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Elevated Cyclic GMP Levels in Rabbit Atria Following Vagal Stimulation and Acetylcholine Treatment[1,2] (39484)

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At the present time, the roles of cyclic nucleotides in the regulation of cardiac contraction are under active investigation. Most of this work is centered upon the connection between catecholamine stimulation and cardiac levels of cyclic 3′,5′ adenosine monophosphate (cAMP). Thus, epinephrine has been shown to increase the levels of cAMP in various heart tissues concomitant with increases in either rate or force of contraction (1-4). Also, cAMP and its dibutyryl derivatives have both been demonstrated to increase rate and/or force of contraction in various isolated cardiac tissue preparations (5, 6).

Comparatively little work has been performed on the elucidation of a possible role for cyclic 3′,5′ guanosine monophosphate (cGMP) in the regulation of the mammalian heart. Several years ago George *et al.* (7) reported that acetylcholine caused a significant rise in cGMP content in isolated perfused spontaneously beating rat hearts, while cAMP content either did not change or decreased slightly. In these studies there was a strong inverse correlation between cGMP content and tension development in the whole rat heart. More recently this work has been further amplified (8) to show that the inverse correlation between cGMP levels and tension development in the isolated perfused rat heart given acetylcholine does not depend upon changes in heart rate, and that the effect of acetylcholine on cGMP levels can be abolished by pretreatment with atropine.

Since the cholinergic innervation of the heart is concentrated primarily within the atria (9), the present investigation deals with *in vitro* and *in vivo* studies into the effect of cholinergic stimulation on the cyclic nucleotide content of rabbit atrial muscle.

Methods. Isolated rabbit atrial preparations. In this series of experiments, atria were obtained from New Zealand white rabbits and prepared according to the method of Levy (10). Rabbits weighing 2-3 kg were sacrificed by cervical dislocation. Their hearts were rapidly excised and placed in cooled, modified Tyrode's solution that was oxygenated with 95% O_2 and 5% CO_2. The composition of the modified Tyrode's solution in mmoles/liter was: NaCl 120, KCl 5, $MgCl_2$ 0.6, $CaCl_2$ 4.0, dextrose 5.0, NaH_2PO_4 0.6, and $NaHCO_3$ 3.0. Each heart was then placed in a dissecting dish containing oxygenated modified Tyrode's solution and the atria were carefully dissected away from the ventricles by cutting along the atrioventricular groove. Before separation of the atria, fat and excess tissue were removed to allow better oxygenation of the tissue. The left atrium was cut open and a suture placed in one end for recording purposes. The other end was held in place in a 50 ml tissue bath containing modified Tyrode's solution at 37°C by a Plexiglas hook secured to a block that contained the punctate electrodes for stimulation. Force of contraction was recorded on a Grass model 7 polygraph with the aid of an FT.03 force-displacement transducer that was attached to the atrium via the aforementioned silk suture. Tension was added to the atria in increments until the peak of the length tension curve was approximated. The muscle was electrically stimulated at 150-180 beats per minute at a voltage approximately 10% above threshold. Following a 30 minute equilibration period, acetylcholine chloride

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at a concentration of 5×10^{-7} *M* (calculated as the salt) was added alone or in combination with atropine sulfate at a concentration of 1×10^{-7} *M* and at various intervals the tissues were frozen in Wollenberger clamps that had been precooled in liquid nitrogen. Cyclic AMP and cGMP content of the tissue was then assayed by a protein binding technique (11) and radioimmunoassay (12), respectively.

In situ rabbit atrium/vagus nerve preparation. In this series of experiments, New Zealand white rabbits weighing between 2–3 kg were anesthetized with sodium pentobarbital (30 mg/kg, iv) and their chests were opened through a mid-sternal incision. Respiration was maintained by applying positive pressure ventilation through a tracheal cannula, such that a constant minute volume of approximately 1 liter was maintained. Following excision of the pericardium, a single fine suture was passed through the apex of the right atrial appendage, to facilitate the rapid removal of the atrium. Heart rate was determined from a standard electrocardiogram taken from needle electrodes placed subcutaneously. The right cervical vagus was isolated, crushed centrally, and placed across shielded bipolar electrodes for stimulation (physiograph model SI-10 stimulator; Palmer shielded electrodes). The cardiac distribution of the right vagus in the rabbit is known to be localized almost exclusively to the sino-atrial node and the right atrium (13). In all experiments, test stimulations were carried out to determine stimulus parameters which would yield approximately a 50% slowing of heart rate from control. Then, following a 10 min rest period, vagal stimulation was reapplied for various time intervals. Then, the right atrium was rapidly excised and frozen in Wollenberger clamps precooled in liquid nitrogen. Control hearts were treated identically except for the final vagal stimulation. In some experiments atropine sulfate, 2 mg/kg, iv, was administered to the rabbit immediately following the test stimulation, and the experiment was completed as before. Cyclic nucleotide content of the atrial tissue was assayed as previously described (11, 12).

Statistical analysis. Differences in group means were determined using the appropriate "*t*" test (14). Regression lines were fitted by means of the method of least squares. Correlation coefficients and the significance of regressions were calculated using standard regression analyses (15).

Results. Isolated rabbit atrial preparation. Table I illustrates the changes in atrial cGMP levels, cAMP levels, and force of contraction in isolated paced rabbit atria following the addition of acetylcholine (5×10^{-7} *M*). As can clearly be seen, the addition of acetylcholine to the tissue bath resulted in increases in atrial cGMP levels from a control value of 92 ± 8 nmole/kg to 129 ± 14 nmole/kg at 5 sec, 195 ± 29 nmole/kg at 10 sec, and 166 ± 18 nmole/kg at 15 sec following drug addition. All values were significantly different from control. At none of these time intervals was the atrial level of cAMP altered significantly ($P > 0.05$) from the control value of 715 ± 50 nmoles/kg. It is of importance here to note that although acetylcholine caused a significant ($P < 0.05$) decrease in the force of

TABLE I. EFFECTS OF ACETYLCHOLINE AND ATROPINE ON RATE, FORCE OF CONTRACTION, AND CYCLIC NUCLEOTIDE CONTENT IN ISOLATED PACED RABBIT ATRIA.

Experimental conditions	*n*	cGMP (nmoles/kg tissue)	cAMP (nmoles/kg tissue)	Heart rate (% control)	Isometric tension (% control)
Control	5	92 ± 8	715 ± 50	100	100 ± 3
Acetylcholine[a] 5 sec	5	129 ± 14[c]	682 ± 88	100	97 ± 3
Acetylcholine 10 sec	5	195 ± 29[c]	642 ± 55	100	90 ± 2[c]
Acetylcholine 15 sec	5	166 ± 18[c]	618 ± 49	100	84 ± 5[c]
Atropine[b]	5	84 ± 11	687 ± 41	100	101 ± 3
Atropine[b] + acetylcholine[a] 5–15 sec	5	96 ± 12	702 ± 23	100	96 ± 3

[a] Acetylcholine concentration = 5×10^{-7} M.
[b] Atropine sulfate concentration = 1×10^{-7} M.
[c] Significantly different from control values ($P < .05$).

atrial contraction at both 10 and 15 sec following its addition to the muscle bath, a significant rise in the atrial levels of cGMP preceded the decrease in force of contraction by approximately 5 sec.

Table I also shows the effects of atropine (1×10^{-7} *M*) on changes in atrial force of contraction and cyclic nucleotide content produced by acetylcholine (5×10^{-7} *M*). Atropine alone caused no significant ($P < 0.05$) alteration in either force of contraction or cyclic nucleotide content of the isolated atria. However, pretreatment of the tissue with atropine successfully attenuated both the decrease in force of contraction and the rise in atrial cGMP content brought about by addition of acetylcholine to the muscle bath.

In situ rabbit atrium/vagus nerve preparation. Table II illustrates the effect of vagus nerve stimulation on right atrial cyclic nucleotide content and heart rate. The atrial levels of cGMP (expressed as percentage of control) were significantly ($P < 0.05$) elevated at 3 sec (125 ± 7%), 5 sec (135 ± 15%), and 15 sec (175 ± 37%) following the onset of vagal stimulation, but had returned to near control levels (105 ± 6%) at the end of 30 sec of stimulation, even in the face of a continued slowing of heart rate. At no time period studied was the atrial content of cAMP found to be significantly ($P > 0.05$) different from that of the control tissues.

Table III shows the effects of pretreatment with atropine (2 mg/kg, iv) on the ability of vagal nerve stimulation to alter heart rate and atrial cGMP content. Atropine pre-treatment was observed to prevent both the decrease in heart rate induced by vagal stimulation as well as the increase in atrial cGMP content associated with this stimulus.

TABLE II. EFFECTS OF VAGAL NERVE STIMULATION ON RABBIT RIGHT ATRIAL CYCLIC NUCLEOTIDE CONTENT AND HEART RATE.

Time (sec)	*n*	Rate (% control)	cGMP (% control)	cAMP (% control)
0	9	100 ± 5	100 ± 4	100 ± 3
3	6	62 ± 8*	125 ± 7*	95 ± 4
5	6	62 ± 11*	135 ± 15*	90 ± 7
15	7	52 ± 6*	175 ± 37*	96 ± 5
30	7	46 ± 5*	105 ± 6	102 ± 2

* Significantly different from control values ($P < .05$).

TABLE III. EFFECTS OF VAGAL NERVE STIMULATION ON RABBIT RIGHT ATRIAL CYCLIC GMP CONTENT AND HEART RATE FOLLOWING ATROPINE PRETREATMENT.

Experimental condition	*n*	Rate (% control)	cGMP (% control)
Atropine[a]	4	100 ± 4	100 ± 7
Atropine[a] + vagal stimulation (15 sec)	4	93 ± 2	89 ± 7

[a] Atropine pretreatment = 2 mg/kg atropine sulfate, iv.

In an attempt to assign a functional role to the alteration in atrial cGMP content observed following cholinergic stimulation, cGMP changes were plotted against either changes in the rate of the *in situ* rabbit atrium during vagal stimulation (Fig. 1), or against the changes in force of contraction of the isolated paced rabbit atrium following addition of acetylcholine (Fig. 2). The results of these plots indicate no correlation between changes in atrial cGMP content and changes in atrial rate produced by vagal stimulation ($r = -0.26$), but a good correlation between alterations in atrial cGMP content and changes in force of contraction brought about by administration of acetylcholine to isolated paced rabbit atria ($r = 0.75$).

Discussion. Results of the present study demonstrate that muscarinic cholinergic stimulation of rabbit atrial muscle, both *in vitro* and *in vivo,* causes an increase in atrial content of cGMP which occurs with, or precedes the mechanical change produced by such stimulation. In addition, the data show that cholinergic stimulation of the rabbit atrium does not significantly alter the atrial level of cAMP. These findings are in agreement with earlier studies involving the isolated whole rat heart (7). However, it is difficult based on current evidence to assign a definite role to cGMP in the regulation of atrial performance. In our studies, we could find no correlation between the increase in atrial cGMP level produced by vagal stimulation and the concomitant decrease in atrial rate of contraction. This result is in disagreement with previous findings (8) utilizing the isolated spontaneously beating rat heart given acetylcholine, where whole heart

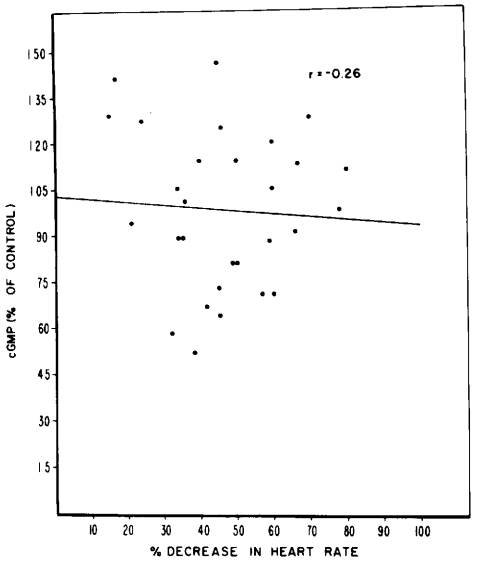

FIG. 1. Relationship between heart rate during vagal stimulation and atrial cGMP content in the *in situ* rabbit atrium. A correlation coefficient of −0.26 was obtained.

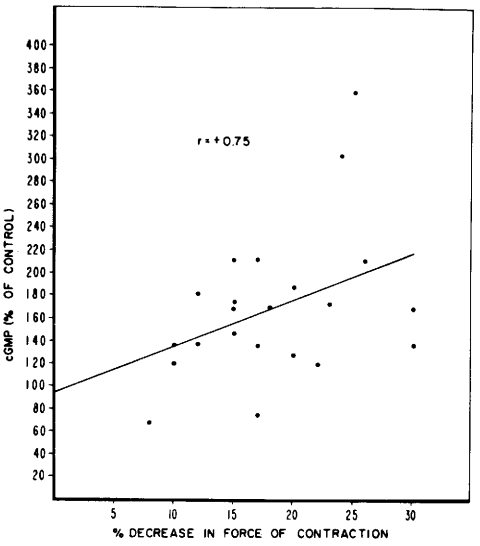

FIG. 2. Relationship between force of contraction and atrial cGMP content in the isolated paced rabbit atrium following addition of acetylcholine ($5 \times 10^{-7}M$). A correlation coefficient of 0.75 was obtained.

cGMP content showed a strong inverse correlation with heart rate. Obviously, nonuniform changes in cyclic nucleotide level within the heart (i.e., atrium vs ventricle, or myocardium vs nodal tissue) could account for the observations, but as yet no such differences have been reported.

In contrast, studies with several different cardiac preparations have shown a decreased inotropic effect associated with an increased tissue content of cGMP (16, 17). In the latter studies (17), increases in myocardial cGMP content preceded the effects of cholinergic stimulation, similar to the results obtained with the atrial preparations of the present report (Tables I and II). Additionally, exogenous cGMP and its derivatives have been demonstrated to decrease force of contraction in preparations of isolated atria (18). Although force of contraction was not determined in our *in situ* rabbit atrium/vagus nerve preparation, the isolated rabbit atria did show a correlation between tissue cGMP content and the decreased isometric force development obtained upon addition of acetylcholine. Even though further *in vivo* studies are required, it seems likely that cGMP may play an important role in the intracellular regulation of cardiac contractility. The mechanism whereby cGMP may exert an effect on cardiac contraction is unclear at present, but interactions with the cAMP system (19) or alterations in calcium fluxes (8) have been previously suggested.

These data provide the first evidence that cardiac cyclic nucleotide levels are subject to alteration through physiological (vagal activation) as well as pharmacological (acetylcholine) intervention.

Summary. In studies designed to investigate the effects of cholinergic stimuli on myocardial cyclic nucleotide content and myocardial performance, an isolated paced rabbit atrial preparation and an *in situ* rabbit right atrium/vagus nerve preparation were utilized. Following vagal stimulation or acetylcholine treatment, increases in atrial cGMP content preceded or occurred at the same time as the mechanical changes associated with cholinergic stimulation. Atrial cAMP content was not affected by these stimuli. Increased cGMP levels were correlated with the decreased force of contraction, but not the decreased chronotropic effects of cholinergic stimulation. These data support the concept that modifications in cardiac performance may be mediated by cGMP. In particular, cGMP may mediate the pharmacological effects of acetylcholine

as well as the physiological effects of vagal stimulation.

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