Br. J. Pharmac. (1981), **73**, 393–399

DIFFERENTIAL RESPONSES TO CARBACHOL, SODIUM NITROPRUSSIDE AND 8-BROMO-GUANOSINE 3′,5′-MONOPHOSPHATE OF CANINE ATRIAL AND VENTRICULAR MUSCLE

MASAO ENDOH & SHUJI YAMASHITA

Department of Pharmacology, Tohoku University School of Medicine, 980 Sendai, Japan

1 The relation between force of contraction and cyclic nucleotide levels during muscarinic receptor stimulation, and after administration of sodium nitroprusside was assessed in canine isolated atrial and ventricular muscle.

2 The pD_2 value (negative logarithm of ED_{50}) for carbachol to decrease force of atrial contraction was similar to that required to inhibit adenosine 3′,5′-monophosphate (cyclic AMP)-mediated positive inotropic responses in ventricular muscle.

3 The cyclic AMP level of atrial muscle did not significantly change during carbachol-induced negative inotropic action, whilst the guanosine 3′,5′-monophosphate (cyclic GMP) level was elevated immediately after administration.

4 Sodium nitroprusside elevated cyclic GMP levels (without changing cyclic AMP levels) both in atrial and ventricular muscle. The force of atrial contraction was significantly reduced by the drug, whilst ventricular contractile force was unaffected.

5 8-Bromo-cyclic GMP markedly decreased contractile force in atrial muscle. In contrast, similar concentrations of 8-bromo-cyclic GMP had no effect on ventricular contractile force.

6 The positive inotropic action of phenylephrine on canine cardiac muscle, which is mediated through β-adrenoceptors, was unaffected either by sodium nitroprusside or by 8-bromo-cyclic GMP.

7 The present results suggest that the effect of muscarinic receptor stimulation in canine atrial and ventricular muscle is related to different changes in intracellular cyclic nucleotide metabolism. The direct myocardial depressant action on atrial muscle seems to be related to an elevation of cyclic GMP level, whilst a reduction of cyclic AMP may be responsible for the indirect action ('accentuated antagonism') in both atrial and ventricular muscle.

Introduction

The force of contraction of mammalian atrial and ventricular muscle changes in response to muscarinic receptor stimulation in a different manner. Muscarinic receptor stimulation causes a profound negative inotropic action on atrial muscle, but scarcely affects force of ventricular contraction. However, muscarinic receptor stimulation exerts a definite negative inotropic action on mammalian ventricular muscle, if the force of contraction has previously been increased by β-adrenoceptor stimulation (Hollenberg, Carrier & Barger, 1965; Levy, Ng, Martin & Zieske, 1966; reviewed by Higgins, Vatner & Braunwald, 1973). Such an effect is termed 'accentuated antagonism' by Levy (1971).

Since adenosine 3′,5′-monophosphate (cyclic AMP) (Sutherland, Robison & Butcher, 1968; reviewed by Tsien, 1977) and guanosine 3′,5′-monophosphate (cyclic GMP) (George, Polson, O'Toole & Goldberg, 1970; George, Wilkerson & Kadowitz, 1973) were proposed as the intracellular mediator of β-adrenoceptor stimulation and muscarinic receptor stimulation, respectively, in the heart, the role of cyclic nucleotides in 'accentuated antagonism' has become of great interest and has been studied intensively. Muscarinic receptor stimulation reduces the cyclic AMP level, elevated previously by β-adrenoceptor stimulation in ventricular (Gardner & Allen, 1976; Keely, Lincoln & Corbin, 1978) and atrial (Brown, 1979; Brown, Polson, Krzanowski & Wiggins, 1980) muscle, although conflicting results have also been reported (Watanabe & Besch, 1975). In previous experiments performed on canine isolated right ventricular trabeculae, we have observed that the cyclic AMP level which had been increased by isoprenaline, papaverine or theophylline was rapidly reduced by carbachol before or at the same time as the force of contraction increased by these agents was suppressed (Endoh & Honma,

0007-1188/81/060393-007 $01.00

1979; Endoh, 1980). Whether or not the 'accentuated antagonism' in ventricular muscle and direct action of muscarinic receptor stimulation on atrial muscle are induced via a similar subcellular mechanism in respect to cyclic nucleotide metabolism is uncertain and the present study was undertaken to settle this question. For this purpose the relation between the negative inotropic action of muscarinic receptor stimulation and cyclic nucleotide responses was assessed in canine isolated left atria under the same experimental conditions as in canine right ventricular muscle. Effects of sodium nitroprusside, which has been shown to increase cyclic GMP levels of various tissues (Katsuki, Arnold & Murad, 1977) and 8-bromo-cyclic GMP, which is supposed to penetrate the myocardial cell membrane (Nawrath, 1976; 1978) were also investigated in order to elucidate the relation between atrial contraction and cyclic GMP.

Methods

Mongrel dogs of either sex weighing 6 to 13 kg were anaesthetized with sodium pentobarbitone (30 mg/kg i.v.) and given heparin sodium (500 units/kg i.v.). The heart was excised and quickly immersed in cold Tyrode solution (3 to 6°C). Trabeculae carneae of the right ventricular wall or strips of the left atrial appendage were isolated and mounted in 20 ml organ baths containing Krebs-Henseleit solution bubbled with 95% O_2 and 5% CO_2 at a temperature of 37°C. Muscles were stretched so that they had a resting tension of about 500 mg and were stimulated by square pulses of 5 ms duration and a voltage twice the threshold (atrial muscle) or 20% above the threshold (ventricular muscle) at a frequency of 0.5 Hz. The composition of the physiological solution used was (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.55, $MgSO_4$ 1.18, KH_2PO_4 1.18, $NaHCO_3$ 24.9, glucose 11.1, ascorbic acid 0.057 and disodium edetate 0.027. During an equilibration period of 1 h, resting tension was readjusted so as to give maximal active tension. Force of contraction was recorded on an ink-writing oscillograph (San-ei Instrument) by the use of force displacement transducers (Grass FT03B; Shikoh UL). After an equilibration period, force of contraction was increased by adding suitable and predetermined amounts of various positive inotropic agents. Cumulative concentration-response curves for carbachol were determined in the absence (atrial muscle) or in the presence of various positive inotropic agents (ventricular muscle). Sodium nitroprusside or 8-bromo-cyclic GMP was allowed to act until the force of contraction reached a steady level and then cumulative concentration-response curves for phenylephrine were determined in their presence. Only one concentration-response curve for phenylephrine was determined in each preparation. One or two muscles excised from the same heart were always used to provide control responses. The maximal response to isoprenaline in the presence of increased extracellular calcium (10 or 12.5 mM) was determined in each preparation at the end of experiments. The positive inotropic actions of cardiotonic agents were expressed as the percentage of the maximal response to isoprenaline.

In some experiments atrial muscle was removed from the organ bath at various times after the administration of 3×10^{-6}M carbachol and immediately frozen in liquid nitrogen. After homogenization by procedures described previously (Endoh & Honma, 1979), cyclic AMP and cyclic GMP were measured by the sensitive radioimmunoassay method described by Honma, Satoh, Takezawa & Ui (1977). One or two muscles isolated from the same heart served as controls.

Concentration-response curves for phenylephrine were described quantitatively by the pD_2 value (negative logarithm of ED_{50}) and the intrinsic activity (Ariëns, Simonis & Van Rossum, 1964). Experimental values are presented as means ± s.e.mean. Statistical significance between mean values was estimated by means of Student's *t* test. A *P* value less than 0.05 was considered to be significant.

Drugs used were: (−)-phenylephrine hydrochloride (Kowa, Nagoya); carbachol chloride (K & K Laboratories, New York); (−)-isoprenaline hydrochloride (Nikken Kagaku, Nagoya); glucagon hydrochloride (Novo Industri, Copenhagen); papaverine hydrochloride (Iwaki Seiyaku, Tokyo); caffeine·H_2O, sodium nitroprusside (Wako, Osaka); 8-bromo-cyclic GMP sodium (Sigma, St. Louis); (±)-pindolol (Sandoz, Basel). For chemical assays, the anti-cyclic AMP and -cyclic GMP sera, [^{125}I]-succinyl cyclic AMP and cyclic GMP tyrosine methyl ester were obtained from Yamasa Shoyu Co. (Choshi). Isoprenaline was dissolved daily in 1% ascorbic acid and diluted with 0.9% w/v NaCl solution (saline). Glucagon was freshly dissolved in saline. These solutions were cooled on ice during the experiments.

Results

Effects of carbachol on atrial and ventricular contraction

Carbachol caused a concentration-dependent negative inotropic action on canine isolated left atrial muscle; the basal force of contraction (474 ± 53 mg; $n=6$) was decreased by 91 ± 2% in the presence of maximal concentrations of carbachol (3×10^{-6} to 10^{-5}M). The pD_2 value for carbachol was 6.84 ± 0.04 ($n=6$). The negative inotropic action of carbachol

Table 1 The positive inotropic actions of various cardiotonic agents, their maximal inhibition by carbachol and the pD_2 values for carbachol in isolated ventricular muscle of the dog

Agonists	n	*PIA (%)*[1]	*Effects of carbachol*	
			Maximal inhibition (%)	*pD_2 values*
Isoprenaline (10^{-7}M)	7	37± 4	83±3	6.64±0.06
Glucagon ($10^{-5}-2\times10^{-5}$M)	6	21± 2	100	7.04±0.07
Papaverine (3×10^{-5}M)	4	42±13	100	6.83±0.06
Caffeine (3×10^{-3}M)	5	32± 5	84±7	6.64±0.14

[1]PIA: the positive inotropic action of agonists is expressed as the percentage of the maximal response to isoprenaline in the presence of increased extracellular calcium (10–12.5 mM). Given are means ± s.e.mean.

was not affected by papaverine (10^{-5}M) known to inhibit cyclic GMP phosphodiesterase (PDE) as well as cyclic AMP-PDE (Lugnier & Stoclet, 1974). Thus, in the presence of 10^{-5}M papaverine the basal force of contraction (472±32mg; n=6) was reduced by 88±3%, and the pD_2 value for carbachol was 6.95±0.07.

Carbachol scarcely affected the basal force of canine ventricular contraction (Endoh & Honma, 1979). However, when the force of contraction had been previously increased by isoprenaline, glucagon, papaverine or caffeine, carbachol had a concentration-dependent negative inotropic action. Table 1 summarizes the positive inotropic actions of these agents, their maximal inhibition by carbachol and the pD_2 values for carbachol in ventricular muscle. The pD_2 values for carbachol determined in the presence of various cardiotonic agents in ventricular muscle paralleled the direct action of carbachol on contractile force in atrial muscle.

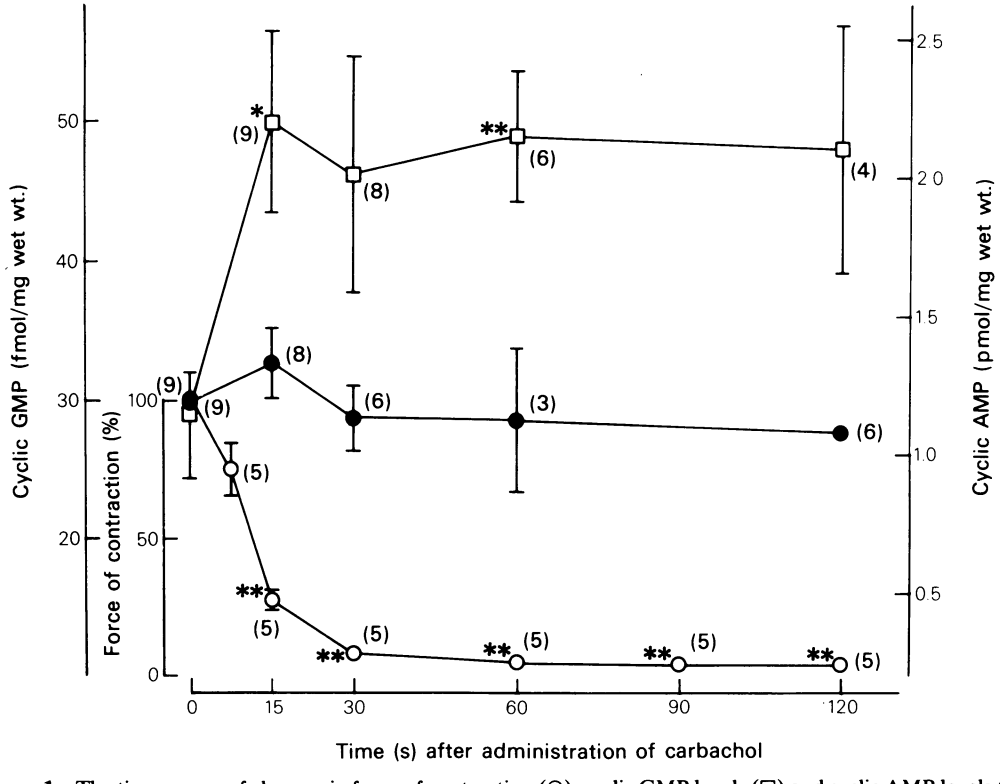

Figure 1 The time course of changes in force of contraction (○), cyclic GMP levels (□) and cyclic AMP levels (●) after the administration of carbachol 3×10^{-6}M in the isolated left atrial muscle of the dog. * $P<0.02$; ** $P<0.01$ vs. the corresponding control values. Values are means, vertical lines show s.e.mean. In some cases s.e.mean is smaller than the size of symbols. Numbers in parentheses represent number of experiments.

Table 2 Influence of sodium nitroprusside on the intracellular cyclic nucleotide levels and force of contraction of canine atrial and venticular muscle

	Cyclic GMP[1]	*Cyclic AMP*[2]	*Force of contraction*[3]
Atrial muscle			
Control	43±5 (7)	1.29±0.04 (8)	—
Sodium nitroprusside			
1×10^{-3}M	1449±178 (5)*	1.44±0.09 (5)	−22±4 (9)*
3×10^{-3}M	1790±344 (5)*	1.49±0.18 (5)	−26±4 (5)*
Ventricular muscle			
Control	16±2 (5)	1.10±0.10 (6)	—
Sodium nitroprusside			
1×10^{-3}M	247±54 (4)*	1.00±0.13 (5)	1±6 (8)
3×10^{-3}M	670±71 (4)*	1.38±0.15 (5)	3±11 (5)

[1]Cyclic GMP (fmol/mg wet wt.) and [2]cyclic AMP (pmol/mg wet wt.) were determined 10 min after the administration of sodium nitroprusside. [3]The percentage changes in basal force of contraction caused by sodium nitroprusside. *$P<0.001$ vs. the corresponding control values. Number of experiments in parentheses. Given are means ± s.e.mean.

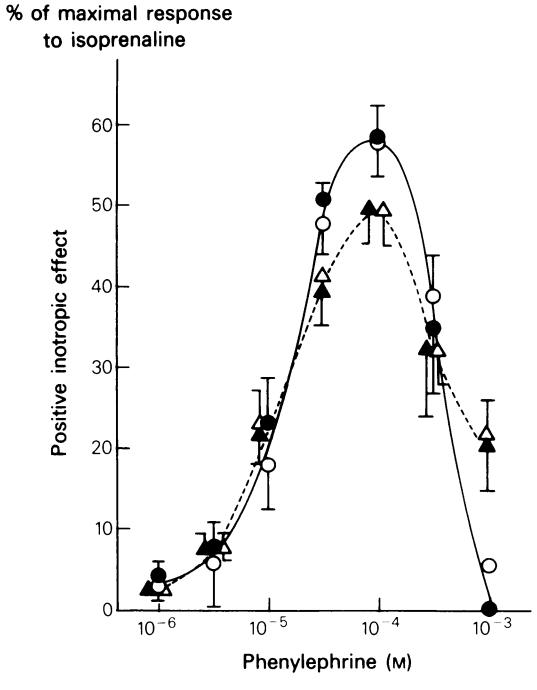

Figure 2 Influence of sodium nitroprusside 10^{-3}M on the concentration-response curve for positive inotropic action of phenylephrine in isolated left atrial and right ventricular muscle of the dog. (●) Control ($n=4$); (○) in the presence of sodium nitroprusside 10^{-3}M ($n=4$) in the left atrial muscle; (▲) control ($n=8$); (△) in the presence of sodium nitroprusside 10^{-3}M ($n=8$) in the right ventricular muscle. Values are means, vertical lines show s.e.mean.

Time course of changes in cyclic nucleotide levels and force of atrial contraction after administration of carbachol

The negative inotropic action of 3×10^{-6}M carbachol was rapid in onset; 15 s after the administration of carbachol the decrease in force of atrial contraction was almost maximal (Figure 1). Force of contraction remained at a decreased stable level in the presence of carbachol. The cyclic GMP level was already maximally increased 15 s after the administration of carbachol, when the force of contraction was significantly reduced. The elevated cyclic GMP level was maintained until 120 s after administration (Figure 1). In contrast, the cyclic AMP level of atrial muscle determined simultaneously after administration of 3×10^{-6}M carbachol was not changed significantly from the control value (Figure 1).

Effects of sodium nitroprusside

The control cyclic GMP level of atrial muscle was higher than that of ventricular muscle (Table 2). Ten minutes after the administration of 10^{-3}M and 3×10^{-3}M sodium nitroprusside the cyclic GMP levels of atrial and ventricular muscle were markedly elevated to levels 15 to 40 times higher than control (Table 2). In contrast, cyclic AMP levels of atrial and ventricular muscle were not changed significantly after administration of sodium nitroprusside (Table 2). The force of atrial contraction was reduced significantly after the administration of 10^{-3}M and 3×10^{-3}M sodium nitroprusside by 22 and 26%, respectively (Table 2) but ventricular contractile force was not significantly affected (Table 2).

The positive inotropic action of phenylephrine (which, in canine cardiac muscle, is caused solely by β-adrenoceptor stimulation; Endoh, Shimizu &

Yanagisawa, 1978), was not influenced by sodium nitroprusside. The concentration-response curves for phenylephrine in the absence and presence of 10^{-3}M sodium nitroprusside in atrial muscle are illustrated in Figure 2. The pD_2 values for phenylephrine were 4.93 ± 0.07 in the absence, and 4.85 ± 0.07 in the presence of 10^{-3}M sodium nitroprusside ($n = 4$, each). The intrinsic activity of phenylephrine was also not affected by sodium nitroprusside (Figure 2).

The concentration-response curves for phenylephrine determined in canine ventricular muscle are also shown in Figure 2. The pD_2 values for phenylephrine were 4.94 ± 0.08 in the absence, and 5.01 ± 0.05 in the presence of 10^{-3}M sodium nitroprusside ($n = 8$, each).

Effects of 8-bromo-cyclic GMP

8-Bromo-cyclic GMP (3×10^{-4}M) caused a definite negative inotropic action on canine isolated left atrial muscle. The negative inotropic action of 8-bromo-cyclic GMP developed gradually to reach a steady level 30 min after administration. The basal force of contraction (508 ± 69 mg; $n = 6$) was reduced by $52 \pm 5\%$, 30 min after the administration of 3×10^{-4}M 8-bromo-cyclic GMP. However, the positive inotropic action of phenylephrine was not affected by 8-bromo-cyclic GMP. Also the threshold concentration (10^{-6}M), the intrinsic activity (0.73 ± 0.05), and the pD_2 value (4.85 ± 0.04) for phenylephrine in the presence of 3×10^{-4}M 8-bromo-cyclic GMP ($n = 5$) were not significantly different from the corresponding control values (10^{-6}M; 0.64 ± 0.07; 4.86 ± 0.05; $n = 5$) in the absence of 8-bromo-cyclic GMP.

We have shown in canine isolated ventricular muscle that 8-bromo-cyclic GMP at a concentration of 10^{-4}M fails to modify contractile force or β-adrenoceptor-mediated positive inotropic responses (Endoh & Shimizu, 1979). In the present study it was confirmed that 8-bromo-cyclic GMP, 3×10^{-4}M, did not affect force of ventricular contraction. These experiments were performed in the presence of a β-adrenoceptor blocking agent, pindolol (3×10^{-8}M), in order to exclude the possibility that the released noradrenaline counteracts the action of 8-bromo-cyclic GMP. The basal force of ventricular contraction (336 ± 60 mg, $n = 4$) was not changed significantly 30 min after the administration of 3×10^{-4}M 8-bromo-cyclic GMP ($98 \pm 6\%$ of the basal value, $n = 4$).

Discussion

It has been shown that the inhibitory action of muscarinic receptor stimulation on cyclic AMP-mediated positive inotropic responses in mammalian ventricular (Keely *et al.*, 1978; Endoh, 1980) and atrial (Brown *et al.*, 1980) muscle is accompanied by a pronounced reduction in cyclic AMP levels previously elevated by cardiotonic agents. Since neither elevation of cyclic GMP level by sodium nitroprusside, nor 8-bromo-cyclic GMP, affected the positive inotropic action of phenylephrine, it appears that reduction of cyclic AMP, rather than the elevation of cyclic GMP, is essential for the indirect inhibitory action of muscarinic receptor stimulation both in the atrial and ventricular muscle. In support of this are the findings of Keeley *et al.* (1978). These workers showed that adrenaline-induced activation of cyclic AMP-dependent protein kinase in the isolated perfused heart of the rat was suppressed by muscarinic receptor stimulation.

The pD_2 values for carbachol to decrease directly the force of contraction of canine isolated left atria (direct action) and to suppress the positive inotropic action of isoprenaline, glucagon, papaverine and caffeine in canine isolated right ventricular muscle (indirect action) were in the same range (between 6.64 and 7.04); both actions were inhibited by atropine. Provided that the direct action of carbachol on atrial contraction and the indirect one on the canine ventricle are mediated via the same intracellular biochemical process triggered by muscarinic receptor stimulation, and that intracellular cyclic nucleotides play an important role in this process (Sutherland *et al.*, 1968; George *et al.*, 1970; 1973; Tsien, 1977), it is to be expected that carbachol-induced changes in cyclic nucleotide levels proceed in a similar manner in both atrial and ventricular muscle. Contrary to this expectation, changes in cyclic nucleotide levels caused by carbachol during induction of negative inotropic action were markedly different in atrial and ventricular muscle. In atrial muscle, the cyclic AMP level did not change significantly during carbachol-induced negative inotropism. On the other hand, cyclic GMP was substantially increased immediately after administration of carbachol and this elevation appeared to parallel the decreased force of contraction. Therefore, the effect of elevating cyclic GMP by means other than muscarinic receptor stimulation was examined. Sodium nitroprusside was used for this purpose and was found to increase markedly the levels of cyclic GMP in atrial muscle and to reduce significantly the force of atrial contraction. A derivative of cyclic GMP, 8-bromo-cyclic GMP, had a pronounced negative inotropic action on atrial muscle. Such a depressant action is consistent with previous observations in guinea-pig atria that 8-bromo-cyclic GMP decreased contractile force (Nawrath, 1976; 1978) and suppressed slow response action potentials (Kohlhardt & Haap, 1978). These results, together with previous findings, favour the view that contractile force in atrial muscle is inversely related to the intracellular cyclic GMP levels (George *et al.*, 1970; 1973). However, the evidence is inadequate that

intracellular levels of cyclic GMP mediate the negative inotropic responses that result from muscarinic receptor stimulation. For example, there are large quantitative differences between the degree of depression of atrial contraction and the accumulation of cyclic GMP caused by muscarinic receptor stimulation on the one hand and by sodium nitroprusside on the other. It has also been reported for other cardiac tissue that cyclic GMP levels are dissociated from the negative inotropic action of carbachol and methacholine (Brooker, 1977; Linden & Brooker, 1979). Thus elucidation of whether cyclic GMP mediates the negative inotropic action resulting from muscarinic receptor stimulation awaits further study.

Increases in intracellular cyclic GMP resulting from administration of sodium nitroprusside exerted different actions on atrial and ventricular muscle under the same experimental conditions: whilst atrial contractile force was significantly reduced by sodium nitroprusside, ventricular contractile force was unaffected. Furthermore, a derivative of cyclic GMP (8-bromo-cyclic GMP) had a marked negative inotropic action on atrial muscle but did not affect the force of ventricular contractions. It is suggested from these results that the subcellular mechanisms responsible for atrial contractions may be different from those of ventricular muscle.

In summary, the present results suggest that in mammalian atrial and ventricular muscle, direct and indirect actions of muscarinic receptor stimulation are related to different changes in intracellular cyclic nucleotides. Such a dual mechanism for inhibitory action via muscarinic receptor stimulation has been proposed recently for avian ventricular muscle (Biegon & Pappano, 1980). The present experiments reveal that the direct myocardial depressant action resulting from muscarinic receptor stimulation may be related to elevation of cyclic GMP; elevation of intracellular cyclic GMP exerts a different action on canine atrial and ventricular muscle.

The authors wish to thank Professor N. Taira, Department of Pharmacology, Tohoku University School of Medicine, for useful advice and encouragement in performing the present experiments. We are grateful to Yamasa Shoyu Co. for a generous supply of the anti-cyclic AMP and -cyclic GMP sera, [^{125}I]-succinyl cyclic AMP and cyclic GMP tyrosine methyl ester.

References

ARIËNS, E.J., SIMONIS, A.M. & VAN ROSSUM, J.A. (1964) Drug receptor interaction: Interaction of one or more drugs with one receptor system. In *Molecular Pharmacology*, ed. Ariëns, E.J. Vol 1, pp. 137–140, 153. New York and London: Academic Press.

BIEGON, R.L. & PAPPANO, A.J. (1980) Dual mechanism for inhibition of calcium-dependent action potentials by acetylcholine in avian ventricular muscle. Relationship to cyclic AMP. *Circulation Res.*, **46,** 353–362.

BROOKER, G. (1977) Dissociation of cyclic GMP from the negative inotropic action of carbachol in guinea pig atria. *J. Cyclic Nucleotide Res.*, **3,** 407–413.

BROWN, J.H. (1979) Cholinergic inhibition of catecholamine-stimulable cyclic AMP accumulation of murine atria. *J. Cyclic Nucleotide Res.*, **5,** 423–433.

BROWN, B.S., POLSON, J.B., KRZANOWSKI, J.J. & WIGGINS, J.R. (1980) Influence of isoproterenol and methylisobutylxanthine on the contractile and cyclic nucleotide effects of methacholine in isolated rat atria. *J. Pharmac. exp. Ther.*, **212,** 325–332.

ENDOH, M. (1980) The time course of changes in cyclic nucleotide levels during cholinergic inhibition of positive inotropic actions of isoprenaline and theophylline in the isolated canine ventricular myocardium. *Naunyn-Schmiedebergs Arch. Pharmac.*, **312,** 175–182.

ENDOH, M. & HONMA, M. (1979) Effects of papaverine and its interaction with isoprenaline and carbachol on the contractile force and cyclic nucleotide levels of the canine ventricular myocardium. *Naunyn-Schmiedebergs Arch. Pharmac.*, **306,** 241–248.

ENDOH, M. & SHIMIZU, T. (1979). Failure of dibutyryl and 8-bromo-cyclic GMP to mimic the antagonistic action of carbachol on the positive inotropic effects of sympathomimetic amines in the canine isolated ventricular myocardium. *Jap. J. Pharmac.*, **29,** 423–433.

ENDOH, M., SHIMIZU, T. & YANAGISAWA, T. (1978). Characterization of adrenoceptors mediating positive inotropic responses in the ventricular myocardium of the dog. *Br. J. Pharmac.*, **64,** 53–61.

GARDNER, R.M. & ALLEN, D.O. (1976). Regulation of cyclic nucleotide levels and glycogen phosphorylase activity by acetylcholine and epinephrine in perfused rat hearts. *J. Pharmac. exp. Ther.*, **198,** 412–419.

GEORGE, W.J., POLSON, J.B., O'TOOLE, A.G. & GOLDBERG, N.D. (1970). Elevation of guanosine 3',5'-cyclic phosphate in rat heart after perfusion with acetylcholine. *Proc. natn. Acad. Sci. U.S.A.*, **66,** 398–403.

GEORGE, W.J., WILKERSON, R.D. & KADOWITZ, P.J. (1973). Influence of acetylcholine on contractile force and cyclic nucleotide levels in the isolated perfused rat heart. *J. Pharmac. exp. Ther.*, **184,** 228–235.

HIGGINS, C.B., VATNER, S.F. & BRAUNWALD, E. (1973). Parasympathetic control of the heart. *Pharmac. Rev.*, **25,** 119–155.

HOLLENBERG, M., CARRIER, S. & BARGER, A.C. (1965). Biphasic action of acetylcholine on ventricular myocardium. *Circulation Res.*, **16,** 527–536.

HONMA, M., SATOH, T., TAKEZAWA, J. & UI, M. (1977). An ultrasensitive method for the simultaneous determination of cyclic AMP and cyclic GMP in small-volume

samples from blood and tissue. *Biochem. Med.*, **18,** 257–273.

KATSUKI, S., ARNOLD, W.P. & MURAD, F. (1977). Effects of sodium nitroprusside, nitroglycerin, and sodium azide on levels of cyclic nucleotides and mechanical activity of various tissues. *J. Cyclic Nucleotide Res.*, **3,** 239–247.

KEELY, S.L., Jr., LINCOLN, T.M. & CORBIN, J.D. (1978). Interaction of acetylcholine and epinephrine on heart cyclic AMP-dependent protein kinase. *Am. J. Physiol.*, **234,** H432–H438.

KOHLHARDT, M. & HAAP, K. (1978). 8-Bromo-guanosine-3′,5′-monophosphate mimics the effect of acetylcholine on slow response action potential and contractile force in mammalian atrial myocardium. *J. mol. cell. Cardiol.*, **10,** 573–586.

LEVY, M.N. (1971). Sympathetic-parasympathetic interactions in the heart. *Circulation Res.*, **29,** 437–445.

LEVY, M.N., NG, M., MARTIN, P. & ZIESKE, H. (1966). Sympathetic and parasympathetic interactions upon the left ventricle of the dog. *Circulation Res.*, **19,** 5–10.

LINDEN, J. & BROOKER, G. (1979). The questionable role of cyclic guanosine 3′:5′-monophosphate in heart. *Biochem. Pharmac.*, **28,** 3351–3360.

LUGNIER, C. & STOCLET, J.-C. (1974). Inhibition by papaverine of cGMP and cAMP phosphodiesterases from the rat heart. *Biochem. Pharmac.*, **23,** 3071–3074.

NAWRATH, H. (1976). Cyclic AMP and cyclic GMP may play opposing roles in influencing force of contraction in mammalian myocardium. *Nature*, **262,** 509–511.

NAWRATH, H. (1978). Evidence for opposing influences of cyclic GMP and cyclic AMP on force of contraction in mammalian myocardium. In *Recent Adv. Stud. Cardiac Struct. Metab.*, Vol 11, ed. Kobayashi, T., Sano, T. & Dhalla, N.S., pp. 419–422. Baltimore: University Park Press.

SUTHERLAND, E.W., ROBISON, G.A. & BUTCHER, R.W. (1968). Some aspects of the biological role of adenosine 3′,5′-monophosphate (cyclic AMP). *Circulation*, **37,** 279–306.

TSIEN, R.W. (1977). Cyclic AMP and contractile activity in heart. *Adv. Cyclic Nucleotide Res.*, **8,** 363–420.

WATANABE, A.M. & BESCH, H.R., Jr. (1975). Interaction between cyclic adenosine monophosphate and cyclic guanosine monophosphate in guinea pig ventricular myocardium. *Circulation Res.*, **37,** 309–317.

(Received August 15, 1980. Revised January 23, 1981.)