Biochemical Pharmacology, Vol. 28, pp. 2601–2606
© Pergamon Press Ltd. 1979. Printed in Great Britain.
0006-2952/79/0901-2601 $02.00/0

VERAPAMIL-INDUCED CHANGES IN MYOCARDIAL CONTRACTILE FORCE AND CYCLIC NUCLEOTIDES IN THE ISOLATED PERFUSED RAT HEART*

ARTHUR A. HANCOCK AND MARILYN E. HESS†

Department of Pharmacology, University of Pennsylvania, School of Medicine, Philadelphia, PA 19104, U.S.A.

(*Received* 10 *July* 1978; *accepted* 1 *March* 1979)

Abstract—Interrelationships among transmembrane flux of calcium ions, cyclic nucleotides, and myocardial contractile force in isolated, perfused rat hearts were studied with verapamil, lanthanum, or zero-calcium solutions to reduce the influx of calcium ions into myocardial cells. Myocardial concentrations of cyclic AMP and cyclic GMP were determined, and changes in contractile force were measured. Perfusion with verapamil, lanthanum, or zero-calcium solutions produced a dose-related decrease in myocardial contractile force and reduced cyclic AMP to levels significantly lower than in control hearts. The reduction in cyclic AMP was independent of the amount of drug used to inhibit calcium influx. Verapamil (6×10^{-8} M) or lanthanum (4×10^{-7} M or 4×10^{-6} M) had no effect on the concentration of cyclic GMP. Zero-calcium solution or verapamil (6×10^{-7} M) decreased significantly myocardial cyclic GMP. 8-Bromo-cyclic GMP (in the presence of inorganic phosphate) and dibutyryl cyclic AMP caused a positive inotropic response which was not inhibited by sotalol. Only dibutyryl cyclic AMP reversed the negative inotropic effect of verapamil. Dibutyryl cyclic GMP alone produced a negative inotropic effect and further depressed the force of contraction induced by verapamil. Since two analogs of cyclic GMP had opposite effects on myocardial contractility, it was concluded that analogs do not necessarily mimic actions ascribed to endogenous parent nucleotides. Although the transmembrane flux of calcium ions remains an important factor controlling myocardial contractile force, the direct mechanism by which cyclic nucleotides may modulate calcium flux in the heart remains unknown.

The relationship between the flux of calcium ions into the myocardial cell and the development of contractile force is well established [1, 2]. However, the control of calcium influx and its mobilization within the cell are less clearly understood. One hypothesis links the positive inotropic effect of catecholamines to the increased generation of cyclic AMP produced by these amines [3]. Several related theories attempt to explain the manner by which cyclic AMP modulates myocardial contractility. For example, it has been suggested that: cyclic AMP promotes the phosphorylation of a protein in the sarcoplasmic reticulum, which subsequently causes an increase in calcium sequestration by the reticulum [4]; the cyclic nucleotide mediates an increase in transmembrane calcium currents [5] and enhances calcium stores [6]. Although there is clear evidence for a correlation between the movement of calcium ions and tissue concentrations of cyclic AMP [7], the exact nature of the underlying events which ultimately result in increased contractile force remains under investigation.

Several aspects of the relationship between cardiac cyclic nucleotides and modifications in myocardial contractility cannot be accounted for by most current models. For instance, the mechanism by which the increase in contractile force is elicited in response to the administration of catecholamines with no elevation in the cyclic AMP in the heart [8–11] remains to be clarified. In addition, the possible role of cyclic GMP as a modulator of contractile force [12–14] has yet to be fully defined.

In our laboratories, Hess [15] and Shanfield *et al.* [16] have investigated the interaction between calcium and cyclic AMP by changing the transmembrane calcium influx with verapamil, a known calcium antagonist [17]. Hess [15] reported that verapamil caused a dose-dependent diminution in the myocardial contractile force of the isolated perfused rat heart, and a decrease in cyclic AMP which was not related to the concentration of drug in the perfusion fluid. In addition, when isolated hearts were perfused with verapamil for prolonged periods of time, contractility returned almost to normal, but the depression in cardial cyclic AMP persisted throughout the duration of verapamil administration [16]. On the basis of these observations, it seemed appropriate to investigate the effect of verapamil on the concentration of cyclic GMP in the heart since from previous reports [14] this nucleotide might be expected to be linked more directly to the negative inotropic action of drugs. As a corollary to this study, we investigated the effects of lanthanum, another calcium antagonist [18], and perfusion with zero-calcium solution on cardiac cyclic nucleotides. In conjunction with these experiments, hearts were perfused with analogs of cyclic AMP and cyclic GMP to evaluate the role of these nucleotides in modulating myocardial contractility at a time when calcium influx was reduced by verapamil. We hypothesized that cyclic AMP analogs, because of their putative inotropic effect, might enhance the transmembrane calcium influx and antagonize the effect of verapamil, whereas the opposite might be expected from a cyclic GMP analog.

* This work was supported by grants from the National Institutes of Health, U.S. Public Health Service 1 R01 HL 16436-03 and GM 07302-02.

† Please address reprint requests to: Marilyn E. Hess, Ph.D., Department of Pharmacology, University of Pennsylvania, School of Medicine/G3, 36th and Hamilton Walk, Philadelphia, PA 19104, U.S.A.

MATERIALS AND METHODS

Perfusion of hearts. Male, albino Wistar rats (200–300 g), fed *ad lib.* with Purina lab chow, were decapitated. The hearts were perfused as described previously [16]. Perfusion solutions were buffered either with $NaHCO_3$ plus KH_2PO_4, or with HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid from CalBiochem, La Jolla, CA). HEPES was used in order to avoid precipitation of lanthanum carbonate or lanthanum phosphate. Perfusion fluids (warmed to 37.5°) were equilibrated with 95% oxygen and 5% carbon dioxide or with 100% oxygen in those experiments in which HEPES was the buffer. The solutions were of the following compositions: (1) Krebs–Ringer–bicarbonate solution (K–R solution): NaCl, 119 mM; KCl, 4.75 mM; $CaCl_2 \cdot 2H_2O$, 2.54 mM; KH_2PO_4, 1.19 mM; $MgSO_4 \cdot 7H_2O$, 1.19 mM; $NaHCO_3$, 25 mM; and glucose, 5.5 mM; (2) HEPES solution: NaCl, 141.16 mM; KCl, 5.94 mM; $CaCl_2 \cdot 2H_2O$, 2.54 mM; $MgSO_4 \cdot 7H_2O$, 1.19 mM; HEPES, 3.0 mM; and glucose 5.5 mM. In some experiments, zero-calcium K–R solution was used and NaCl was increased to 122.81 mM to maintain osmolarity. Several hearts were perfused with a HEPES solution containing phosphate anions in which the KCl concentration was reduced to 4.75 mM and KH_2PO_4 was added to give a final concentration of 1.19 mM.

The Langendorff apparatus was equipped with a three-way stopcock, which allowed the rapid change of perfusion medium from control to solution containing appropriate drugs. Calcium influx into myocardial cells was reduced by perfusing hearts either with solutions containing verapamil or lanthanum or with zero-calcium medium. Several analogs of endogenous cyclic nucleotides were perfused in the presence or absence of verapamil. These analogs were dibutyryl cyclic AMP, dibutyryl cyclic GMP, and 8-bromo-cyclic GMP. At the end of the experiment, the hearts were frozen with aluminum Wollenberger tongs precooled in liquid nitrogen and the tissues were stored in liquid nitrogen. Tissue analysis for the myocardial concentration of cyclic nucleotides was performed within 2 weeks following the experiment.

Myocardial force of contraction was measured as described previously [16] and changes were recorded on a Sanborn oscillograph calibrated so that a one mm pen deflection resulted from 1 g of developed tension. In some experiments, heart rate was controlled by stimulating the right ventricle with stainless steel electrodes at a rate of 5 Hz with a Grass model S44 stimulator, which generated square wave pulses of 1-msec duration and 3V amplitude (approximately 1V greater than threshold).

Myocardial water content and extracellular space were measured in one series of experiments with mannitol [^{3}H] according to the method of Randle and Smith [19]. Mannitol [^{3}H], used to determine extracellular space, was measured in extracts of hearts (digested in 0.1 N HCl) perfused with mannitol [^{3}H] at a concentration of 0.005 μCi/ml for the final five minutes of the experiment.

Cyclic nucleotide assay. The tissue content of cyclic AMP was measured according to the method of Gilman [20] with minor modifications suggested by Shanfeld *et al.* [16]. Cyclic GMP was assayed according to a radioimmunoassay technique [21], as modified by Frandsen and Krishna [22]. Tissue content of the nucleotides is expressed as pmoles cyclic AMP or fmoles cyclic GMP/mg of tissue wet weight.

Drugs. Cyclic AMP, cyclic GMP, dibutyryl cyclic AMP, dibutyryl cyclic GMP and 8-bromo-cyclic GMP were purchased from the Sigma Chemical Co., St. Louis, MO. Cyclic AMP[^{3}H] was obtained from Schwartz-Mann, Orangeburg, NY, and cyclic GMP[^{3}H] from ICN Pharmaceuticals, Irvine, CA. Mannitol[^{3}H] was purchased from New England Nuclear, Boston, MA. Sotalol hydrochloride was obtained from the Regis Chemical Co., Chicago, IL. Cyclic GMP antiserum and cyclic GMP[^{125}I] were obtained from Collaborative Research, Waltham, MA. Verapamil hydrochloride was generously given by Knoll A-G., Chemische Fabriken, Ludwigshafen am Rhein, Germany.

Statistical analysis. Data were subjected to either one-way or two-way analysis of variance [23]. A P value of less than 0.05 was accepted as the critical level of significance.

RESULTS

Effect of perfusion with verapamil or zero-calcium solution on myocardial contractile force, cardiac cyclic AMP and cyclic GMP. Two concentrations (6×10^{-8} and 6×10^{-7} M) of the calcium antagonist, verapamil, were administered to perfused rat hearts. At the lower concentration, verapamil decreased contractile force by 40 percent in 4–5 min. The 10-fold higher dose of verapamil caused a more rapid and marked depression

Table 1. Effects of perfusion with verapamil and zero-calcium solutions on contractile force (CF) and cyclic nucleotides in perfused rat hearts*

	Pretreatment CF (g)	Changes in CF (g)	Cyclic AMP (pmoles/mg)	Cyclic GMP (fmoles/mg)	Cyclic AMP / Cyclic GMP
Controls (K–R)† (36)	11.9 ± 0.40	−0.21 ± 0.10	0.77 ± 0.02	21.9 ± 0.90	39.2 ± 2.00
Verapamil, 6×10^{-8} M (11)	12.3 ± 0.80	−4.9 ± 0.43‡	0.68 ± 0.02‡	21.5 ± 1.7	33.1 ± 2.4
Verapamil, 6×10^{-7} M (7)	12.7 ± 0.6	−10.1 ± 0.6‡, §	0.59 ± 0.04‡	14.8 ± 1.0‡, §	40.9 ± 3.9
Zero-calcium (9)	12.7 ± 0.7	−12.3 ± 0.7‡, §, ‖	0.63 ± 0.03‡	17.0 ± 1.5‡	38.7 ± 2.9

* Data are presented as means ± S.E.M. Numerals enclosed in parentheses indicate the number of hearts in each group.
† K–R = Krebs–Ringer–bicarbonate solution.
‡ Significantly different from controls.
§ Significantly different from verapamil (6×10^{-8} M).
‖ Significantly different from verapamil (6×10^{-7} M).

of tension development. Within 2–3 min, the contractile force of these hearts had decreased by 80 percent. Neither concentration of verapamil produced a significant alteration in coronary flow. Perfusion with zero-calcium K–R solution caused nearly complete loss of contractile force within 15–30 sec. These results are presented in Table 1.

When the decrease in contractile force caused by verapamil or the calcium-free solution reached a maximum, the hearts were frozen immediately for subsequent assay of nucleotides. Verapamil, at either concentration, reduced the cardiac concentration of cyclic AMP significantly, but not in a dose-related manner (Table 1). These results are in agreement with those reported by Hess and Gabel [24]. Using a wide dose range of verapamil, these investigators found that the decrease in cardiac cyclic AMP caused by verapamil, unlike the negative inotropic action of the drug, was independent of the concentration of verapamil in the fluid perfusing the heart. Our data are expressed as pmoles of cyclic AMP/mg of tissue wet weight in order to facilitate comparison with values reported previously by other investigators [25] in similar studies and because we found that verapamil, perfused at a concentration of 6×10^{-8} M, did not significantly alter coronary flow, tissue water content or extracellular space, when measured with mannitol[^{3}H].

When verapamil (6×10^{-7} M) was used, there was a slight but insignificant increase in coronary flow from 8.1 ± 0.59 ml/min to 9.4 ± 0.42 ml/min ($N = 7$). In these experiments in which the higher concentration of verapamil was used, tissue water content and extracellular space (mannitol [^{3}H] space) were not determined. Therefore, the possibility exists that, with 6×10^{-7} M verapamil in the perfusion fluid, a slight increase in cell water may have occurred, thereby making a small contribution to the large reduction in cyclic AMP that we measured in these hearts. Perfusion with zero-calcium medium also reduced cyclic AMP to approximately the same degree as verapamil at either concentration.

Verapamil (6×10^{-8} M) had no effect on the concentration of cardiac cyclic GMP. A higher dose of verapamil (6×10^{-7} M) or perfusion with zero-calcium K–R solution reduced the cyclic GMP concentration significantly (Table 1). Neither perfusion with verapamil solution nor perfusion with zero-calcium medium had a significant effect on the ratio of cyclic AMP to cyclic GMP.

Effect of perfusion with lanthanum on myocardial contractile force, cardiac cyclic AMP and cyclic GMP. Control hearts perfused with the HEPES-buffered solution had a somewhat lower initial contractile force after equilibration (Table 2) than those hearts perfused with K–R solution (Table 1). However, force of contraction in the former was equally stable during the subsequent period of perfusion when no drugs were administered.

Lanthanum ions, at a concentration of 4×10^{-7} M, decreased the contractile force of the heart by 66 percent in 2.5 to 3.5 min (Table 2). A 10-fold increment in the concentration of lanthanum (4×10^{-6} M) caused the developed tension of the heart to fall to less than 1 g (greater than 90 percent loss of force) in 30–45 sec.

Lanthanum, like verapamil and zero-calcium K–R solution, caused a reduction of myocardial cyclic AMP, and, like verapamil, lanthanum-induced depression in the nucleotide was not dose-dependent (Table 2). Unlike verapamil, perfusion with either 4×10^{-7} M or 4×10^{-6} M lanthanum, although causing a severe reduction in the force of contraction, had no effect on myocardial cyclic GMP (Table 2). Neither concentration of lanthanum changed significantly the ratio of cyclic AMP to cyclic GMP (Table 2).

Effect of prolonged perfusion with verapamil in the absence or presence of cyclic nucleotide analogs. The positive inotropic effect of dibutyryl cyclic AMP [26] has been ascribed to its ability to enhance transmembrane calcium flux [27, 28]. If endogenous cyclic nucleotides modulate calcium mobilization in cells [7], then a model for the role played by these nucleotides might be found in studying the effects of synthetic nucleotide analogs on cardiac contractile force [29]. The observation that dibutyryl cyclic GMP caused decreased myocardial contractility [30–33] has interesting implications not only for the role of this nucleotide in modulating calcium flux, but also in terms of the Yin-Yang hypothesis [14]. We perfused hearts simultaneously with verapamil and one of several cyclic nucleotide analogs, expecting to observe antagonisms of the depressant effects of verapamil on force of contraction with cyclic AMP analogs, and augumentation of the verapamil-induced negative inotropic effect with cyclic GMP analogs. Hearts were perfused with the HEPES-buffered solution of cyclic nucleotide analogs for at least 15 min to allow sufficient time for the development of inotropic changes. In addition, heart rate was controlled as described in Materials and Methods. This variation in procedure resulted in a more constant depression of contractile force with verapamil (Fig. 1), rather than the transient reduction of force previously reported [16].

Dibutyryl cyclic AMP (5×10^{-4} M) alone produced a significant increase in the development of tension in

Table 2. Effects of perfusion with lanthanum on contractile force (CF) and cyclic nucleotides in perfused rat hearts*

	Pretreatment CF (g)	Changes in CF (g)	Cyclic AMP (pmoles/mg)	Cyclic GMP (fmoles/mg)	Cyclic AMP / Cyclic GMP
Controls (HEPES) (11)	9.5 ± 0.72	-0.93 ± 0.23	0.73 ± 0.03	23.7 ± 1.2	31.6 ± 2.1
Lanthanum, 4×10^{-7} M (12)	8.9 ± 0.78	-5.9 ± 0.60†	0.63 ± 0.03†	21.7 ± 1.9	31.3 ± 2.6
Lanthanum, 4×10^{-6} M (13)	10.0 ± 0.66	-9.2 ± 0.64†,‡	0.64 ± 0.03†	21.0 ± 1.5	32.8 ± 3.0

* Data are presented as means ± S.E.M. Numerals enclosed in parentheses indicate the number of hearts in each group.
† Significantly different from controls.
‡ Significantly different from Lanthanum (4×10^{-7} M).

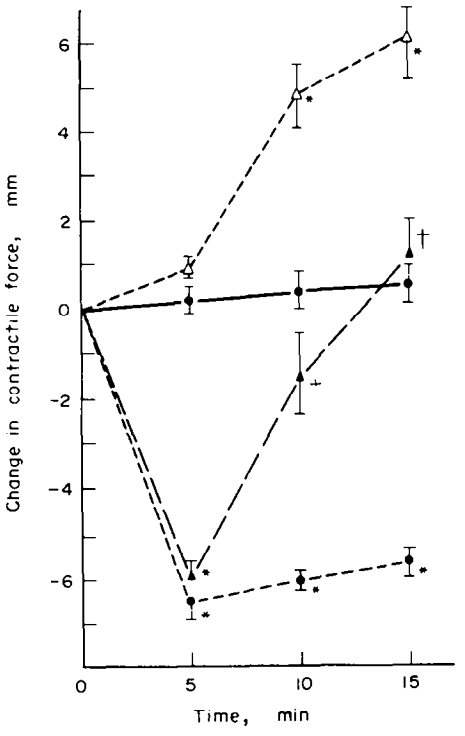

Fig. 1. Effects of perfusion with verapamil, dibutyryl cyclic AMP, or the combination of both drugs in HEPES-buffered solution on contractile force in paced, isolated rat hearts. Key: (● ——●) controls in HEPES (n = 9); (△ – – – △) dibutyryl cyclic AMP (5×10^{-4} M) in HEPES (n = 8); (● – · – ●) verapamil (6×10^{-8} M) in HEPES (n = 6); and (▲ – – – ▲) Verapamil (6×10^{-8} M) plus dibutyryl cyclic AMP (5×10^{-4} M) in HEPES (n = 9). The number of animals per experimental group is indicated by n. Vertical bars indicate the S.E.M. All drugs were given beginning at time 0. The asterisk (*) indicates significantly different from corresponding control. The single dagger (†) indicates significantly different from verapamil.

the heart (Fig. 1). When hearts were perfused with both verapamil and dibutyryl cyclic AMP, the nucleotide analog did not alter the initial negative inotropic effect of the calcium antagonist. However, in the presence of dibutyryl cyclic AMP, there was a rapid reversal of the depression in contractile force caused by verapamil (Fig. 1). By the end of the 15-min perfusion period with both agents, contractile force had recovered to control.

In a second series of experiments, the hearts were perfused with two analogs of cyclic GMP in a manner similar to that described above. A preliminary group of experiments using 8-bromo-cyclic GMP in K–R solution revealed that at concentrations between 1×10^{-10} M and 1×10^{-5} M, this analog had no effect on myocardial contractile force in the isolated, perfused rat heart. At a concentration of 1×10^{-4} M in K–R solution, however, a positive inotropic effect was observed that was not reduced by simultaneous infusion of sotalol (1×10^{-3} M, data not shown).

In hearts perfused with 8-bromo-cyclic GMP in HEPES solution, this increase in contractile force could not be demonstrated (even with five times the concentration). When 8-bromo-cyclic GMP was perfused with HEPES fluid to which phosphate ions had been added to give the same final concentration as contained in the K–R medium, the positive inotropic effect of the nucleotide was again demonstrable and approximated that observed with dibutyryl cyclic AMP. Unlike the latter analog, however, 8-bromo-cyclic GMP did not reverse the depression of myocardial contractile force caused by verapamil (Fig. 2) or, to express it another way, the positive inotropic effect of 8-bromo-cyclic GMP was not elicited in the presence of verapamil. A further difference between 8-bromo-cyclic GMP and dibutyryl cyclic AMP was that the latter analog did not require phosphate anions in the HEPES solution in order for the positive inotropic effect caused by the nucleotide to occur.

A second analog of cyclic GMP (dibutyryl cyclic GMP) was used in additional experiments. This form of cyclic GMP did not affect contractile force when perfused at concentrations between 1×10^{-6} M and 5×10^{-5} M in phosphate-containing HEPES solution (data not shown). However, at a dose of 1×10^{-4} M, dibutyryl cyclic GMP, unlike the 8-bromo-cyclic GMP derivative, caused a significant decrease in myocardial contractile force (Fig. 3). In combination with verapamil (6×10^{-8} M), dibutyryl cyclic GMP augmented the decline in contractile force caused by verapamil (Fig. 3).

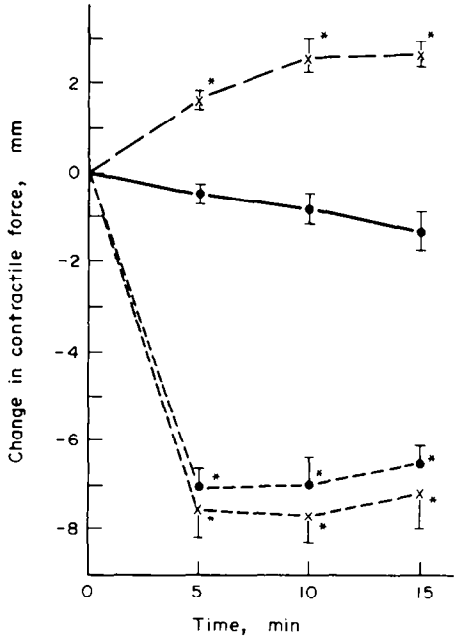

Fig. 2. Effects of perfusion with verapamil, 8-bromo-cyclic GMP, or the combination of both drugs on contractile force in HEPES-phosphate (HR–P) in paced, isolated rat hearts. Key: (●——●) controls in HR–P (n = 7); (X – – – X) 8-bromo-cyclic GMP (1×10^{-4} M) in HR–P (n = 6); (● – – – ●) verapamil (6×10^{-8} M) in HR–P (n = 5); and (X – – – X) verapamil (6×10^{-8} M) plus 8-bromo-cyclic GMP (1×10^{-4} M) in HR–P (n = 5). The number of animals per experimental group is indicated by n. Vertical bars represent the S.E.M. All drugs were given beginning at time 0. The asterisk (*) indicates significanly different from corresponding control.

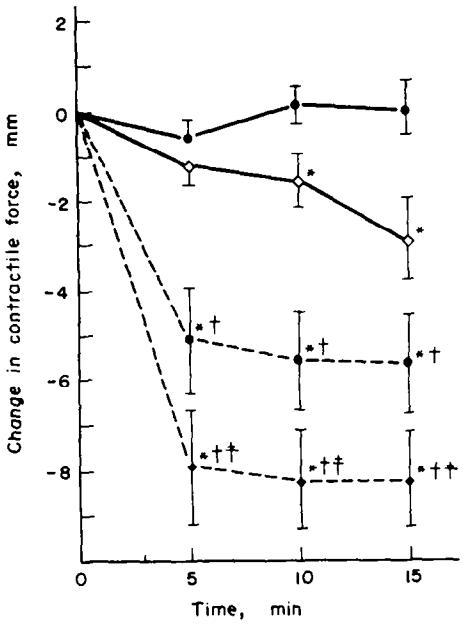

Fig. 3. Effects of perfusion with verapamil, dibutyryl cyclic GMP, or the combination of both drugs in HEPES-phosphate (HR–P) on contractile force in paced, isolated rat hearts. Key: (●——●) controls in HR–P (n = 6); (● – – – ●) verapamil (6×10^{-8} M) in HR–P (n = 4); (◇——◇) dibutyryl cyclic GMP (1×10^{-4} M) in HR–P (n = 4); and (◆ – – – ◆) verapamil (6×10^{-8} M) plus dibutyryl cyclic GMP (1×10^{-4} M) in HR–P (n = 3). The number of animals per experimental group is indicated by n. Vertical bars indicate the S.E.M. All drugs were given beginning at time 0. The asterisk (*) indicates significantly different from corresponding control. The single dagger (†) indicates significantly different from dibutyryl cyclic GMP. The double dagger (‡) indicates significantly different from verapamil.

DISCUSSION

The results of our experiments clearly revealed that in the rat heart no simple relationship exists between the negative inotropic effect caused by the inhibition of transmembrane calcium influx and the changes in the concentrations or ratios of cardiac cyclic nucleotides. For example, perfusing rat hearts with zero-calcium solution completely inhibited the development of contractile force within 30 sec. However, the depression of myocardial cyclic AMP, albeit significant, was no greater than the decrease in the nucleotide caused by the various doses of calcium antagonists which reduced myocardial tension development in proportion to the dose perfused. Although no attempt was made to measure shifts in the intracellular locations of the cyclic nucleotides, in no instance did the perfusion of hearts with calcium antagonists raise the concentration of cyclic GMP when cyclic AMP was decreased. Therefore, we find no support for the applicability of the Yin-Yang hypothesis to the control of myocardial contractile force under conditions where calcium influx is reduced.

The results obtained when we perfused hearts with zero-calcium solution differ from those described by several other investigators [34–37]. In previously reported studies, perfusion with zero-calcium was associated with increased myocardial concentrations of cyclic AMP. These elevations in cardiac cyclic AMP were observed in hearts which had been perfused with zero-calcium medium for periods of 30 min [34–37]. In contrast, the hearts used in our experiments were exposed to zero-calcium solution for less than 30 sec.

In a different study with D600, a more potent analog of verapamil [17], an enhancement of the isoproterenol-induced increase in cyclic AMP was measured after the administration of D600 to noncontracting hearts depolarized with 22 mM external potassium [38]. In the same study [38], D600 administered alone to hearts depolarized by perfusion with high potassium solution did not increase myocardial cyclic AMP. Results from our experiments showed a reduction in heart cyclic AMP with perfusion of calcium antagonists (verapamil or lanthanum) or with zero-calcium medium.

Since there was a difference in the species from which we obtained the hearts, as well as variations in tissue preparation, it is difficult to attribute the divergence of our results from those of other investigators to a single factor. It should be emphasized that the concentrations of the calcium antagonist agents we used were considerably lower than those employed in other laboratories. Because we used such small amounts of verapamil and lanthanum and because the hearts were exposed to these drugs for very short periods, we believe that our results are due to direct effects of reduced availability of calcium to the contractile apparatus and the cyclic nucleotide systems rather than to secondary effects resulting from altered membrane potential, cellular pH or cell damage. This hypothesis is supported by the observation that the effects of calcium antagonists can be rapidly and completely reversed by increasing the concentration of calcium in the perfusion fluid [17, 24] or by administering a catecholamine [16, 17].

Robison *et al.* [29] originally suggested several criteria which would establish the role of cyclic nucleotides in cellular processes. One of these was the proposal that synthetic analogs of the cyclic nucleotides could serve as models for the postulated action of the endogenous nucleotide. Since previous investigators [4–6] provided data which supported the concept that increases in cyclic AMP are associated with the positive inotropic effect of drugs, while elevations in cyclic GMP are more likely to be observed with decreases in force of contraction [12, 14], our results with the dibutyryl analogs of these nucleotides are not unexpected. Administered alone, dibutyryl cyclic AMP increased, and dibutyryl cyclic GMP decreased, myocardial contractility. When perfused with verapamil, dibutyryl cyclic GMP acted synergistically to depress further myocardial contractile force, whereas the effect of verapamil on force contraction was reversed by dubutyryl cyclic AMP.

However, 8-bromo-cyclic GMP appears to possess unique characteristics. Although a cyclic GMP analog, it produced an unanticipated positive inotropic effect. This observation does not support the proposal that analogs usually produce results similar to those of the parent endogenous nucleotide. The unusual nature of 8-bromo-cyclic GMP to increase force of contraction is further demonstrated by the necessity for phosphate anions in the perfusion fluid in order for the development of this response. Since phosphate ions are known to play a role in calcium sequestration by sarcoplasmic

reticulum [39] and mitochondria [40], it is possible that 8-bromo-cyclic GMP may act in combination with phosphate ions to increase calcium stores in one of both of these organelles, thereby enhancing myocardial contractility.

In summary, we believe that our experiments shed new light on the underlying complexity of the development of myocardial contractility and its modification by pharmacological agents. Clearly, the calcium ion is of primary importance to the development of tension. Recent experiments [5, 6] have shown that cyclic AMP probably modulates calcium availability by altering the transmembrane flux of this ion. However, the importance of cyclic GMP, if any, in regulating myocardial contractile force remains unclear.

REFERENCES

1. A. Grossman and R. F. Furchgott, *J. Pharmac. exp. Ther.* **143**, 120 (1964).
2. A. Grossman and R. F. Furchgott, *J. Pharmac. exp. Ther.* **145**, 162 (1964).
3. E. W. Sutherland, I. Oye and R. W. Butcher, *Recent Prog. Horm. Res.* **21**, 623 (1965).
4. M. A. Kirchberger, M. Tada, D. I. Repke and A. M. Katz, *J. Molec. cell. Cardiol.* **4**, 673 (1972).
5. H. Reuter, *J. Physiol., Lond.* **242**, 429 (1974).
6. H. Reuter, *Rec. Adv. Stud. Cardiac Struct. Metab.* **5**, 13 (1975).
7. H. Rasmussen and D. B. P. Goodman, *Physiol. Rev.* **57**, 421 (1977).
8. J. Shanfeld, A. Frazer and M. E. Hess, *J. Pharmac. exp. Ther.* **169**, 315 (1969).
9. A. Langslet and I. Oye, *Eur. J. Pharmac.* **12**, 137 (1970).
10. B. G. Benfey, *Br. J. Pharmac.* **43**, 757 (1971).
11. E. H. Hu and J. C. Venter, *Molec. Pharmac.* **14**, 237 (1978).
12. W. J. George, J. B. Polson, A. C. O'Toole and N. D. Goldberg, *Proc. natn. Acad. Sci. U.S.A.* **66**, 398 (1970).
13. A. Wollenberger, E. B. Babskii, E. G. Krause, S. Genz, D. Blohm and E. V. Bogdanova, *Biochem. biophys. Res. Commun.* **55**, 446 (1973).
14. N. D. Goldberg, M. K. Haddox, D. K. Hartle and J. W. Hadden, *Proc. Fifth Int. Congr. Pharmac.* **5**, 146 (Eds. R. A. Maxwell and G. H. Acheson), Karger, Basel (1973).
15. M. E. Hess, *Sixth Int. Congr. Pharmac.* (Abstr.) p. 226 (1975).
16. J. Shanfeld, M. E. Hess and N. R. Levine, *J. Pharmac. exp. Ther.* **193**, 317 (1975).
17. A. Fleckenstein, in *Calcium and the Heart* (Eds. P. Harris and L. Opie), p. 135. Academic Press, New York (1971).
18. G. A. Langer, S. D. Serena and L. M. Nudd, *Am. J. Physiol.* **229**, 1003 (1975).
19. P. J. Randle and G. H. Smith, *Biochem. J.* **70**, 501 (1958).
20. A. G. Gilman, in *Advances in Cyclic Nucleotide Research* (Eds. P. Greengard and G. A. Robison), p. 9. Raven Press, New York (1972).
21. A. L. Steiner, R. E. Wehrmann, C. W. Parker and D. M. Kipnis, in *Advances in Cyclic Nucleotide Research* (Eds. P. Greenard and G. A. Robison), p. 51. Raven Press, New York (1972).
22. E. K. Frandsen and G. Krishna, *Life Sci.* **18**, 529 (1976).
23. G. W. Snedecor and W. G. Cochran, *Statistical Methods*, p. 258. Iowa State University Press, Ames, IA. (1967).
24. M. E. Hess and B. E. Gabel, *Cardiology* **64**, 75 (1979).
25. A. M. Watanabe and H. R. Besch Jr., *J. Pharmac. exp. Ther.* **191**, 241 (1974).
26. W. R. Kukovetz and G. Poch, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **266**, 236 (1970).
27. R. W. Tsien, W. Giles and P. Greengard, *Nature New Biol.* **240**, 181 (1972).
28. T. Meinertz, H. Nawrath and H. Scholz, *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **279**, 327 (1973).
29. G. A. Robison, R. W. Butcher and E. W. Sutherland, in *Cyclic AMP*, p. 44. Academic Press, New York (1971).
30. A. M. Watanabe and H. R. Besch Jr., *Circulation Res* **37**, 309 (1975).
31. K. Goshima, *J. Molec. cell Cardiol.* **8**, 713 (1976).
32. R. D. Wilkerson, R. J. Paddock and W. J. George, *Eur. J. Pharmac.* **36**, 247 (1976).
33. H. Nawrath, *Nature, Lond.* **262**, 509 (1976).
34. D. H. Namm, S. E. Mayer and M. Maltbie, *Molec. Pharmac.* **4**, 522 (1968).
35. J. G. Dobson, J. Ross, Jr. and S. E. Mayer, *Circulation Res.* **39**, 388 (1976).
36. M. Endoh, O. E. Brodie, D. Reinhardt and H. J. Schumann, *Nature, Lond.* **261**, 716 (1976).
37. I. Harary, J-F. Renaud, E. Sato and G. A. Wallace, *Nature, Lond.* **261**, 60 (1976).
38. A. M. Watanabe and H. R. Besch Jr., *Circulation Res.* **35**, 316 (1974).
39. W. G. Nayler, *Am. Heart J.* **73**, 379 (1967).
40. N. Haugaard, E. S. Haugaard, N. H. Lee and R. S. Horn, *Fedn. Proc.* **28**, 1657 (1969).