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

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Abstract—Effects of carbachol, dbcGMP and 8-bromo-cyclic GMP on the positive inotropic actions of sympathomimetic amines and dbcAMP were studied on the canine isolated right ventricular myocardium. Carbachol alone did not substantially change the developed tension of the muscle, but did markedly shift the dose response curve for isoprenaline on the developed tension to the right and depressed the maximal response to the drug in a concentration dependent manner. The positive inotropic action of phenylephrine was affected by carbachol in the same manner as that of isoprenaline. DbcGMP in concentrations of 10^{-3} M and higher produced a significant increase in the developed tension. The positive inotropic action of dbcGMP was partly inhibited by a β-adrenoceptor blocking agent, pindolol. In the presence of dbcGMP, isoprenaline produced an action similar to that seen in the control experiment, but this action was not maintained on such a steady level as in the control. This rapid decline of the effect of isoprenaline in the presence of dbcGMP was prevented by adding ascorbic acid to the organ bath. The positive inotropic actions of phenylephrine and of dbcAMP were not substantially affected by dbcGMP. 8-Bromo-cyclic GMP in a concentration of 10^{-4} M did not change either the basal developed tension or the positive inotropic actions of noradrenaline and of isoprenaline. The present results indicate that these cyclic GMP derivatives are not able to mimic the antagonistic action of cholinergic stimulation on the positive inotropic action of adrenergic stimulation on the canine ventricular myocardium.

Substantial evidence has accumulated that 3',5'-cyclic adenosine monophosphate (cyclic AMP) may mediate the positive inotropic action of certain hormones in the heart of various species of animals (1-4). It has been observed that dibutyryl cyclic AMP (dbcAMP), a derivative of cyclic AMP, which is assumed to penetrate into myocardial cells more easily than cyclic AMP and to be resistant to hydrolysis by cyclic nucleotide phosphodiesterase, mimicked the positive inotropic action of catecholamines in various isolated heart preparations (5-8).

It has been proposed that the inotropic action of cholinergic stimulation may be mediated by 3',5'-cyclic guanosine monophosphate (cyclic GMP), since the intracellular cyclic GMP level was elevated by cholinergic stimulation concurrently with the inhibitory action on the contractility of the isolated perfused rat heart (9, 10). In the mammalian ventricular myocardium, cholinergic stimulation by ACh or via vagus nerve had little effect on the basal contractile force, while during sympathetic stimulation or noradrenaline infusion this
stimulation produced a pronounced myocardial depression (11–15). This complex interaction between adrenergic and cholinergic actions on the mammalian ventricular contractility has been termed accentuated antagonism by Levy (16). Watanabe and Besch suggested that in the isolated guinea pig ventricle, cyclic GMP mediates the anti-adrenergic effects of ACh by specifically antagonizing the inotropic actions of cyclic AMP. Dibutyryl cyclic GMP (dbcGMP) mimicked the antagonistic action of ACh on the inotropic effect of isoprenaline in these experiments (17). Other workers have reported that other cyclic GMP derivatives, monobutyryl cyclic GMP (18) and 8-bromo-cyclic GMP (19–21) could also mimic the action of cholinergic stimulation on the contractile force or on the action potential in the guinea pig, rat and cat hearts.

The present study was an attempt to determine whether or not in canine isolated ventricular myocardium the derivatives of cyclic GMP, dbcGMP and 8-bromo-cyclic GMP, are able to simulate the accentuated antagonism induced by carbachol.

MATERIALS AND METHODS

Mongrel dogs of either sex (6–13 kg) were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and given heparin (500 U/kg i.v.). The heart was removed and immediately immersed into cold Tyrode solution (3–6°C) bubbled with 95% O₂ and 5% CO₂. Trabeculae carneae of the right ventricular wall (<1 mm in diameter) were dissected, fixed in a 20 ml organ bath containing Krebs-Henseleit solution equilibrated with 95% O₂ and 5% CO₂ at a temperature of 37°C and stretched with a tension of 0.5 g. The composition of the solution used is as follows: NaCl, 118; KCl, 4.7; CaCl₂, 2.55; MgSO₄, 1.18; KH₂SO₄, 1.18; NaHCO₃, 24.9; glucose, 11.1 (mM). The muscle was electrically stimulated by square wave pulses of 5 msec duration and a voltage of just above the threshold at a rate of 0.5 Hz. The developed tension of the muscle was recorded isometrically on an ink-writing oscillograph (San-ei Instrument) by the use of the force displacement transducers (Grass FT03B; Shinkoh UL). During an equilibration period of 1 hr, the muscle length was adjusted to Lₘₐₓ, where the developed tension was maximal. The cumulative dose response curves for sympathomimetic amines or dbcAMP were determined in the paired muscles isolated from the same heart, in the absence and in the presence of various concentrations of carbachol, dbcGMP or 8-bromo-cyclic GMP. Cyclic GMP derivatives were allowed to act for 30 or 60 min prior to determination of the dose response curve for the agonists, and carbachol, for 15–20 min. The concentration of drugs in the bath was increased by 0.5 log units until the maximal response was obtained. Only one dose response curve for the drug was determined in each preparation. The maximal contraction was induced in each muscle by injecting CaCl₂ after the determination of the cumulative dose response curve for the agonists. The positive inotropic action of the drug was expressed as the percentage of the maximal response to calcium. The pD₂-value (−log ED₅₀) was calculated as described by Van Rossum (22). Experimental values are presented as mean ± S.E. Significant differences between mean values were estimated by the use of Student’s t-test. A p-value smaller than 0.05 was considered to be significant.
In some experiments muscles were removed quickly from the organ bath, frozen immediately in liquid nitrogen and homogenized as described in detail in a previous paper (3). After homogenization the supernatant was extracted five times with 1 ml of ether after adding 10 μl 1N HCl. The cyclic GMP content was assayed by the radioimmunoassay methods after succinylation of the nucleotide (23). The cyclic GMP level was expressed as pmol/mg wet weight of the tissue.

Drugs used were (−)-isoprenaline hydrochloride (Nikken Kagaku, Nagoya); carbachol chloride (K & K Laboratories, New York); (−)-noradrenaline base (Fluka, Buchs) (+)-pindolol (Sandoz, Basel); (−)-phenylephrine hydrochloride (Kowa, Nagoya); dibutryl cyclic GMP sodium (Sigma, St. Louis; Boehringer, Mannheim; Yamasa Shoyu, Choshi); 8-bromo-cyclic GMP sodium (Sigma, St. Louis); dibutyryl cyclic AMP sodium (Daiichi Seiyaku, Tokyo). The solution of isoprenaline was prepared in 1% ascorbic acid on each experimental day, and diluted with 0.9% saline solution. The solutions of isoprenaline, noradrenaline and carbachol were kept ice-cooled.

RESULTS

Influence of carbachol on the cumulative dose response curves for isoprenaline and phenylephrine

Effects of $3 \times 10^{-6}$ M carbachol on the basal developed tension and the cumulative dose response relationship for isoprenaline in a typical experiment are shown in Fig. 1. Carbachol, $3 \times 10^{-6}$ M, produced a slight negative inotropic action followed by a slight increase in the developed tension. The developed tension 15–20 min after the administration of carbachol, when the dose response curve for isoprenaline was determined, was not significantly different from the value before application of carbachol. The threshold concentration of isoprenaline required to increase the developed tension was raised by carbachol (Fig. 1). The dose response curve for isoprenaline was shifted to the right and the maximal response to isoprenaline was significantly reduced by carbachol (Fig. 2 and Table 1). Shift of the dose response curve for isoprenaline was dependent on the concentration of carbachol: the pD$_{25}$ values for isoprenaline were reduced significantly in the presence of carbachol in concentrations of $10^{-6}$ M or higher (Table 1).

The positive inotropic action of phenylephrine was affected by carbachol in the same

![Fig. 1.](image-url)
manner as that of isoprenaline: the dose response curve for phenylephrine was shifted to the right and the maximal response was diminished markedly in the presence of $3 \times 10^{-6}$ M carbachol (Fig. 3). The pD$_2$-value for and the maximal response to phenylephrine in the canine isolated right ventricular myocardium. Ordinate: positive inotropic effect of phenylephrine expressed as the percentage of the maximal response to calcium; abscissa: molar concentration of phenylephrine on logarithmic scale; numbers in parentheses: number of experiments; vertical bars: ±S.E. of the mean.

**Fig. 2.** Influence of $3 \times 10^{-6}$ M and $10^{-5}$ M carbachol on the cumulative dose response curves for isoprenaline on the developed tension of the canine isolated right ventricular myocardium. Ordinate: positive inotropic effect of isoprenaline expressed as the percentage of the maximal response to calcium; abscissa: molar concentration of isoprenaline on logarithmic scale; numbers in parentheses: number of experiments; vertical bars: ±S.E. of the mean.

**Fig. 3.** Influence of $3 \times 10^{-6}$ M carbachol on the cumulative dose response curve for phenylephrine on the developed tension of the canine isolated right ventricular myocardium. Ordinate: positive inotropic effect of phenylephrine expressed as the percentage of the maximal response to calcium; abscissa: molar concentration of phenylephrine on logarithmic scale; numbers in parentheses: number of experiments; vertical bars: ±S.E. of the mean.

**Table 1.** Influence of carbachol on the pD$_2$-values for and the maximal responses to isoprenaline and phenylephrine in the canine isolated right ventricular myocardium. Given are mean ± S.E.

(A) Isoprenaline

|                  | n  | pD$_2$-value | Maximal response (%) | Developed tension (mg) |
|------------------|----|--------------|----------------------|------------------------|
|                  |    |              | Basal                | Maximal                |
| Control          | 7  | 6.82 ± 0.08  | 97.7 ± 3.5           | 626 ± 68               | 2011 ± 238             |
| Carbachol        |    |              |                      |                        |                       |
| $1 \times 10^{-6}$ M | 4  | 6.13 ± 0.08**| 79.0 ± 5.2*          | 820 ± 89               | 1685 ± 211            |
| $3 \times 10^{-6}$ M | 5  | 5.91 ± 0.08**| 82.3 ± 1.9*          | 666 ± 117              | 1956 ± 257            |
| $1 \times 10^{-5}$ M | 6  | 5.80 ± 0.13**| 56.2 ± 2.1**         | 720 ± 68               | 2133 ± 96             |

(B) Phenylephrine

|                  |    |              |                      |                        |                       |
|                  |    |              |                      |                        |                       |
| Control          | 5  | 4.96 ± 0.03  | 56.9 ± 6.4           | 956 ± 154              | 3008 ± 531            |
| Carbachol        |    |              |                      |                        |                       |
| $3 \times 10^{-6}$ M | 5  | 4.68 ± 0.02**| 25.0 ± 1.0*          | 780 ± 116              | 2568 ± 483            |

Maximal response was expressed as the percentage of the maximal increase induced by calcium in each preparation. *p<0.02, **p<0.001 vs. the control values.
presence of carbachol were significantly lower than the value and response in the presence of phenylephrine alone (Table 1).

Influence of dbcGMP on the cumulative dose response curves for isoprenaline and phenylephrine

DbcGMP in concentrations of $10^{-3}$ M and higher increased slightly but definitely the developed tension in the canine isolated right ventricular muscle as shown in Fig. 4. The positive inotropic action induced by $10^{-3}$ M dbcGMP was significant: the percentage increase amounted to $12.7 \pm 1.8$ (basal developed tension: $616 \pm 59$ mg; $n=6$; $p<0.001$). Since a $\beta$-adrenoceptor blocking agent, pindolol, inhibited partly the positive inotropic action of dbcGMP, the effect appears to be partly mediated via release of adrenergic transmitters from the tissue: pindolol, $3 \times 10^{-8}$ M, by itself did not affect the basal developed tension (Fig. 4B), but did cause a prominent negative inotropic action, when administered in the presence of $3 \times 10^{-8}$ M dbcGMP (Fig. 4A); dbcGMP, $3 \times 10^{-3}$ M, caused a slight positive inotropic action even in the presence of $3 \times 10^{-8}$ M pindolol (Fig. 4B).

The cumulative dose response relationship for isoprenaline determined in the absence and in the presence of $10^{-3}$ M dbcGMP in a typical experiment is shown in Fig. 5. In the presence of $10^{-3}$ M dbcGMP the positive inotropic action of isoprenaline was not maintained in the steady level and the developed tension declined rapidly to the control level. The $pD_2$-values calculated from the maximal level of the developed tension reached in each concentration of isoprenaline in the presence of $10^{-4}$ M and $10^{-3}$ M dbcGMP were slightly less than the $pD_2$-value in the control experiments, but the difference was not statistically significant; the maximal response to isoprenaline was not changed by dbcGMP (Table 2A).

Since the type of depression of the positive inotropic action of isoprenaline by dbcGMP (Fig. 5) was completely different from that produced by carbachol (Fig. 1), we considered the possibility that the autoxidation of isoprenaline in the organ bath is accelerated by the presence of dbcGMP. Therefore, the next series of experiments were conducted in the presence of ascorbic acid in order to prevent the autoxidation of isoprenaline. Ascorbic acid in a concentration which did not affect the basal developed tension (the final concen-

**Fig. 4.** Influence of $3 \times 10^{-8}$ M pindolol on the positive inotropic action of dbcGMP in the canine isolated right ventricular myocardium. Pindolol was administered in the presence of dbcGMP (A), and vice versa (B).

**Fig. 5.** Influence of $10^{-3}$ M dbcGMP on the basal developed tension and on the cumulative dose response relationship for isoprenaline on the developed tension of the canine isolated right ventricular myocardium.
tration in the organ bath: 0.284 mM) was given 5 min prior to the determination of the dose response curve for isoprenaline. The positive inotropic action of isoprenaline did not decline and was maintained in the same steady level seen in the control experiments, even in the presence of dbcGMP. The pD₂-value for, and the maximal response to isoprenaline were not affected by the presence of 10⁻⁴M and 10⁻³M dbcGMP (Table 2B).

The cumulative dose response relationship for phenylephrine was not affected by 10⁻³ M dbcGMP even in the absence of ascorbic acid (Fig. 6). The pD₂-value for, and the maximal developed tension induced by phenylephrine in the presence of 10⁻³ M dbcGMP were not significantly different from those in the control experiments (Table 2C).

Table 2. Influence of dbcGMP on the pD₂-values for and the maximal responses to isoprenaline and phenylephrine in the canine isolated right ventricular myocardium. Given are mean±S.E.

(A) Isoprenaline

|        | n   | pD₂-value     | Maximal response (%) | Developed tension (mg) |
|--------|-----|----------------|----------------------|------------------------|
|        |     |                | Basal                | Maximal                |
| Control| 7   | 7.13±0.23      | 97.2±1.5             | 576±143                | 2577±275               |
| DBCGMP |     |                |                      |                        |                        |
| 1×10⁻⁴M| 4   | 6.64±0.17      | 98.0±1.2             | 825±132                | 3680±743               |
| 1×10⁻³M| 4   | 6.89±0.22      | 94.8±2.0             | 685±170                | 2205±161               |

(B) Isoprenaline in the presence of ascorbic acid (0.284 mM)

|        | n   | pD₂-value     | Maximal response (%) | Developed tension (mg) |
|--------|-----|----------------|----------------------|------------------------|
|        |     |                | Basal                | Maximal                |
| Control| 8   | 7.19±0.19      | 94.8±2.9             | 750±48                 | 1980±183               |
| DBCGMP |     |                |                      |                        |                        |
| 1×10⁻⁴M| 5   | 7.01±0.18      | 96.5±3.6             | 716±61                 | 2432±408               |
| 1×10⁻³M| 5   | 7.47±0.15      | 99.2±0.9             | 928±137                | 2376±277               |

(C) Phenylephrine

|        | n   | pD₂-value     | Maximal response (%) | Developed tension (mg) |
|--------|-----|----------------|----------------------|------------------------|
|        |     |                | Basal                | Maximal                |
| Control| 4   | 5.13±0.08      | 1330±84*             | 538±103                | —                      |
| DBCGMP |     |                |                      |                        |                        |
| 1×10⁻³M| 4   | 5.17±0.14      | 1245±130             | 488±120                | —                      |

*Since calcium max. was not determined in this series, the maximal response to phenylephrine was given in mg. Values are not significantly different from each other.

The cumulative dose response relationship for phenylephrine was not affected by 10⁻³ M dbcGMP even in the absence of ascorbic acid (Fig. 6). The pD₂-value for, and the maximal developed tension induced by phenylephrine in the presence of 10⁻³ M dbcGMP were not significantly different from those in the control experiments (Table 2C).

![Fig. 6. Influence of 10⁻³ M dbcGMP on the basal developed tension and on the cumulative dose response relationship for phenylephrine on the developed tension of the canine isolated right ventricular myocardium.](image-url)
Influence of 8-bromo-cyclic GMP on the cumulative dose response curves for noradrenaline and isoprenaline

8-Bromo-cyclic GMP in a concentration of $10^{-4}$ M did not change the basal developed tension of the canine ventricular muscle. Thirty or sixty minutes after the administration of $10^{-4}$ M 8-bromo-cyclic GMP, the cumulative dose response curves for noradrenaline and isoprenaline were determined. The dose response curves for both catecholamines were not affected by the concurrent presence of $10^{-4}$ M 8-bromo-cyclic GMP. The pD$_2$-values for noradrenaline and isoprenaline in this series of experiments are shown in Table 3. The positive inotropic action of noradrenaline was also not modified by $10^{-4}$ M dbcGMP (Table 3A).

| (A) Noradrenaline |
|-------------------|
| n     | pD$_2$-value | Maximal response (mg or %) | Developed tension |
| Control | 6 | $5.75 \pm 0.14$ | $2363 \pm 369^*$ | $763 \pm 165$ |
| 8-Bromo-cyclic-GMP | $1 \times 10^{-4}$ M | $5.60 \pm 0.17$ | $2985 \pm 519$ | $994 \pm 169$ |
| DbcGMP | $1 \times 10^{-4}$ M | $5.74 \pm 0.07$ | $2420 \pm 275$ | $765 \pm 120$ |

| (B) Isoprenaline |
|-------------------|
| n     | pD$_2$-value | Maximal response (mg or %) | Developed tension |
| Control | 4 | $6.91 \pm 0.15$ | $90.0 \pm 4.5$ | $885 \pm 244$ | $2260 \pm 125$ |
| 8-Bromo-cyclic-GMP | $1 \times 10^{-4}$ M | $6.84 \pm 0.08$ | $88.2 \pm 2.7$ | $795 \pm 17$ | $2290 \pm 121$ |

*Since calcium max. was determined only in one series, in which the response to noradrenaline was the same as that to calcium, the maximal response was given as absolute value in mg. These experiments were performed in the presence of ascorbic acid, 0.057 mM (3).

Influence of dbcGMP on the positive inotropic action of dbcAMP

The effects of $10^{-3}$ M and $3 \times 10^{-3}$ M dbcGMP on the cumulative dose response curve for, and the time course of the development of the action of dbcAMP are shown in Fig. 7. DbcGMP, $10^{-3}$ M, did not affect the effect of dbcAMP on the developed tension. In the presence of $3 \times 10^{-3}$ M dbcGMP, the action of a lower concentration ($3 \times 10^{-4}$ M) of dbcAMP was enhanced and that of $3 \times 10^{-3}$ M dbcAMP was reduced (Fig. 7).

Cyclic GMP levels of the tissue in the presence of dbcGMP

The cyclic GMP level determined in the control muscles was $0.0697 \pm 0.0366$ pmoles/mg w.w. (n=6). The cyclic GMP level determined 1 hr after the administration of dbcAMP ($10^{-3}$ M), when the developed tension was increased significantly, was $0.1279 \pm 0.0256$ pmoles/mg w.w. (n=4); the value being not significantly different from the control. When dbcGMP ($10^{-3}$ M) was allowed to act for 1 hr in the presence of $10^{-3}$ M dbcAMP, the cyclic...
GMP level of the muscle was markedly elevated to 55.31 ± 6.93 pmoles/mg w.w. (n=6).

DISCUSSION

Carbachol in a concentration that left the basal developed tension virtually unchanged shifted the dose response curve for isoprenaline on developed tension to the right and depressed the maximal response to the drug in a concentration dependent manner in the canine isolated right ventricular muscle. The positive inotropic action of phenylephrine was also antagonized prominently by the same concentration of carbachol. The accentuated antagonism on the mammalian ventricular myocardium (16), as described usually for whole animal experiments (11–15) as well as in the isolated ventricular muscle (17), was confirmed to be operative also in the canine isolated ventricular muscle.

George and coworkers (9, 10) suggested that cyclic GMP, which is increased intracellularly during induction of the negative inotropic action of ACh in the rat heart, may play a role in mediating the cardiac actions of cholinergic stimulation. Since the antagonistic action of cholinergic stimulation on the positive inotropic actions of catecholamines in the mammalian ventricular muscle appears to be closely linked to the elevation of the intracellular cyclic AMP, it has been proposed that the regulation of intracellular cyclic AMP and cyclic GMP levels by the autonomic stimulations may be the subcellular regulatory mechanism of the adrenergic-cholinergic interaction on the contractility (17). In this regard, cyclic GMP may mediate the antagonistic action of cholinergic stimulation on the adrenergic stimulation. Watanabe and Besch (17) reported in guinea pig heart that dbcGMP, a derivative of cyclic GMP can permeate the cell membrane and shift the dose response curve of isoprenaline to the right.

The present study conducted on the canine isolated ventricular myocardium failed to
confirm the findings in the guinea pig heart. It was found that dbcGMP in concentrations of $10^{-3}$ M and higher caused a significant positive inotropic action on the canine ventricular muscle and that the action was partly blocked by a $\beta$-adrenoceptor blocking agent, pindolol. The positive inotropic action of isoprenalin declined rapidly to the basal level in the presence of dbcGMP (Fig. 5). It is not considered, however, that these results are evidence for the existence of the intracellular cyclic AMP-cyclic GMP interaction, for the following reasons: (1) the maximal developed tension induced by each concentration of isoprenalin was not changed by dbcGMP: (2) the addition of ascorbic acid to the organ bath prevented the decline of the action of isoprenalin: (3) the action of phenylephrine, a sympathomimetic amine that is structurally more stable than isoprenalin, was not affected by dbcGMP: (4) the action of dbcAMP which is probably induced by a direct increase in the intracellular cyclic AMP level was little affected by dbcGMP. In the present study, we used dbcGMP, products of three different companies, in order to avoid the possibility that our dbcGMP was inactive because of the breakdown. The results obtained with these substances from different origins, however, were the same. We concluded, therefore, that dbcGMP accelerated the autoxidation of isoprenalin in the organ bath, but did not affect the positive inotropic actions of sympathomimetic amines intracellularly, which may be mediated by cyclic AMP in the canine ventricular myocardium.

The other cyclic GMP derivative, 8-bromo-cyclic GMP, was also inactive in modifying the positive inotropic actions of catecholamines. The reasons for the difference in our results compared with those of others (17-21) are not readily apparent, but the following two possibilities should be considered: (1) the species differences in the cyclic nucleotide metabolism and (2) the intracellular cyclic GMP accumulation is not essential for and secondary to the action of cholinergic stimulation. Schmitz and Kruse (24) have shown that the myocardial phosphodiesterase activity is different among mammalian species: the basal phosphodiesterase activity was 3-5 times higher in the guinea pig than in rat hearts. This suggests that the action of cyclic nucleotide derivatives may be influenced by the difference in the basal phosphodiesterase activity in each species. A similar concentration of dbcAMP, however, produced a prominent positive inotropic action on the canine ventricular muscle: this shows that in the cyclic AMP system, dbcAMP could penetrate the cell membrane and produce an action before the nucleotide is metabolized. The intracellular cyclic GMP level was markedly elevated 1 hr after the administration of $10^{-3}$ M dbcGMP. These results, however, are not decisive since there is a possibility that dbcGMP and mono-butyryl cyclic GMP in both the intracellular and extracellular spaces may cross-react with cyclic GMP-antiserum and disturb the determination (23).

In support of the second possibility, there are observations not consistent with the cyclic GMP second messenger theory: (1) cholinergic stimulation did not activate the guanylate cyclase in the broken cell preparation (26): (2) some drugs including sodium nitroprusside and nitroglycerin elevated considerably the intracellular cyclic GMP level but had no effect on cardiac contractility (27).

In the canine ventricular muscle, antagonism by carbachol of the positive inotropic
actions mediated via cyclic AMP was accompanied by elevation of the level of intracellular cyclic GMP (25). In these experiments carbachol increased the cyclic GMP level during antagonism of the positive inotropic actions of isoprenaline, histamine, glucagon, theophylline and papaverine. The cyclic GMP level increased most in the presence of papaverine, but the antagonistic action of carbachol was not influenced by papaverine: the action of cholinergic stimulation was not enhanced by inhibiting cyclic GMP phosphodiesterase.

There may exist a complex subcellular biochemical process operative during the adrenergic-cholinergic interaction on the contractile force of the mammalian ventricular myocardium. Whatever the underlying mechanism, the present study indicates that in the canine ventricular myocardium, the cyclic GMP derivatives, dbcGMP and 8-bromo-cyclic GMP are not able to mimic the antagonistic action of cholinergic stimulation on the positive inotropic effects of sympathomimetic amines and of dbcAMP.

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