic AMP and 3',5'-cyclic GMP levels given in legend to Fig. 3.

The changes in intracellular 3',5'-cyclic AMP and 3',5'-cyclic GMP (values obtained after treatment with dibutyryl 3',5'-cyclic AMP minus control levels) for ventricles exposed to different concentrations of dibutyryl 3',5'-cyclic AMP are shown in Fig. 4. The relationship approximates to a linear one, with a slope of around +0.02 pmole increase in 3',5'-cyclic GMP·pmole^{-1} increase in 3',5'-cyclic AMP (regression data: slope ± S.E.: = +0.018 ± 0.002 pmole 3',5'-cyclic GMP·pmole^{-1} increase in 3',5'-cyclic AMP; $n=12$; $P < 0.001$).

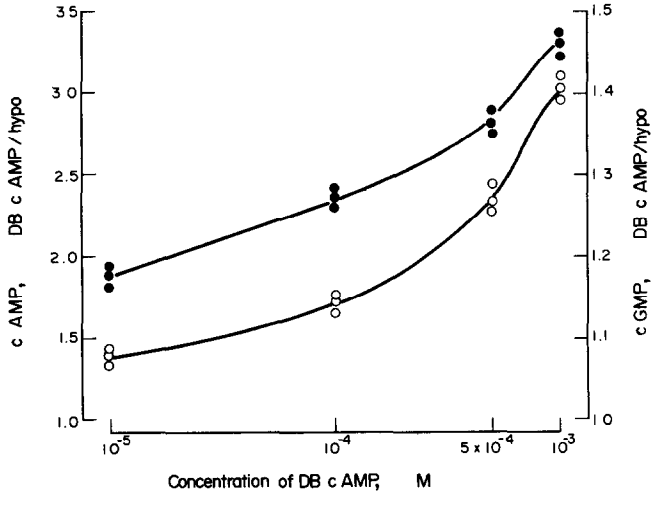

Fig. 3. Log-dose–response curves for concentrations of dibutyryl 3',5'-cyclic AMP ranging from 10^{-5} to 10^{-3} M. Open circles: 3',5'-cyclic AMP (left abscissa); filled circles: 3',5'-cyclic GMP (right abscissa). Preparations superfused for 25 min prior to freezing – clamping. Both parameters are expressed as multiples of control values. The latter were: 3',5'-cyclic AMP, 8.62 ± 0.17 pmoles·mg^{-1} protein, 3',5'-cyclic GMP, 1.17 ± 0.04 pmole·mg^{-1} protein ($n=12$).

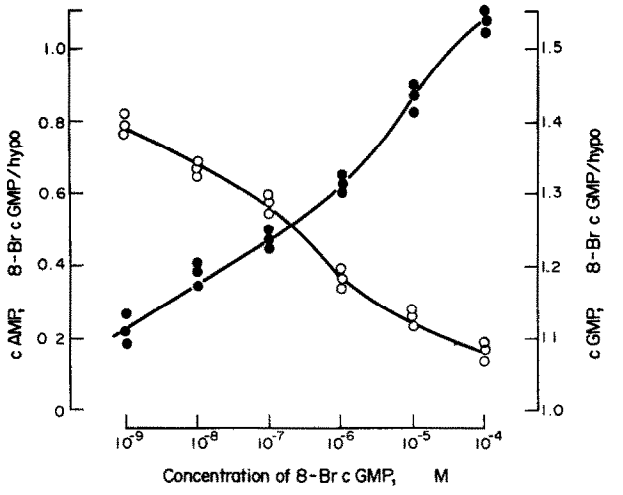

Fig. 5. Log-dose–response curves for changes in intracellular 3′,5′-cyclic AMP (open circles, left abscissa) and 3′,5′-cyclic GMP (filled circles, right abscissa) following stimulation with concentrations of 8-br 3′,5′-cyclic GMP ranging from 10^{-9} to 10^{-4} M. Preparations superfused for 25 min prior to freezing–clamping. Cyclic nucleotide levels expressed as multiples of control values. The latter were: 3′,5′-cyclic AMP, 8.24 ± 0.23 pmoles·mg^{-1} protein; 3′,5′-cyclic GMP, 1.22 ± 0.04 pmole·mg^{-1} protein (n=18).

The changes in 3′,5′-cyclic AMP and 3′,5′-cyclic GMP resulting from treatment with different concentrations of 8-br 3′,5′-cyclic GMP are shown in Fig. 5. There are two points to be emphasised here. First, 8-br 3′,5′-cyclic GMP produces a dose-related increase in 3′,5′-cyclic GMP levels (closed circles), as expected, but second (and more importantly) the entry of 8-br 3′,5′-cyclic GMP into the fibres is seen to be accompanied by a *decrease* in endogenous 3′,5′-cyclic AMP levels (open circles).

The inverse relationship between changes in 3′,5′-cyclic GMP and 3′,5′-cyclic AMP is depicted in Fig. 6. The changes in intracellular 3′,5′-cyclic nucleotides are expressed in the same way as those in Fig. 4; that is to say, the values shown are the levels observed following treatment with 8-br 3′,5′-cyclic GMP minus those found in control preparations. The important point to notice is that whereas dibutyryl 3′,5′-cyclic AMP had a comparatively small stimulatory effect on endogenous 3′,5′-cyclic GMP levels, 8-br 3′,5′-cyclic GMP had a much greater, depressant effect on endogenous 3′,5′-cyclic AMP levels. The results show that the effect of artificially raising 3′,5′-cyclic GMP on the levels of endogenous 3′,5′-cyclic AMP is around 600 times greater than the effect on endogenous 3′,5′-cyclic GMP which resulted from elevating intracellular 3′,5′-cyclic AMP. Thus, the regression analysis for the data appearing in Fig. 6 yields a slope of −10.79 ± 0.70 pmole 3′,5′-cyclic AMP·pmole^{-1} increase in 3′,5′-cyclic GMP (n = 18; P < 0.001).

The relationship between changes in intracellular 3′,5′-cyclic nucleotides and ventricular contractility

Earlier work [14–18] has established a clear correlation between changes in the contractile performance of the ventricle (ΔP) and changes in the relative proportion of 3′,5′-cyclic AMP : 3′,5′-cyclic GMP (ΔR) present in the fibres, where:

$$P = \frac{\text{tension produced by test half-ventricle}}{\text{tension produced by control half-ventricle}}$$

and

$$R = \frac{\text{3′,5′-cyclic AMP : 3′,5′-cyclic GMP (test half-ventricle)}}{\text{3′,5′-cyclic AMP : 3′,5′-cyclic GMP (control half-ventricle)}}$$

This relationship applied equally to agents which evoked positive or negative inotropic responses and to one, adenosine 5′triphosphate [14], which pro-

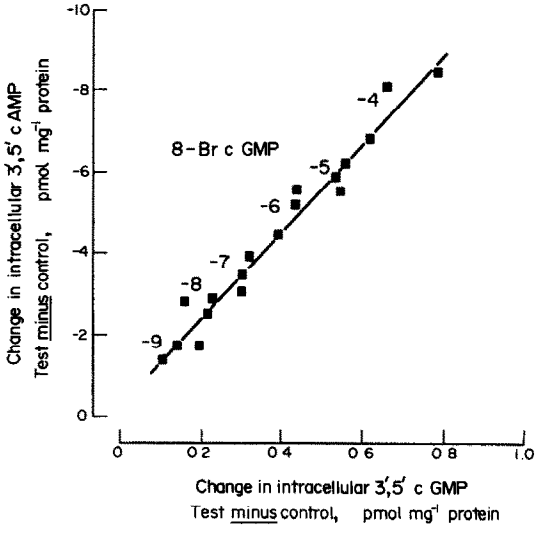

Fig. 6. Inverse relationship between changes in intracellular 3′,5′-cyclic AMP and 3′,5′-cyclic GMP (treated ventricle – control ventricle) following treatment with 8-br 3′,5′-cyclic GMP (10^{-9} to 10^{-4} M). The slope of the line relating the reduction in 3′,5′-cyclic AMP to the corresponding increase in 3′,5′-cyclic GMP is fitted by linear regression analysis (regression data: −10.79 ± 0.60 pmoles 3′,5′-cyclic AMP·pmole^{-1} 3′,5′-cyclic GMP; P < 0.001, n=18). Control 3′,5′-cyclic AMP and 3′,5′-cyclic GMP levels given in legend to Fig. 5.

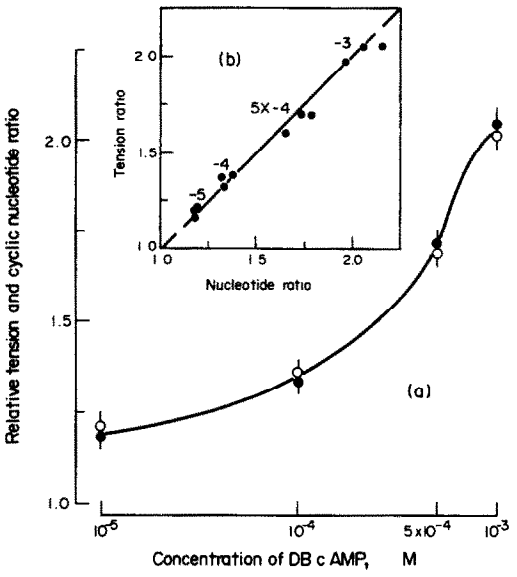

Fig. 7. (a) Changes in isometric force (open circles) and 3′,5′-cyclic nucleotide ratio (closed circles) for different concentrations of dibutyryl 3′,5′-cyclic AMP. Both parameters expressed as multiples of control values. Each point is mean value ± S.E. (n=3). (b) Relationship between isometric twitch tension and 3′,5′-cyclic nucleotide ratio for varying concentrations of dibutyryl 3′,5′-cyclic AMP (indicated by numbers beside each point). Correlation coefficient ± S.E.: 0.99 ± 0.05, 5=12, P < 0.001. Continuous line denotes a 1:1 correlation. Note that changes in twitch tension are closely paralleled by changes in the 3′,5′-cyclic nucleotide ratio.

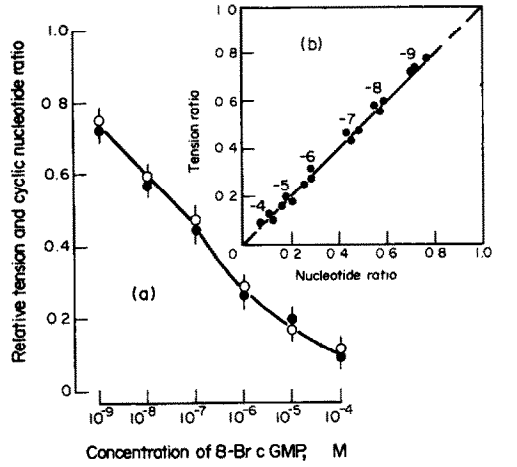

Fig. 8. (a) The effect of different concentrations of 8-br 3′,5′-cyclic GMP (10^{-9} to 10^{-4} M) on the steady-state contractile force (open circles) and 3′,5′-cyclic nucleotide ratio (filled circles). Each point is mean value ± S.E. (n=3). (b) Relation between isometric force and 3′,5′-cyclic nucleotide ratio (multiples of control values) for responses evoked by different concentrations of 8-br 3′,5′-cyclic GMP (indicated by numbers beside each point). Correlation coefficient ± S.E.: 0.99 ± 0.02, n=18, P < 0.001. Note that the decline in twitch tension is accompanied by a corresponding decrease in the ratio 3′,5′-cyclic AMP: 3′,5′-cyclic GMP. Continuous line drawn with a slope of 1.0.

duced a complex response, comprising both positive and negative components.

The same relationship is observed under the present experimental conditions, in which the levels of intracellular 3′,5′-cyclic nucleotides were manipulated directly. Figure 7(a) shows log-dose–response curves for the steady-state twitch tension (open circles) and the accompanying increase in the 3′,5′-cyclic nucleotide ratio (solid circles) elicited by different concentrations of dibutyryl 3′,5′-cyclic AMP. There is a close correlation between these two parameters (correlation coefficient ± S.E. : 0.99 ±0.05, n=12, P < 0.001), which is best illustrated by plotting the change in ventricular contractility as a function of the change in the 3′,5′-cyclic nucleotide ratio (Fig. 7(b)).

The decrease in the 3′,5′-cyclic nucleotide ratio (open circles), with varying concentrations of 8-br 3′,5′-cyclic GMP, and the corresponding fall in isometric twitch tension (solid circles), measured at the time of freeze-clamping each preparation, are shown in Fig. 8(a). The data again reveal a clear correlation (Fig. 8(b); correlation coefficient ± S.E.: 0.99 ± 0.02, n=18, P < 0.001) between changes in the relative proportion of 3′,5′-cyclic AMP : 3′,5′-cyclic GMP, and changes in contractile force.

DISCUSSION

It has been suggested that 3′,5′-cyclic AMP and 3′,5′-cyclic GMP act in a reciprocal manner to regulate the contractile performance of the heart, 3′,5′-cyclic AMP augmenting contraction and 3′,5′-cyclic GMP acting in an antagonistic fashion [6, 15, 20]. The results of recent experiments with the isolated frog ventricle, in which responses to a wide range of pharmacologically-different agents were studied [14–18], support this view, in so far as they reveal that changes in twitch amplitude are invariably accompanied by corresponding changes in the relative proportion of 3′,5′-cyclic AMP : 3′,5′-cyclic GMP present in the fibres. These observations can of course be interpreted in a different way. The effects on the twitch may be causally related to the changes in 3′,5′-cyclic AMP and 3′,5′-cyclic GMP, but an equally plausible explanation is that the two effects arise from a common cause, in which case they are themselves only incidentally related.

The aim in making the present experiments was to see if manipulating the levels of 3′,5′-cyclic AMP and 3′,5′-cyclic GMP directly, by superfusing preparations with their more permeant analogues, would result in the appropriate change in contractility, and also reveal a similar correlation between changes in twitch tension and changes in 3′,5′-cyclic nucleotide ratio. The results which have been obtained affirm both of these points: qualitatively, the effects of dibutyryl 3′,5′-cyclic AMP and of 8-br 3′,5′-cyclic GMP on the twitch are in the direction to be anticipated from our earlier studies; and quantitatively, they show that the same relationship exists between changes in ventricular contractility and changes in the ratio 3′,5′-cyclic AMP : 3′,5′-cyclic GMP. These observations must, however, be interpreted cautiously, because it has been shown that dibutyryl 3′,5′-cyclic AMP [28, 29] and 8 br 3′,5′-cyclic GMP

[21] affect the action potential, in a way which suggests that they are able to modulate Ca^{2+} entry into the fibres during activation. This is an important point to mention, since Ca^{2+} can markedly influence 3′,5′-cyclic nucleotide metabolism by activating the Ca^{2+}-binding modulator protein, calmodulin [30]. What is not clear from the literature is whether the reported effects on the action potential precede any changes in 3′,5′-cyclic nucleotide levels, or whether they lag behind them. The crucial experiments do not appear to have been made, and until this point is clarified, it is reasonable to accept the principal finding at face value, as being indicative of a direct involvement of both 3′,5′-cyclic nucleotides in regulating contractility, at least for the time being.

The evidence concerning the apparent depressant effect of 3′,5′-cyclic GMP on 3′,5′-cyclic AMP levels is important, because it suggests one means whereby 3′,5′-cyclic GMP could exert its antagonistic effects on the twitch—namely by regulating 3′,5′-cyclic AMP metabolism, and thereby influencing the rate at which 3′,5′-cyclic AMP-dependent protein kinases phosphorylate regulatory proteins. The possibility that such a mechanism exists was raised earlier, following an investigation into the effects of ATP on the frog ventricle [14]. The results obtained suggested that an early, abrupt increase in 3′,5′-cyclic AMP levels, observed shortly after commencing treatment with exogenous ATP, was terminated by the intervention of a somewhat slower and delayed increase in 3′,5′-cyclic GMP levels. We drew attention at the time [14] to two important features concerning the relationship between the time course of these changes: first, that the maximum rate of fall of 3′,5′-cyclic AMP coincided with the peak increase in 3′,5′-cyclic GMP levels; and second, that when the latter eventually subsided, 3′,5′-cyclic AMP levels again rose.

The existence of a 3′,5′-cyclic GMP-dependent mechanism for regulating 3′,5′-cyclic AMP levels has some interesting implications. It is easy to see, for example, that agents which temporarily lower 3′,5′-cyclic GMP would remove (or at least reduce) its depressant effect on 3′,5′-cyclic AMP levels; if this were to coincide with a drug-induced stimulation of adenylate cyclase activity, then it would serve to facilitate the production of 3′,5′-cyclic AMP. In fact, three responses that we have studied recently (refs. 15 and 16, and unpublished experiments with adrenaline) show this feature: the early increase in 3′,5′-cyclic AMP was accompanied by an initial, transient fall in 3′,5′-cyclic GMP levels.

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