ARE INCREASES IN CYCLIC GMP LEVELS RESPONSIBLE FOR THE NEGATIVE INOTROPIC EFFECTS OF ACETYLCHOLINE IN THE HEART?

by

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It has been suggested that increases in cyclic GMP levels are responsible for the negative inotropic effects of acetylcholine in the heart. This hypothesis was tested by monitoring the effects of acetylcholine and sodium nitroprusside on tension and cyclic nucleotide levels in strips of cat atrial appendage. Sodium nitroprusside markedly increased atrial cyclic GMP levels but did not decrease the twitch tension developed by the atrial strips. Low concentrations of acetylcholine, on the other hand, decreased twitch tension without increasing myocardial cyclic GMP levels. No significant change in cyclic AMP levels was observed in any of these experiments. These results are not consistent with the proposed role for cyclic GMP as the mediator of the negative inotropic effects of acetylcholine.

Since 1970 a number of papers have concluded that there is a good correlation between the ability of acetylcholine to decrease cardiac contractility and its ability to increase cyclic GMP levels in the heart (1-6). This was first noted by George et al. (1) in perfused, spontaneously-beating rat hearts where the relationship between tissue levels of cyclic GMP and force of contraction after acetylcholine treatment was found to be approximately linear. Similar results were subsequently reported in electrically-paced rat hearts (2,3), electrically-paced rabbit atria (4,5) and spontaneously-beating cultured rat heart cells (6). In electrically-paced rabbit atria, the increase in cyclic GMP caused by acetylcholine appeared to precede the decrease in contractile force caused by the drug (4,5). A recent report (7), suggesting that

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pretreatment of rabbit atria with an inhibitor of guanylate cyclase can prevent the cardiodepressant effects of acetylcholine, implies that the synthesis of cyclic GMP is necessary for acetylcholine's effects on the heart. It has also been noted that the 8 bromo derivative of cyclic GMP can itself decrease contractile force in various cardiac muscle preparations (8,9,10). All of these results support the hypothesis, originally suggested by George et al. (3), that increases in cardiac cyclic GMP levels are responsible for the negative inotropic effects of acetylcholine.

If the above hypothesis is true, it might be expected that any drug which will increase cyclic GMP levels in the heart will also cause a negative inotropic response. We have previously reported that nitroglycerin can markedly increase cyclic GMP levels in uterine and vascular smooth muscle (11,12) and other workers have shown that both nitroglycerin and sodium nitroprusside can elevate cyclic GMP levels in other tissues as well (13,14). However, preliminary experiments in our laboratory had indicated that neither nitroglycerin nor sodium nitroprusside had any negative inotropic effect on cat atrial strips. Therefore, we felt it was of interest to determine whether these drugs could in fact increase cyclic GMP levels in the atrial preparations as they did in other tissues. If cyclic GMP levels could be increased in atrial myocardium without decreasing contractile force, it would tend to argue against a role for cyclic GMP as a mediator of the negative inotropy. For the sake of comparison, cyclic nucleotide levels were also measured in cat atrial strips during exposure to negatively inotropic concentrations of acetylcholine. The results of these experiments suggest that increases in cyclic GMP levels are not responsible for the decreased force of contraction caused by acetylcholine in this preparation.

METHODS

Male and female cats weighing 2.5 to 4.5 kg were anesthetized with diethyl ether, and their hearts were excised and immediately placed in a conventional Tyrodes solution oxygenated with 95% O_2 - 5% CO_2. Atrial appendages were dissected free from the rest of the heart and each appendage

was then cut along its longitudinal axis into a caudal and cephalic half. Experiments could then be done on a paired basis with one-half of each appendage serving as a control for the other half.

The atrial strips were transferred to 25 ml organ baths and equilibrated at 37°C in Tyrodes solution with the following composition (in mM/liter): NaCl, 140; KCl, 4; $MgCl_2$, 0.5; $CaCl_2$, 2.7; $NaHCO_3$, 12; NaH_2PO_4, 1.8 and dextrose, 5.5. The muscles were oxygenated with 95% O_2 - 5% CO_2 which maintained the pH of the solutions at approximately 7.4. Silk sutures, which had been tied to each end of a muscle, were connected to a stationary hook and a Grass FT-03 force transducer, respectively. Resting tension, recorded by means of a Grass polygraph, was added until it was approximately 90% of the value at the peak of the length-twitch tension curve. The muscles were driven electrically, at a rate of 120/min, via field stimulation with square wave pulses of 5 msec duration and an amplitude approximately 10% above threshold. After equilibration of the muscles for approximately 45 min, freshly prepared solutions of acetylcholine chloride or sodium nitroprusside were added directly to the muscle baths. Test muscles were then frozen, at predetermined times after addition of drugs, using a Wollenberger-type clamp precooled in isopentane at -80°C. Control muscles were equilibrated and frozen in the same way but were not exposed to drugs.

Cyclic nucleotide levels in the frozen muscles were determined essentially as previously described (12). Briefly, the muscles were homogenized in 5% trichloroacetic acid and the trichloroacetic acid removed by washing with diethyl ether. After removal of the ether, the aqueous extracts were acetylated as suggested by Harper and Brooker (15) to increase the sensitivity of the assay. Cyclic nucleotide levels were then determined using the radioimmunoassay techniques of Steiner et al. (16). Levels of cyclic nucleotides are expressed as picomoles per g wet weight of tissue.

RESULTS

EFFECTS OF SODIUM NITROPRUSSIDE ON CYCLIC NUCLEOTIDE LEVELS AND CONTRACTILE FORCE.

Paired strips of cat atrial appendage were suspended in isolated organ baths for recording of isometric tension as described in the methods section. After equilibration, one strip from each pair was clamp-frozen without further treatment and was used as the control. The other strip from each pair was exposed to 10^{-4} M sodium nitroprusside for 60 sec before freezing. This concentration of sodium nitroprusside produced a small but consistent increase in the contractile force developed by the atrial strips (Figure 1 and Table 1). Cyclic GMP levels were increased more than 17 fold by sodium nitroprusside, but cyclic AMP levels were unchanged (Table 1).

EFFECTS OF ACETYLCHOLINE ON CYCLIC NUCLEOTIDE LEVELS AND CONTRACTILE FORCE.

Atrial strips were frozen at different times after addition of either

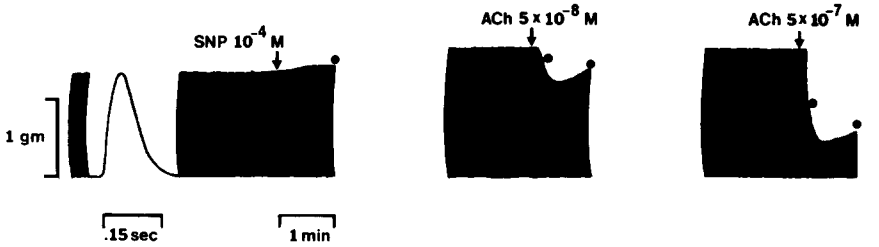

FIGURE 1. Representative tracing illustrating the effects of sodium nitroprusside (SNP) and acetylcholine (ACh) on contractile force of cat atrium. In experiments in which cyclic nucleotide levels were measured, muscles were frozen at points indicated by dots.

5×10^{-8} M or 5×10^{-7} M acetylcholine to the muscle baths. These concentrations of acetylcholine decreased the atrial twitch tension by an average of 25% and 70% respectively (when measured at the time of maximum depression). See Figure 1 for representative tracings illustrating the effects of these concentrations of acetylcholine on tension developed by the atrial strips. As indicated by dots in the figure, muscle samples were frozen at either 15 sec or 60 sec after administration of the two concentrations of acetylcholine. No significant change in cyclic GMP levels was observed at either time after 5×10^{-8} M acetylcholine (Table 1). However, cyclic GMP levels were significantly increased (by about 3.5 fold) 15 sec after 5×10^{-7} M acetylcholine. Even with this concentration of the drug, the levels of cyclic GMP had returned to normal within 60 sec, although contractile force was still markedly depressed at that time. No significant change in cyclic AMP levels was observed in any of these experiments.

EFFECT OF SODIUM NITROPRUSSIDE ON THE NEGATIVE INOTROPIC EFFECT OF ACETYLCHOLINE.

The possibility of an interaction between sodium nitroprusside and acetylcholine on atrial contractile activity was studied by monitoring the effects of 5×10^{-8} M acetylcholine, in the same muscles, in the presence and absence of 10^{-4} M sodium nitroprusside. In the absence of sodium nitroprusside, 5×10^{-8} M acetylcholine decreased the force of contraction by $31.0 \pm 6.2\%$ (N = 8). After

TABLE 1

EFFECTS OF SODIUM NITROPRUSSIDE (SNP) AND ACETYLCHOLINE (ACh) ON CYCLIC NUCLEOTIDE LEVELS AND CONTRACTILE FORCE IN CAT ATRIUM

Treatment	N	Cyclic GMP (pmoles/g tissue)	Cyclic AMP (pmoles/g tissue)	Contractile Force (% change)
Control	7	12.7 ± 2.3	382 ± 88	
SNP (10^{-4}M, 60 sec)	7	218.3 ± 30.9*	383 ± 77	+11.4 ± 1.5*
Control	8	6.8 ± 1.1	750 ± 80	
ACh ($5x10^{-8}$M, 15 sec)	8	6.8 ± 1.5	607 ±103	-14.9 ± 3.3*
Control	6	9.5 ± 1.4	484 ± 48	
ACh ($5x10^{-8}$M, 60 sec)	6	8.3 ± 1.5	394 ± 59	-19.5 ± 7.7*
Control	8	7.6 ± 0.9	471 ± 116	
ACh ($5x10^{-7}$M, 15 sec)	8	26.9 ± 8.4*	463 ± 94	-41.8 ± 3.3*
Control	8	14.5 ± 2.9	343 ± 44	
ACh ($5x10^{-7}$M, 60 sec)	8	13.3 ± 2.3	332 ± 38	-63.0 ± 6.5*

*Significantly different from the corresponding controls ($p < 0.05$ or better). Results are expressed as means ± S.E. of the number of samples indicated (N). Percent change in contractile force represents the difference between pre-drug twitch tension and twitch tension at the time the preparations were frozen.

pre-exposure of the muscles for 1 min to 10^{-4} M sodium nitroprusside, the same concentration of acetylcholine decreased contractile force by 30.3 ± 6.3% (N = 8). Thus, the pretreatment of the atrial strips with sodium nitroprusside had no effect on the negative inotropic response of the muscles to acetylcholine.

DISCUSSION

As mentioned earlier, if an increase in cyclic GMP levels is actually responsible for the negative inotropic effect of acetylcholine in atrial myocardium, then any agent which can directly increase atrial cyclic GMP levels should also have a negative inotropic action. The results of the present study indicate that this is not the case. Marked increases in cyclic GMP levels could be produced in cat atrial strips by concentrations of sodium nitroprusside that caused a slight increase, rather than a decrease, in con-

tractile force. The magnitude of the cyclic GMP increase caused by sodium nitroprusside in our experiments was much greater than that caused by a dose of acetylcholine which decreased atrial contractile force by more than 70%. If the 3.5 fold increase in cyclic GMP levels produced by 5×10^{-7} M acetylcholine is in fact causing the negative inotropic effect of the drug, then the 17 fold increase in cyclic GMP levels produced by sodium nitroprusside should have resulted in an even greater negative inotropic response. However, as shown in Results, sodium nitroprusside had no negative inotropic effect in our experiments. It might be argued that acetylcholine, in addition to elevating cyclic GMP levels, has some other action which allows the cyclic GMP to exert its depressant effect on the heart, and that sodium nitroprusside lacks this additional action. If this is so, pretreatment of atrial strips with sodium nitroprusside (which would markedly increase cyclic GMP levels in the muscles) should enhance the cardiodepressant effects of small doses of acetylcholine. However, in the experiments described above (see Results), 5×10^{-8} M acetylcholine produced the same percent decrease in atrial contractile force in the presence and absence of 10^{-4} M sodium nitroprusside. It might also be argued that sodium nitroprusside and acetylcholine cause cyclic GMP levels to increase in separate intracellular pools and that only the acetylcholine-sensitive pool has any effect on cardiac contractile force. Evidence against this possibility is provided by our experiments with acetylcholine itself. In agreement with previous reports in rabbit atrium (4,5), twitch tension was decreased and cyclic GMP levels were increased in cat atrial strips exposed to 5×10^{-7} M acetylcholine for 15 sec. However, a lower concentration of acetylcholine (5×10^{-8} M) also consistently depressed contractile force of the atrial strips in our experiments, but had no effect on cyclic GMP levels in the muscles. Concentrations of acetylcholine lower than 5×10^{-7} M had not been studied in the earlier experiments with rabbit atrium (4,5). Thus, in the case of sodium nitroprusside we could markedly increase cardiac cyclic GMP levels without depressing contractile force, and in the case of low doses

of acetylcholine we could depress contractile force without increasing cyclic GMP levels. These results do not appear to be consistent with a role for cyclic GMP as a mediator of decreased contractile force in the heart. They do not rule out other possible roles for cyclic GMP in cardiac function such as mediation of acetylcholine's antagonism of the cardiac effects of isoproterenol (17,18). However, in our opinion, increases in cyclic GMP levels are probably not responsible for the negative inotropic effects of acetylcholine on the heart.

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