Dissociation between the Electrophysiological Properties and Total Tissue Cyclic Guanosine Monophosphate Content of Guinea Pig Atria

MICHAEL J. MIRRO, JOHN C. BAILEY, AND AUGUST M. WATANABE

SUMMARY The purpose of this study was to investigate the role of cyclic guanosine monophosphate (cyclic GMP) in mediating the direct electrophysiological effects of acetylcholine in guinea pig atria. Acetylcholine significantly diminished spontaneous rate of right atria without increasing cyclic GMP content. Reductions in rate following acetylcholine were augmented by pretreatment with physostigmine, but cyclic GMP levels remained unchanged. In left atria, acetylcholine significantly shortened action potential duration within 5 seconds (both with and without physostigmine pretreatment), but cyclic GMP content was not significantly elevated. Cyclic GMP levels in right atria were significantly increased in response to acetylcholine when the Ca\textsuperscript{2+} content of the buffer was elevated from 1.25 mM to 2.5 mM; however, reductions in automaticity in the right atria were not augmented in the high Ca\textsuperscript{2+} buffer. Marked increases in cyclic GMP content were produced by Na nitroprusside superfusion without changing automaticity of right atria or action potential duration of left atria. Finally, both right and left atria were superfused with cyclic GMP analogs (8-bromo cyclic GMP and dibutyryl cyclic GMP) at high concentrations (10^{-4}) for 15 minutes without producing significant effects on spontaneous rate or action potential duration. These results failed to show a correlation between total tissue cyclic GMP content and the electrophysiological effects of acetylcholine on guinea pig atria. The reasons for this are either that cyclic GMP does not mediate directly the electrophysiological effects of acetylcholine, or that small changes in cyclic GMP concentrations, undetectable when total tissue nucleotide levels are measured, occur in discrete effector pools of the cardiac cell to mediate the intracellular effects of the choline ester.
Effects on ventricular muscle. By contrast, acetylcholine and its analogs have marked direct electrophysiological effects on atria. Stimulation of atrial muscarinic receptors results in shortening of action potential duration and hyperpolarization, independent of changes in rate (Hoffman and Suckling, 1953; Webb and Holland, 1956; Furchgott et al., 1960; Hoffman and Cranefield, 1969). In right atria, cholinergic agonists decrease automaticity primarily by reducing the slope of phase 4 depolarization in the sinoatrial node (Hoffman and Suckling, 1953; Hoffman and Cranefield, 1969; Vassalle, 1971). These changes in electrophysiological properties are accompanied by marked increases in membrane conductance to potassium (Harris and Hunter, 1956; Fozzard and Sleator, 1967).

The few published studies regarding cyclic GMP levels and electrophysiological properties of atria both support and vitiate the hypothesis that cyclic GMP mediates the electrophysiological effects of muscarinic cholinergic agonists (Nawrath, 1976; Nawrath, 1977). For example, Nawrath recently demonstrated that 8-bromo cyclic GMP shortened action potential duration of rat left atria. On the other hand, however, the same author showed that 8-bromo cyclic GMP did not change the spontaneous rate of depolarization of rat right atria (Nawrath, 1976). Furthermore, the ionic conductance changes observed with cholinergic agonists were not mimicked by administration of cyclic GMP analogs (Nawrath, 1976). Thus, although muscarinic cholinergic agonists produce their most marked electrophysiological effects on atria, the relationship between the changes in electrophysiological properties and cyclic GMP levels that result from these agonists has been studied to a limited extent, and the role of this nucleotide in mediating the effects of acetylcholine remains uncertain. The purpose of the present study was to assess the role of cyclic GMP in mediating the electrophysiological effects of muscarinic receptor agonists on guinea pig atria.

Methods

Electrophysiological Studies

Guinea pigs of either sex, weighing 400–600 g, were injected intraperitoneally with heparin sulfate (500 U) 30 minutes prior to use. Each guinea pig was stunned with a blow to the head, after which the heart was removed rapidly and placed in cool oxygenated Tyrode’s solution. The left atrial appendage and right atrium (including the appendage and the area of the sinus node) were removed and the ventricles discarded. The atrial tissues were affixed to the floor of a wax-bottomed Lucite muscle chamber that was superfused constantly with Tyrode’s solution, gassed with 95% O2-5% CO2, and maintained at a temperature of 37 ± 0.5°C. The composition of the Tyrode’s solution was (mM): Na+, 138; K+, 4.0; Cl−, 128; Ca2+, 1.25 (or 2.5 when noted); HCO3−, 20.0; H2PO4−, 0.9; Mg2+, 0.5; and glucose, 5.5. The osmolarity of this solution was 285 mOsm/liter and the pH was 7.4.

Conventional microelectrode techniques were used to record transmembrane action potentials of the atrial tissues. The action potentials were displayed on a Tektronix 5100 series oscilloscope and were photographed with a Tektronix C-59 Polaroid oscilloscope camera. In addition, the experiments were recorded continuously with an eight-channel Honeywell 7600 tape recorder. To record the rate of spontaneous discharge of the right atrium, an action potential-triggered tachometer (MECA CLC-1) was used. The tachometer produced a linear ramp that was triggered by the upstroke of each successive action potential. The tachometer was calibrated by stimulating the preparation at known constant, basic cycle lengths and measuring the height of the ramp. The tissue was superfused in the muscle chamber for 15 minutes (while recording control spontaneous cycle length) prior to starting any experimental intervention. Left atria were paced at a basic cycle length of 500 msec (bipolar extracellular stainless steel electrode) for a control period of 15 minutes. Electrophysiological parameters (transmembrane resting potential, action potential amplitude, and action potential duration at 50% and 75% repolarization) were measured from Polaroid photographs obtained at specific times, during the control period and after beginning the drug superfusion. Shortening of action potential duration at 75% repolarization was the most reproducible change noted in action potential configuration following exposure to cholinergic agonists; therefore, this parameter was measured to quantify the electrophysiological response of left atria to muscarinic cholinergic agonists.

Drugs were administered by continuous infusion into the superfusant along with an initial bolus of drug into the muscle chamber to attain the desired drug concentration at time zero. In the experiments in which the effect of acetylcholinesterase inhibition was investigated, the tissue was pretreated with physostigmine (10^{-6} M) for 5 minutes, and then physostigmine (10^{-6} M) plus acetylcholine were administered. Following drug exposure, the tissue was frozen at specific times (5, 10, 15, 30, and 60 seconds of drug exposure) and stored in liquid nitrogen at −196°C until assay. The time interval between removing the tissue from the muscle chamber and freezing it was no greater than 1 second.

Cyclic GMP Assay

Cyclic GMP concentrations were measured in the same tissues that were studied electrophysiologically. Tissues in which cyclic GMP levels were to be measured were frozen quickly (≤ 1 second) by immersing them in Freon, which had been cooled to the temperature of liquid nitrogen. The frozen atria were stored in liquid nitrogen until assayed. In preparation for assay, the tissues were pulverized.
with a mortar and pestle, which had been cooled to the temperature of liquid nitrogen. The powdered tissue was homogenized in 5% trichloracetic acid, which was removed by extraction with ether. The extracted homogenate was introduced into a 5 x 25-mm ion exchange column (Dowex AG 1X8, 200-400 mesh, formate form) that had been equilibrated previously with 0.1 N formic acid. Cyclic GMP was eluted with 4 N formic acid. After the eluate was freeze-dried to concentrate cyclic GMP and the formic acid was removed, the lyophilized nucleotide was resuspended in 50 mM sodium acetate buffer (pH 6.2) for assay. Prior to homogenization of the tissue, tracer amounts of tritiated cyclic GMP (4400 dpm or 0.13 pmol) were added to the powdered hearts to check for recoveries (subtracted from the total cyclic GMP measured). A portion of the resuspended nucleotide was counted to quantify recoveries, which were usually in the range of 60-75% for cyclic GMP. The values reported for cyclic GMP have been corrected for recoveries. Cyclic GMP concentrations were determined by radioimmunoassay, as previously described, except that the cyclic nucleotide was succinylated to increase the sensitivity of the assay (Watanabe and Besch, 1975).

**Drugs**

Acetylcholine chloride, physostigmine sulfate, Na nitroprusside, dibutyryl cyclic GMP, and 8-bromo cyclic GMP were purchased from Sigma Chemical Company. [3H]cyclic GMP (specific activity, 19 Ci/mmol) was purchased from Amersham-Searle. [35S]sc-cyclic GMP was purchased from Collaborative Research.

**Statistical Analysis**

The electrophysiological data were analyzed by the paired Student’s t-test since, in each experiment, a continuous single cell impalement was maintained, and each observation was dependent on its own control. The cyclic nucleotide data were analyzed by the Student’s unpaired t-test and analysis of variance. All results are expressed as the mean ± standard error of the mean. The difference between the means were considered significant when P < 0.05 (Dixon and Massey, 1969).

**Results**

**Effect of Acetylcholine on Electrophysiological Properties and Cyclic GMP Content of Guinea Pig Atria**

Acetylcholine produced concentration-dependent reductions in automaticity of spontaneously depolarizing guinea pig right atria, as expected. The threshold concentration for this effect was 10⁻⁶ M. The effect of this concentration of acetylcholine is shown in Figure 1. Spontaneous rate began to slow within a few seconds of the beginning of superfusion and maximal slowing occurred after 15 seconds, after which the rate remained stable (Fig. 1). Similarly, 10⁻⁵ M acetylcholine produced marked slowing of spontaneous rate within 10 seconds after beginning superfusion, and the rate remained slowed through 60 seconds of drug treatment (control rate, 236 ± 10 depolarization per minute; rate after 60 seconds, 175 ± 19; P < 0.01). Cyclic GMP concentrations were not significantly elevated after 5, 10, 15, 30, or 60 seconds of 10⁻⁶ M acetylcholine superfusion (Fig. 1). After 60 seconds of superfusion with 10⁻⁵ M acetylcholine, cyclic GMP levels also were not elevated (Table 1).

In guinea pig left atria, which were paced at a constant basic cyclic length of 500 msec, acetylcholine produced shortening of action potential duration, as has been described previously by many investigators (Hoffman and Suckling, 1953; Webb and Hollander, 1956; Furchgott et al., 1960; Hoffman and Cranefield, 1960). The threshold concentration after which the rate remained stable (Fig. 1). Similarly, 10⁻⁵ M acetylcholine produced marked slowing of spontaneous rate within 10 seconds after beginning superfusion, and the rate remained slowed through 60 seconds of drug treatment (control rate, 236 ± 10 depolarization per minute; rate after 60 seconds, 175 ± 19; P < 0.01). Cyclic GMP concentrations were not significantly elevated after 5, 10, 15, 30, or 60 seconds of 10⁻⁶ M acetylcholine superfusion (Fig. 1). After 60 seconds of superfusion with 10⁻⁵ M acetylcholine, cyclic GMP levels also were not elevated (Table 1).

**Table 1** Effect of Acetylcholine Alone or Combined with Physostigmine on Cyclic GMP Content of Guinea Pig Atria

<table>
<thead>
<tr>
<th>Cyclic GMP content (fmol/mg wet wt)</th>
<th>Basal</th>
<th>Acetylcholine (10⁻⁶ M)</th>
<th>Acetylcholine (10⁻⁵ M) + physostigmine (10⁻⁶ M)</th>
<th>Acetylcholine (10⁻⁵ M)</th>
<th>Acetylcholine (10⁻⁴ M) + physostigmine (10⁻⁵ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left atria</td>
<td>14 ± 1 (36)</td>
<td>15 ± 2 (12)</td>
<td>12 ± 2 (11)</td>
<td>11 ± 2 (9)</td>
<td>17 ± 3 (6)</td>
</tr>
<tr>
<td>Right atria</td>
<td>17 ± 1 (32)</td>
<td>19 ± 2 (16)</td>
<td>17 ± 3 (12)</td>
<td>13 ± 2 (12)</td>
<td>18 ± 3 (9)</td>
</tr>
</tbody>
</table>

Tissues were frozen after 60 seconds of drug superfusion. (Numbers in parentheses indicate n.)
tration of acetylcholine’s effect was $10^{-7} \text{M}$ at which concentration action potential duration (75%) was significantly reduced from $58 \pm 3 \text{msec}$ to $47 \pm 4 \text{msec}$ after only 15 seconds of superfusion. Acetylcholine, $10^{-6} \text{M}$ produced greater effects, as illustrated in Figure 2. Action potential duration was significantly shortened after 5 seconds and then remained stable for up to 60 seconds of superfusion. Acetylcholine, $10^{-5} \text{M}$, produced the most marked effects: action potential duration was reduced from $59 \pm 2 \text{msec}$ to $26 \pm 4 \text{msec}$ after 15 seconds and $17 \pm 3 \text{msec}$ after 60 seconds of superfusion. In spite of these dramatic effects of the choline ester on action potential duration which occurred within 5 seconds of drug exposure, in the same tissues $10^{-6} \text{M}$ acetylcholine produced no significant change in cyclic GMP levels after 5, 10, 15, 30, and 60 seconds of superfusion. There appeared to be a small transient rise in cyclic GMP levels after 5 and 10 seconds of superfusion, but these changes were not statistically significant. Acetylcholine, $10^{-5} \text{M}$, which produced the most marked effects on action potential duration, did not significantly increase cyclic GMP concentrations even after 60 seconds of superfusion (Table 1).

Effect of Acetylcholine Plus Physostigmine on Electrophysiological Properties and Cyclic GMP Content of Guinea Pig Atria

It is likely that exogenous acetylcholine was hydrolyzed rapidly by tissue acetylcholinesterases, and therefore, the effects of the administered neurotransmitter were probably attenuated by this enzyme. This situation was used to explore further the relationship between changes in tissue cyclic GMP levels and electrophysiological effects of muscarinic cholinergic agonists. As previously reported (Webb and Hollander, 1956), the efficacy of acetylcholine in reducing spontaneous rate was enhanced when it was given during inhibition of acetylcholinesterase; the threshold concentration of acetylcholine was reduced to $10^{-7} \text{M}$ (control rate, $158 \pm 15$ depolarizations per minute; rate after 15 seconds of acetylcholine with physostigmine, $138 \pm 16$ depolarizations per minute ($P < 0.05$)). The effects of $10^{-6} \text{M}$ acetylcholine also were augmented significantly by physostigmine (Fig. 3). The most marked effects were seen with $10^{-5} \text{M}$ acetylcholine plus physostigmine when action potential duration was shortened from $46 \pm 4$ to $10 \pm 2 \text{msec}$ after 60 seconds of superfusion.

Although the electrophysiological effects of acetylcholine were potentiated markedly by physostigmine, there were no significant changes in cyclic GMP content of right atria with $10^{-6} \text{M}$ or $10^{-5} \text{M}$ acetylcholine; in left atria, cyclic GMP content was

of $10^{-6} \text{M}$ acetylcholine plus physostigmine is shown in Figure 4. The most marked effects were seen with $10^{-5} \text{M}$ acetylcholine plus physostigmine when action potential duration was shortened from $46 \pm 4$ to $10 \pm 2 \text{msec}$ after 60 seconds of superfusion.

Like the effects of physostigmine on rate changes induced by acetylcholine, the cholinesterase inhibitor also potentiated acetylcholine’s effects on action potential duration in paced left atria; however, the threshold concentration for acetylcholine’s effect on action potential duration remained at $10^{-7} \text{M}$. With $10^{-7} \text{M}$ acetylcholine, action potential duration was shortened significantly from $47 \pm 3 \text{msec}$ to $37 \pm 2 \text{msec}$ after 15 seconds of superfusion. The effect

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Time course of the effect of acetylcholine $10^{-6} \text{M}$ on action potential duration (measured at 75% repolarization) and cyclic GMP content in guinea pig left atria. Values are means ± SE for 10-36 hearts. *$P$ < 0.05 compared to control.

![Figure 3](http://circres.ahajournals.org/)

**Figure 3.** Time course of the effect of acetylcholine $10^{-6} \text{M}$ (●) and acetylcholine $10^{-5} \text{M}$ plus physostigmine $10^{-6} \text{M}$ (○) on spontaneous rate in guinea pig right atria. Values are means ± SE for 18-24 hearts. *$P$ < 0.05 compared to control (paired t-test). +$P$ < 0.05 compared to same time point without physostigmine (unpaired t-test).
only minimally elevated after 60 seconds of treatment with $10^{-5} \text{ M}$ acetylcholine (Table 1).

**Effect of Acetylcholine on Electrophysiological Properties and Cyclic GMP Content of Guinea Pig Atria Superfused with "High" Calcium Buffer**

Increased $\text{Ca}^{2+}$ concentrations in incubation media or perfusion buffers potentiates cholinergic-induced elevations of tissue cyclic GMP levels (Schultz et al., 1973). Accordingly, experiments were performed with buffer containing 2.5 mM, rather than the normal 1.25 mM $\text{Ca}^{2+}$, to attempt to augment the cholinergic-induced elevation of atrial cyclic GMP content. All experiments were performed in the presence of physostigmine. The basal levels of cyclic GMP in right atria were the same in buffer containing 1.25 mM or 2.5 mM $\text{Ca}^{2+}$ (Fig. 5). However, the biochemical response of the tissues to acetylcholine was magnified in buffer containing 2.5 mM $\text{Ca}^{2+}$; cyclic GMP levels in "high" $\text{Ca}^{2+}$ buffer increased from 16 ± 2 to 34 ± 9 fmol/mg wet weight with $10^{-6}$ M acetylcholine plus physostigmine (Fig. 5). Atria treated identically, except that the buffer contained only 1.25 mM $\text{Ca}^{2+}$, did not demonstrate a significant increase in cyclic GMP content. In spite of the augmented formation of cyclic GMP in the atria bathed in "high" $\text{Ca}^{2+}$ buffer, however, the change in spontaneous rate in the two groups of tissues was not significantly different (Fig. 5). Thus, exposure of right atria to cholinergic agonists in the presence of 2.5 mM $\text{Ca}^{2+}$ produced an augmented biochemical response (increased cyclic GMP) without augmenting the measured electrophysiological response (reduction in spontaneous rate).

**Effect of Na Nitroprusside on Electrophysiological Properties and Cyclic GMP Content of Guinea Pig Atria**

Recently it has been demonstrated that Na nitroprusside is a potent activator of guanylate cyclase in a variety of tissue, including the heart (Katsuki et al., 1977). Thus, this agent allowed us to elevate intracellular cyclic GMP levels without activating muscarinic cholinergic receptors and thereby provided another means of examining the relationship between electrophysiological properties and cyclic GMP content of guinea pig atria.

Na nitroprusside produced marked increases in tissue cyclic GMP levels of both right and left atria (Fig. 6). With $10^{-3} \text{ M}$ Na nitroprusside treatment for 60 seconds, cyclic GMP levels were increased 20-fold in right atria and 32-fold in left atria. In spite of these marked changes in cyclic GMP concentrations, the electrophysiological properties of the atria were unchanged (Table 2). Furthermore, longer exposure to Na nitroprusside ($10^{-3} \text{ M}$ for 3 minutes) resulted in significant prolongation of action potential duration in left atria from 46 ± 3 to 51 ± 3 msec ($P < 0.01$) and a significant increase in spontaneous rate of right atria from 256 ± 15 to 277 ± 21 depolarizations per minute ($P < 0.05$). That is, this high concentration of Na nitroprusside produced electrophysiological effects opposite to those observed with acetylcholine. In these tissues, cyclic GMP levels were elevated markedly after 3 minutes.
of exposure to Na nitroprusside (10^{-3} M) (left atria: 308 ± 50 fmol/mg wet weight; right atria: 274 ± 33 fmol/mg wet weight). When Na nitroprusside (10^{-3} M) was given in combination with acetylcholine (10^{-6} M), it did not augment or attenuate the shortening in action potential duration produced by the choline ester in left atria (Fig. 7).

**Lack of Effect of Cyclic GMP Derivatives on Electrophysiological Properties of Guinea Pig Atria**

Both dibutryl cyclic GMP and 8-bromo cyclic GMP were administered to guinea pig atria as another means of increasing tissue levels of cyclic GMP without activating muscarinic cholinergic receptors. Neither of these compounds in a concentration of 10^{-4} M produced significant changes in either spontaneous rate (right atria) or action potential duration (left atria) after superfusion for 15 minutes.

**Table 2: Lack of Effect of Na Nitroprusside on the Electrophysiological Properties of Guinea Pig Atria**

<table>
<thead>
<tr>
<th>Concentration of Na nitroprusside (M)</th>
<th>Right atria</th>
<th>Left atria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rate*</td>
<td>Rate after 60 seconds of drug</td>
<td>Control APD after 60 seconds of drug</td>
</tr>
<tr>
<td>10^{-5}</td>
<td>193 ± 11</td>
<td>49 ± 4</td>
</tr>
<tr>
<td>10^{-4}</td>
<td>204 ± 7</td>
<td>54 ± 4</td>
</tr>
<tr>
<td>10^{-3}</td>
<td>207 ± 17</td>
<td>45 ± 3</td>
</tr>
</tbody>
</table>

*Rate expressed as depolarizations per minute (mean ± SE) for 10-13 hearts.

† Action potential duration (APD), measured at 75% repolarization, expressed in milliseconds (mean ± SE) for 11-13 hearts.

**Discussion**

Stimulation of vagal nerves or administration of cholinergic agonists produces marked electrophysiological effects on cardiac atria (Hoffman and Suckling, 1953; Webb and Hollander, 1956; Furchgott et al., 1960; Hoffman and Cranefield, 1960; Vassalle, 1971; Harris and Hunter, 1956; Fozzard and Sleator, 1967; Higgins et al., 1973). Among these effects, perhaps the most reproducible and easily demonstrable are reductions in spontaneous rate of right atria and shortening of action potential duration of either atria. These electrophysiological parameters therefore were chosen in an attempt to determine whether cyclic GMP mediates the electrophysiological effects in atria of muscarinic cholinergic receptor stimulation.

In the present study, there was not a correlation between spontaneous rate or action potential duration and total tissue cyclic GMP concentration in guinea pig atria. In the first series of experiments, stimulation of muscarinic cholinergic receptors produced significant electrophysiological effects without changing tissue cyclic GMP levels. Acetylcholine significantly slowed spontaneous rate of right atria and shortened action potential duration of left atria, but cyclic GMP levels remained unchanged. The foregoing electrophysiological effects of acetylcholine were augmented substantially by the cholinesterase inhibitor, physostigmine, but cyclic GMP levels still were not uniformly increased.

In the second series of experiments, increases in cyclic GMP levels were produced without any accompanying changes in the measured electrophysiological properties of the atria. In the presence of unphysiologically high free ionized Ca^{2+} concentra-
tions (2.5 mM), acetylcholine significantly increased tissue cyclic GMP levels. However, the electrophysiological effects of the cholinergic receptors were the same when the tissues were bathed with buffer containing 2.5 mM Ca²⁺ or 1.25 mM Ca²⁺. Na nitroprusside produced marked elevations in tissue cyclic GMP levels, presumably by directly activating the soluble form of cardiac guanylate cyclase (Kimura and Murad, 1974). This agent, which has no effect on muscarinic cholinergic receptors, after 60 seconds did not change the electrophysiological properties of the atria. Finally, two analogs of cyclic GMP, given in high concentrations for a relatively long period of time, failed to produce any electrophysiological effects on either right or left atria. These latter data must be interpreted with caution, particularly because the results were negative. It is not known for certain that the analogs of cyclic GMP penetrated into the atrial cell membranes and then actually increased cyclic GMP levels (Miller et al., 1973). However, taken together with the results of other experiments, these findings appeared to show a dissociation between tissue cyclic GMP content and the measured electrophysiological properties of atria. Thus, the results of the present study extend our earlier observations (Watanabe and Besch, 1975) and two other recently published studies, which failed to show a correlation between total tissue cyclic GMP levels and the negative inotropic effects of acetylcholine (Brooker, 1977; Diamond et al., 1977).

From the present results it might be concluded that cyclic GMP does not play a role in mediating the intracellular effects of acetylcholine. It is possible that activation of muscarinic cholinergic receptors leads directly to changes in membrane conductance to certain ions and that the associated increases in tissue cyclic GMP levels, sometimes seen with muscarinic cholinergic receptor activation, are merely parallel and unrelated phenomena. On the other hand, it is possible that cyclic GMP does mediate the intracellular effects of acetylcholine, but that the levels of the nucleotide need change only a small amount in a discrete effector compartment of the cardiac cell (e.g., sarcolemma), and that this small regional increase was not detectable when total tissue cyclic GMP levels were measured. In this latter formulation, when experiments were conducted so that changes in total tissue cyclic GMP levels could be measured, it could be concluded that the electrophysiological responses were unchanged because the augmented tissue cyclic GMP concentrations were superfluous because a critical (but unmeasurable) change in concentration of the nucleotide had already occurred in a discrete effector compartment of the cell. Similarly, it is possible that even though Na nitroprusside markedly elevated total tissue cyclic GMP levels, this increase occurred in an electrophysiologically noncritical area of the cell (e.g., cytoplasm), and therefore, the nucleotide did not evoke any electrophysiological changes. That such compartmentation of cyclic GMP in cardiac cells may occur is suggested by recent evidence from our laboratory, which indicates that cardiac tissue has both particulate and soluble guanylate cyclase and that the particulate form of the enzyme is localized to the sarcolemma (Revtyak et al., 1978). Furthermore, the two forms of the enzyme are regulated differentially: the particulate, but not the soluble form of the enzyme, is activated by lubrol, whereas only the soluble form is activated by Na nitroprusside. Other investigators have observed similar results with other tissues (Kimura and Murad, 1974).

Moreover, it has been shown in intact tissues that the concentration of cyclic GMP can increase in one compartment while simultaneously decreasing in another compartment of the cell, so that total tissue nucleotide levels do not change (Koide et al., 1978). It is not possible with presently available data to decide whether (1) cyclic GMP does not mediate the measured electrophysiological effects of acetylcholine, or (2) small, but undetectable, changes in levels of the nucleotide occur in discrete effector pools of the cardiac cell, and that these localized changes mediate the effects of acetylcholine. However, it is clear that there is not a straightforward relationship between total tissue cyclic GMP levels and electrophysiological effects of acetylcholine. Additional studies in which nucleotide levels in cellular compartments are measured are needed before it can be concluded definitively whether cyclic GMP is involved in mediating the direct electrophysiological effects of acetylcholine.

A possible explanation for the differing results regarding cyclic GMP and cardiac effects of acetylcholine which exist in the literature may be the difference in Ca²⁺ concentrations of the buffers used to perfuse or superfuse the tissues studied. It is well established that Ca²⁺ is required for muscarinic cholinergic stimulation of guanylate cyclase activity in intact tissues (Schultz et al., 1973). Results in the present study have demonstrated that doubling the free ionized Ca²⁺ concentration from 1.25 mM to 2.5 mM augmented the effects of acetylcholine on cyclic GMP accumulation. Most of the early studies that attempted to relate cyclic GMP levels and functional effects of acetylcholine in the heart used 4.0 mM Ca²⁺ in the buffers (George et al., 1970; Kuo et al., 1972; George et al., 1975). Therefore, in these latter experiments, muscarinic cholinergic agonists simultaneously increased cyclic GMP levels while they produced negative inotropic effects. By contrast, in the present study and that of Brooker (1977), the Ca²⁺ concentration in the buffers was 1.25 mM and 1.50 mM, respectively, and under these conditions, functional changes occurred in the cardiac tissues without any elevation of cyclic GMP levels.

When the biochemical basis for the electrophysiological effects of acetylcholine are investigated, the specific electrophysiological effect under study...
must be defined clearly because the choline ester can produce multiple and varied effects on the heart depending upon the tissue in question. Although in the present study there was not a correlation between the direct electrophysiological effects of acetylcholine and cyclic GMP levels in atria, other possible actions of cyclic GMP have not been eliminated. It is possible that cyclic GMP is involved in the cellular mechanisms of adrenergic-cholinergic antagonism in cardiac ventricles. Several lines of evidence accumulated over the past 2 decades indicate that activation of muscarinic cholinergic receptors antagonizes the inotropic (Levy, 1971; Watanabe and Besch, 1975; Levy, 1977), electrophysiological (Kent et al., 1973; Watanabe et al., 1978a; Bailey et al., 1979), and metabolic (Gardner and Allen, 1976; Hess et al., 1962; Murad et al., 1962; LaRaia and Sonnenblick, 1971; Wilkerson et al., 1976; Keeley et al., 1978; Lindemann et al., 1979; Watanabe et al., 1978b) effects of β-adrenergic receptor stimulation. We previously suggested that this effect of acetylcholine might be mediated partially through cyclic GMP, because the choline ester antagonized the inotropic effects of isoproterenol without attenuating the amount of cyclic AMP formed in response to β-adrenergic receptor stimulation, and these effects of acetylcholine were mimicked by dibutyryl cyclic GMP (Watanabe and Besch, 1975). These results subsequently have been confirmed by several other investigators. Wilkerson et al. (1976) showed that dibutyryl cyclic GMP antagonized the positive inotropic effects of dibutyryl cyclic AMP in cat papillary muscles. Keeley et al. (1978) recently demonstrated that acetylcholine attenuated the positive inotropic effects of isoproterenol to a greater degree than could be accounted for by the attenuation in the generation of cyclic AMP. Finally, recent studies of A23187 performed in our laboratory suggest that cyclic GMP may antagonize the intracellular effects of cyclic AMP (Lindemann et al., 1979).

Although such a mechanism involving cyclic GMP in the cholinergic antagonism of adrenergic effects is possible, it is unlikely that this is the only mechanism for the reciprocal cellular interaction between the two limbs of the autonomic nervous system. Results from several laboratories, including our own, have demonstrated that muscarinic cholinergic agonists attenuate the amount of cyclic AMP formed in response to β-adrenergic receptor stimulation (Murad et al., 1962; LaRaia and Sonnenblick, 1971; Watanabe et al., 1978b). The mechanism for this effect appears to be an inhibition of the catecholamine stimulation of adenylate cyclase activity (Murad et al., 1962; Watanabe et al., 1978b). Thus, it appears likely that this also may be a cellular mechanism for cholinergic antagonism of adrenergic effects on the heart.

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