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Adenosine Cyclic 3',5'-Monophosphate Concentrations during the Positive Inotropic Response of Cat Cardiac Muscle to Polymeric Immobilized Isoproterenol

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SUMMARY

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Changes in cardiac contractility and concentrations of cyclic 3',5'-AMP in isolated cat papillary muscles in response to isoproterenol diazotized to a 12,800 mol wt random copolymer of hydroxypropylglutamine with *p*-aminophenylalanine (copoly-Iso) have been examined. It has been previously shown that copoly-Iso retains positive chronotropic and inotropic effects in perfused guinea pig hearts and isolated cat papillary muscles, respectively, without isoproterenol dissociation. In the present experiments, in isometrically contracting cat papillary muscles copoly-Iso (equivalent to 0.37 μmole of isoproterenol) resulted in prompt increases in the force and velocity of contraction within 20 sec. The copoly-Iso responses reached maximal levels by 180 sec and were similar in magnitude and time course to peak *l*-isoproterenol responses. Cat papillary muscles frozen 30-180 sec subsequent to copoly-Iso addition were subjected to cyclic AMP radioimmune assay. No detectable change in cyclic AMP concentrations were observed during increases in contractility (e.g., cyclic AMP control level was 5.54 ± 0.49 pmoles/mg of protein, $n = 5$). In contrast, papillary muscles frozen 60 sec after soluble *l*-isoproterenol addition had markedly increased cyclic AMP levels (11.56 ± 2.49 pmoles/mg of protein, $n = 6$). Gel permeation chromatography of muscle bath contents at the time of papillary muscle freezing indicated that no isoproterenol dissociated from the matrix during the experiments. These results confirm our previous findings for glass bead immobilized isoproterenol, where nearly maximal inotropic responses were obtained without detectable changes in cyclic AMP concentrations, and place further into question the role of cyclic AMP in catecholamine-induced *beta* receptor-stimulated cardiac contractility.

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INTRODUCTION

Catecholamines interacting with *beta* adrenergic receptors in the myocardium produce well-characterized positive ino-

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tropic and chronotropic responses. Since the work of Sutherland and colleagues (1) it has become a general premise that the cardiac contractile changes produced in response to *beta* adrenergic receptor stimulation are a result of cyclic 3',5'-AMP formation (2). The evidence linking cyclic AMP to inotropic action is circumstantial in nature, as has been discussed in two recent review articles (3, 4). Those authors suggested that the "cyclic AMP hypothesis" may have to be modified. Much of the early experimental data relating cyclic AMP increases to cardiac positive inotropic responses were based on the time course of increases in cyclic AMP and contractility in response to catecholamines (5, 6). Cyclic AMP reaches an apparent maximum within 15 sec of isoproterenol addition to papillary muscle baths (7). This 2–3-fold increase in cyclic AMP levels (1, 7) occurs prior to changes in contractility. The time course of the cyclic AMP increase is also in apparent disparity with the diffusion rate of isoproterenol into the muscle (8).

Venter and co-workers have reported that glass beads containing covalently bound isoproterenol produced a positive inotropic response in isolated cardiac muscle that is similar in magnitude and proceeds with the same time course as that produced with soluble isoproterenol (7). The increases in the contractile force in response to both the glass bead isoproterenol and soluble isoproterenol approached the maximal inotropic response, obtained with paired electrical stimulation (7). However, in contrast to soluble isoproterenol effects, there is no detectable change in cyclic AMP concentration in response to glass bead-immobilized isoproterenol (7). These and other results suggested a propagation throughout the tissue of the signal responsible for the inotropic response from the site of the isoproterenol–*beta* adrenergic receptor interaction (7) and indicated that cyclic AMP was not involved in the actual propagation of the inotropic response. Further support for these findings has come from a recent study by Ingerbretsen *et al.* (9) on cat papillary muscles under normal conditions and in guinea pig muscles partly depolarized with 22 mM potassium. Those workers observed an increase in the inotropic state of the muscle in response to isoproterenol and to isoproterenol-glass beads, as well as an increase in the activation of phosphorylase, with no concomitant change in the formation of cyclic AMP. In conforming our earlier study, they also reached the conclusion that the localized stimulation of receptors at or near the point of contact between isoproterenol-glass beads and muscles resulted directly in the initiation and propagation of an increased inotropic state. Cyclic AMP was clearly not the mediator of this propagated response (9).

In order to decipher the role of cylic AMP in the positive inotropic response to both soluble and immobilized isoproterenol, isoproterenol diazotized to a 12,800 mol wt random copolymer of hydroxypropylglutamine with *p*-aminophenylalanine was prepared. Copoly-Iso[3] is soluble in physiological solutions, but because of its size, its diffusion rate is much slower than that of isoproterenol (8). In addition, it provides for a more homogeneous interaction with the surface of papillary muscles than can isoproterenol immobilized on glass beads. We have previously demonstrated that copoly-Iso elicits a positive inotropic and chronotropic response in cat papillary muscles and isolated perfused guinea pig hearts, respectively, without dissociation of isoproterenol from the matrix (10).

In the present study, the copoly-Iso positive inotropic response in cat papillary muscles was examined in detail. The copoly-Iso response follows the same time course and achieves the same peak tension as responses to the parent isoproterenol. Comparison of the contractile responses to copoly-Iso with those obtained with paired electrical stimulation indicates that the majority of the muscle is involved in the contractile process. However, in contrast to the parent isoproterenol, no changes in

[3] The abbreviations used are: copoly-Iso, isoproterenol diazotized to a 12,800 mol wt random copolymer of hydroxyglutamine with *p*-aminophenylalanine: PES, paired electrical stimulation.

cyclic AMP could be detected during the contractile response to copoly-Iso. This study provides further evidence for a propagated inotropic effect in response to isoproterenol stimulation of superficial layers of cardiac muscle, and raises further questions concerning the exact role of cyclic AMP in cardiac contraction.

MATERIALS AND METHODS

Synthesis and purification of copoly-Iso. *l*-Isoproterenol *d*-bitartrate (Sigma) containing *dl*-[7-^{3}H]isoproterenol (New England Nuclear) as a tracer was diazotized as described (10) to a random copolymer of hydroxypropylglutamine with *p*-aminophenylalanine; ratio, 4.5:1, mol wt 12,800 (Miles Laboratories).

Copoly-Iso was purified by gel chromatography at 4° on a Bio-Gel P-2 (Bio-Rad) column (3 × 120 cm) with 1 mM ammonium bicarbonate as the eluent. The polymeric peak was chromatographed repeatedly until the parent isoproterenol was no longer detectable (four times). The copoly-Iso, without traces of parent isoproterenol (less than 0.01%) or 6-aminoisoproterenol (less than 1.5%), was lyophilized in the dark and stored at −85° until use. Copoly-Iso was repurified every 2 weeks.

Contamination by isoproterenol and 6-aminoisoproterenol was determined as previously described (10, 11).

Biological characterization of copoly-Iso. Copoly-Iso, assayed for biological activity in isolated, perfused guinea pig hearts as previously described (8, 10), produces positive chronotropic and inotropic effects in a dose-related manner. Maximal responses to copoly-Iso and *l*-isoproterenol were identical. The dose-response curve for copoly-Iso displayed a rightward shift of two orders of magnitude from the *l*-isoproterenol curves, as described (10).

Cat papillary muscles. Papillary muscles approximately 1 mm in diameter or less were quickly dissected from the right ventricles of cats (2–3.5 kg) anesthetized with intraperitoneal sodium pentobarbital (40 mg/kg). Muscles were positioned horizontally in Lucite baths. One end of the muscle was held by a Lucite clip attached to a force transducer (Statham), and the tendinous end was tied by a silk thread to the wall of a Lucite bath containing Krebs solution (20 ml) at 30° maintained at pH 7.4 when bubbled continuously with 95% O_2 and 5% CO_2. The muscles were contracted isometrically at 12 times/min with transverse field stimulation provided by a Grass stimulator and two platinum electrodes situated parallel to the muscle. The stimulus voltage was maintained at 2 V above threshold (usually 9–15 V) for 5 msec. Peak isometric force and rate of force development (df/dt) were recorded with a time marker on a forced-ink oscillographic recorder (model 7pl, Grass Instruments). The muscles were stretched to obtain peak isometric tension (L_{max}) by performing a length-tension curve. Muscle length was then adjusted as to obtain peak force equal to one-half the L_{max} force. The muscle lengths were maintained at one-half L_{max} throughout the experiments.

After muscle stabilization, the maximal positive inotropic response was obtained utilizing paired electrical stimulation (10). The stimulus voltage and frequency were maintained as described above. The second stimulus was added at a delay of 340 ± 16 msec (n = 27) after the first stimulus. The exact delay that resulted in the maximal isometric force was determined by increasing the delay in 50-msec steps between 50 and 800 msec. Following muscle restabilization either 1 mg of copoly-Iso (equivalent to 0.37 μmole of isoproterenol) or 0.1 μM (final bath concentration) *l*-isoproterenol was added to the muscle bath to obtain the time course of drug action. This concentration of copoly-Iso produces the maximal inotropic response to this agent.

Cyclic AMP measurement. Papillary muscles were rapidly frozen at the times indicated by freeze-clamping with hemostats that had been cooled in liquid nitrogen. Muscles were maintained at −85° prior to rapid homogenization in 5% trichloracetic acid at 0°. Samples were centrifuged for 5 min in a Microfuge, and protein was determined on the trichloracetic acid precipitate by the method of Lowry *et al.* (12). Supernatants were ether-extracted and then lyophilized. Cyclic AMP was

determined by the radioimmune assay of Steiner *et al.* (13) as supplied by Schwarz/Mann.

Assay bath contents. The papillary muscle baths were attached via a drain to a suction flask under vacuum. At the time of papillary muscle freezing, the bath contents were rapidly sucked into the flask, which was cooled with a mixture of Dry Ice and isopropyl alcohol. Bath contents were stored at −85° until assay by chromatography as described above.

RESULTS

Purification of copoly-Iso. The proposed structure of copoly-Iso is shown in Fig. 1. Amino acid analysis has shown that the ratio of hydroxypropylglutamine to *p*-aminophenylalanine is 4.5:1. The molecular weight was determined by analytical ultracentrifugation in dimethylformamide to be 12,800.[4]

The initial elution profile of copoly-Iso from the diazotization mixture following synthesis was essentially identical with that previously described (8, 10), with a copoly-Iso peak followed by a 6-aminoisoproterenol peak and a peak of parent *l*-isoproterenol.

Repeated gel chromatography of the copoly-Iso peak was performed. A typical elution profile of copoly-Iso prior to lyophilization and bioassay is depicted in Fig. 2. The inset in Fig. 2 (40-fold increased sensitivity) illustrated a peak of 6-aminoisoproterenol. Thin-layer chromatographic identification of the peaks indicated that peak A was copoly-Iso; peak B, 6-aminoisoproterenol; and peak C, *l*-isoproterenol standard.

Copoly-Iso was repurified until isoproterenol was undetectable (limit of sensitivity, 0.01%). Slight, recurrent contamination by 6-aminoisoproterenol was observed at each purification and was assumed to be due to photolysis of the azo bond linking isoproterenol to the polymer. We have previously shown that azo bond reduction produces an isoproterenol derivative with an amino group at position 6 of the catechol ring (11). As the 6-aminoisoproterenol derivative is significantly less active than

[4] Performed by Miles Laboratories.

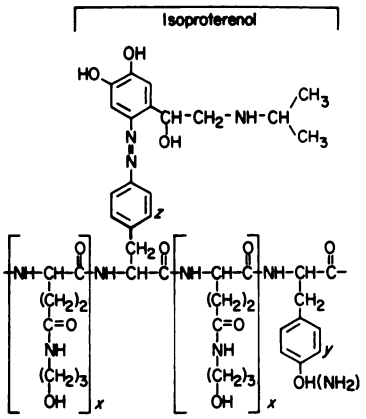

FIG. 1. *Proposed structure of isoproterenol following diazotization to p-aminophenylalanine (z) in a random copolypeptide of hydroxypropylglutamine (x) with p-aminophenylalanine (y) (x:y = 4.5:1)*

The molecular weight is 12,800, estimated from ultracentrifugation in dimethylformamide (sedimentation and diffusion). There is 2.7% (by weight) incorporation of isoproterenol into the polymer. Since the diazotized aminophenylalanine residues are not fully substituted, we assume that the remainder are hydrolyzed to tyrosyl residues.

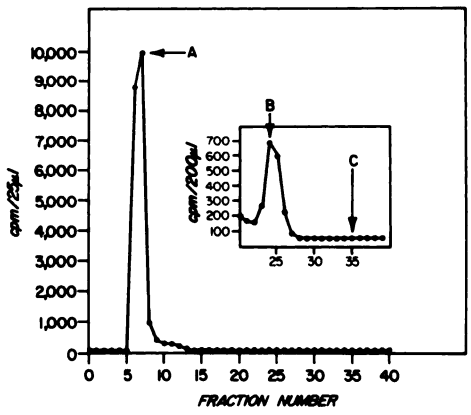

FIG. 2. *Gel permeation chromatography purification of isoproterenol diazotized to a random 12,800 mol wt copolymer of hydroxypropylglutamine with p-aminophenylalanine (copoly-Iso)*

The elution profile for the separation of the copoly-Iso is shown in the main figure. The inset (40-fold increase in sensitivity) indicates a peak of 6-aminoisoproterenol. The peaks identified by thin-layer chromatography are copoly-Iso (A), 6-aminoisoproterenol (B), and *l*-isoproterenol standard (C). Copoly-Iso was eluted from a column (Bio-Gel P-2, 3 × 120 cm) at 4° with 1 mM ammonium bicarbonate. Fractions (13.9 ml) were collected and monitored by radioactivity.

copoly-Iso itself (10), the degree of contamination by this compound (always less than 1.5%) was deemed acceptable.

Positive inotropic response to copoly-Iso. The addition of copoly-Iso to muscle baths containing isometrically contracting cat papillary muscles resulted in increases in the contractile force. The time course and magnitude of this positive inotropic response are depicted in Fig. 3. The mean control isometric force was 1.78 ± 0.42 g (SE) (n = 30).

In response to copoly-Iso the force of contraction was augmented within 10 sec of drug addition (i.e., on the second contraction). The force continued to increase, reaching a maximum at 180 sec. When force measurements were continued beyond 180 sec, no further increases were noted (Fig. 3). The average percentage change in isometric force (Δ peak force/0.5 L_{max} force × 100) at 180 sec was 117% ± 24% (n = 7).

The maximal attainable force was determined on each muscle, utilizing paired electrical stimulation. In response to PES, the force of contraction increased rapidly, reaching a maximum in 20–60 sec (Fig. 3). The average percentage change in force response to PES was 141.7% ± 23% (n = 23). The addition of isoproterenol or copoly-Iso during PES did not elicit a further increase in contractile force. We therefore assume that the peak force obtained with PES represents 100% of the force attainable by each muscle with inotropic stimulation under the conditions described. The peak response to copoly-Iso represents 81.3% of the peak PES response, a value comparable to that obtained with *l*-isoproterenol [82.9% (7)]. These results are summarized in Table 1.

Rate of force development (df/dt). In addition to the change in contractile force development, the rate of change in tension (df/dt) was recorded. The change in df/dt with time, in response to PES and copoly-Iso, is illustrated in Fig. 4. Copoly-Iso produced a change in df/dt within 10 sec of its addition to the muscle bath; df/dt continued to increase, reaching a maximum within 180 sec. No further changes occurred beyond 180 sec. The peak copoly-Iso response was a 94.1% ± 17.8% (n = 7) change. PES produced a prompt increase in df/dt, with a maximum level of 89.3% ± 10.3% (n = 23) being achieved by 60 sec. Unlike the tension response to copoly-Iso, which was 81% of PES, the peak df/dt response to copoly-Iso was essentially identical with the response to PES. These results are summarized in Table 1.

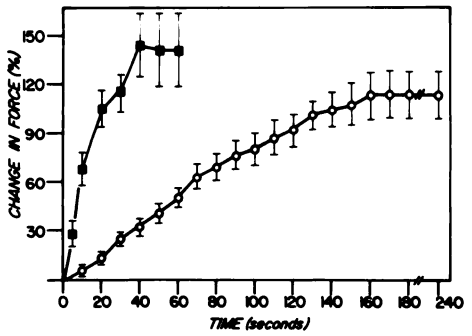

FIG. 3. *Change in isometric contractile force (percentage of control force) with time in response to paired electrical stimulation* (■) *and copoly-Iso* (○) The force achieved with PES is assumed to represent the maximum force obtainable by inotropic stimulation for each muscle. Papillary muscles were dissected from right ventricles of cats and were 1 mm or less in diameter. Muscles were placed horizontally in a bath containing Krebs solution bubbled with 95% O_2 and 5% CO_2. Muscles were stimulated to contract isometrically with transverse field stimulation. Vertical bars denote standard errors of the mean. The number of experiments for each point is indicated in Table I.

Cyclic AMP concentration during inotropic response to copoly-Iso. Cyclic AMP was measured in papillary muscles frozen at various intervals following the addition of copoly-Iso to the muscle baths. Contraction was monitored on each muscle up to the point of freezing. In contrast to the effect of soluble isoproterenol, but in agreement with the effects of isoproterenol-glass beads (7), the addition of copoly-Iso to papillary muscles produced no detectable changes in cyclic AMP levels during the positive inotropic response (Fig. 5).

Dose-response curves (not shown) for soluble isoproterenol showed that maximal inotropic responses were obtained with a bath concentration of 0.1 μM isoproterenol. As a control, a series of muscles

TABLE 1

Summary of contractile and cyclic AMP responses to copoly-Iso, isoproterenol, and paired electrical stimulation

Values represent means ± standard errors; the range is in parentheses; n = number of papillary muscles.

Time	Increase in peak force with Copoly-Iso[a]	Increase in *df/dt* with copoly-Iso[b]	Increase in peak force with PES[a]	Increase in *df/dt* with PES[b]	Percent of PES_{max}[c]	Percent of PES_{max}[d]	Cyclic AMP due to copoly-Iso	Significance[e]
sec	%	%	%	%	%	%	*pmoles/mg protein*	
0							5.54 ± 0.49 (4.19–7.79) n = 6	
30	24.7 ± 3.1 (0–64.7) n = 30	20.4 ± 2.7 (0–74.9) n = 30	116.8 ± 14.8 (34.4–240.0) n = 30	83.4 ± 6.6 (18.7–185.7) n = 30	17.4 ± 2.2 (0–45.7) n = 30	22.9 ± 3.1 (0–84.0) n = 30	4.94 ± 0.51 (2.76–6.72) n = 7	$p \leqq 0.5$
60	50.9 ± 4.0 (14.3–88.2) n = 23	47.5 ± 4.9 (3.0–95.8) n = 23	141.6 ± 23.2 (72.7–262.5) n = 23	89.3 ± 10.3 (22.2–207.1) n = 23	35.9 ± 2.9 (10.1–62.3) n = 23	53.2 ± 5.6 (3.4–107.4) n = 23	5.66 ± 0.43 (4.48–6.62) n = 5	$p \leqq 0.5$
120	92.6 ± 10.3 (28.6–166.7) n = 13	72.8 ± 10.9 (18.2–166.7) n = 13			65.4 ± 7.3 (20.2–117.7) n = 13	81.6 ± 12.3 (20.4–186.7) n = 13	4.91 ± 0.65 (2.43–6.75) n = 6	$p \leqq 0.5$
180	115.1 ± 15.1 (66.7–180.0) n = 7	94.1 ± 17.8 (55.5–175.0) n = 7			81.3 ± 10.6 (47.1–127.1) n = 7	105.5 ± 19.9 (65.7–196.1) n = 7	4.79 ± 0.72 (3.27–7.53) n = 5	$p \leqq 0.5$
							l-Isoproterenol	
60							11.56 ± 2.49 (6.26–20.6) n = 6	$p \leqq 0.05$

[a] Calculated as Δ force/0.5 L_{max} force × 100.

[b] Calculated as Δ *df/dt*/0.5 L_{max} *df/dt* × 100.

[c] Calculated as Δ force (copoly-Iso)/Δ force (PES_{max}) × 100.

[d] Calculated as Δ *df/dt* (copoly-Iso)/Δ *df/dt* (PES_{max}) × 100.

[e] Comparison of cyclic AMP levels obtained with copoly-Iso or isoproterenol with zero-time control level by a two-tailed Student's *t*-test.

was tested for cyclic AMP increases in response to this agent. The cyclic AMP response to 0.1 μM *l*-isoproterenol was measured at 60 sec, a time previously shown to be clearly maximal for the cyclic AMP response to isoproterenol (7). Cyclic AMP concentration at 60 sec subsequent to isoproterenol addition averaged 11.56 ± 2.49 pmoles/mg of protein (n = 6), a value significantly different from the control ($p \leqq 0.05$). In contrast, at no point (30, 60, 120, and 180 sec) were the concentrations of cyclic AMP in the copoly-Iso-treated muscles significantly different from those in the control muscles ($p < 0.5$ by Student's *t*-test). The control cyclic AMP concentration, as determined by radioimmune assay, was 5.54 ± 0.49 pmoles/mg of protein (n = 6). Following copoly-Iso addition, cyclic AMP levels were 4.94 ± 0.51 (n = 7), 5.66 ± 0.43 (n = 5), 4.91 ± 0.65 (n = 6), and 4.79 ± 0.72 (n = 5) at 30, 60, 120, and 180 sec, respectively. These results are summarized in Table 1.

Assay of muscle bath contents. The contents of the baths were collected at the time of muscle freezing as described under MATERIALS AND METHODS. Samples were lyophilized and chromatographed on the Bio-Gel P-2 column. No parent isoprotere-

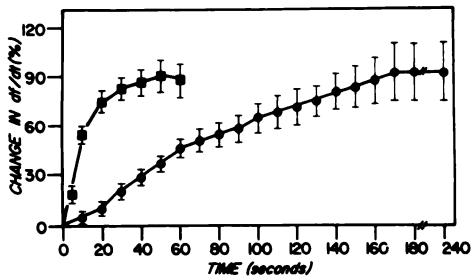

FIG. 4. *Change in rate of isometric force development (df/dt) of cat papillary muscles in response to paired electrical stimulation* (■) *and copoly-Iso* (●)
Vertical bars denote standard errors of the mean. The number of experiments for each point is indicated in Table 1.

nol was ever detectable, and the 6-aminoisoproterenol concentration was always less than 1.5% but was also usually undetectable.

DISCUSSION

The use of covalently "immobilized" catecholamines to investigate the mechanism of catecholamine-induced responses in the heart has provided evidence for the propagation of inotropic responses throughout isolated cardiac muscle from a finite, localized site of drug-receptor interaction (7, 9).

In the present study the use of a soluble amino acid copolymer to which isoproterenol had been covalently attached allowed us to investigate the effects of a "high molecular weight isoproterenol." This soluble polymer-drug system has certain advantages over isoproterenol-glass beads. For example, arguments that a localized concentration of drug is "trapped" at the bead-muscle interface cannot be applied here. Each polymer molecule has on the average 1.5 molecules of isoproterenol covalently attached, and thus classical bimolecular reactions can occur between the *beta* adrenergic receptors and the covalently attached isoproterenol. In addition, the soluble system allows for complete quantitation and characterization of any "release products" that may be formed during the testing of the complex. These controls are extremely important, as they provide strong evidence that the observed responses are due to the covalently coupled isoproterenol.

We reported above that the copoly-Iso dose-response curve is shifted two orders of magnitude to the right of *l*-isoproterenol. This means that 1% contamination of the polymer by the parent isoproterenol would be adequate to explain the responses. As illustrated in Fig. 2, no parent isoproterenol could be detected in our system which was capable of detecting traces of isoproterenol greater than 0.01%. 6-Aminoisoproterenol, produced by azo bond reduction (11), appeared at practically every purification step of the copoly-Iso. However, 6-aminoisoproterenol is substantially less active than copoly-Iso itself (10, 11). We have calculated that it would take contamination by this agent on the order of 300% to explain the biological activity of copoly-Iso.

The time course and magnitude of the positive inotropic response to copoly-Iso are remarkably similar to those reported previously for soluble isoproterenol and for isoproterenol-glass beads (7). The maximal copoly-Iso response (Fig. 3) was found to be 81.3% of the PES force; the force with PES provides an internal standard for each muscle with which chemically

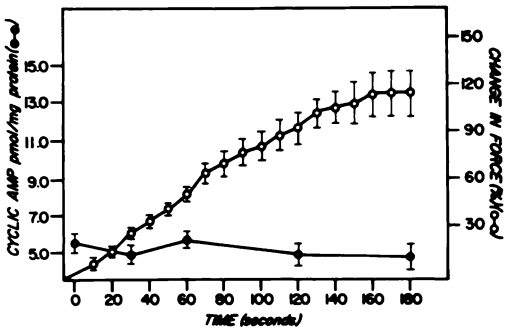

FIG. 5. *Time course and magnitude of change in isometric force* (○) *and cyclic AMP concentration* (●) *in response to copoly-Iso in cat papillary muscle*
Following the addition of copoly-Iso to the muscle baths, the papillary muscles were frozen at intervals of 30, 60, 120, and 180 sec. Cyclic AMP was determined by radioimmune assay subsequent to muscle homogenization. Control values are indicated at zero time. Vertical bars denote standard errors of the mean. The number of experiments for each point is indicated in Table 1.

induced inotropic responses can be compared. In a previous study it was demonstrated that the maximal isoproterenol response was 82.9% of the PES response whereas the response to isoproterenol-glass beads was 63% of the PES response (7). These studies suggested that the isoproterenol-glass beads cannot supply enough isoproterenol to a sufficient number of cells to routinely produce maximal drug responses. However, comparison of the isoproterenol-glass beads response with the PES response indicated that a majority of the muscle was involved in the contractile process even though only a small portion of the muscle cells was exposed to isoproterenol (7).

Our previous study has shown that copoly-Iso responses are completely and immediately reversible by one bath wash following a 10-min incubation with papillary muscles. In contrast the time required for complete reversal of the isoproterenol responses averaged 20 times longer under the same conditions (10). These studies indicate a very limited diffusion of copoly-Iso into muscles. The full drug response (81.3% of PES) obtained with copoly-Iso can therefore best be explained by assuming that the copoly-Iso interacts with adrenergic receptors on or near the outer layers of the papillary muscles.

Isoproterenol (mol wt 211), copoly-Iso (mol wt 12,800), and isoproterenol-glass beads (300 μm in diameter) produce qualitatively and quantitatively similar contractile responses (7) (Fig. 4). However, only the response to isoproterenol itself is preceded by a detectable increase in the concentration of cyclic AMP (7). While it is possible that the free isoproterenol acts via a different mechanism in producing inotropic responses than do the immobilized forms of isoproterenol, the evidence to date indicates that responses to all three agents are mediated via the *beta* adrenergic receptor (7-10). Only the *l* enantiomers of catecholamines are biologically active when covalently "immobilized" (7-9), and responses to the soluble and solid-phase isoproterenol are all antagonized by propranolol, a potent *beta* adrenergic blocking agent (7, 8). Although copoly-Iso increased the peak isometric force development in the muscles, cyclic AMP was not elevated throughout the course of the experiments (Figs. 3-5).

In that there are no apparent quantitative or qualitative differences in the inotropic response to copoly-Iso, which proceeded without detectable increase in cyclic AMP, or to parent isoproterenol, which was preceded by a 2-3-fold increase in cyclic AMP (7), it becomes difficult to assign a definite cause-effect relationship between cyclic AMP increases and isoproterenol-induced positive inotropic responses. Like our previous study using isoproterenol-glass beads (7), the present study does not "prove" that cyclic AMP is not involved at all in catecholamine-induced inotropic effects. However, we feel that our data clearly show, for both isoproterenol-glass beads and copoly-Iso, that cyclic AMP is not involved in the *propagation* of the inotropic response that must have occurred in these studies. This conclusion further supports the hypothesis of response propagation proposed for the "immobilized" isoproterenol (7).

Based upon studies on the time course of isoproterenol diffusion into papillary muscles, approximately 10 min are required for equilibrium to be approached (8). In response to isoproterenol, cyclic AMP is at a maximum in 15 sec or less and contraction has peaked in 120-180 sec. These results and the apparent response propagation seen with the immobilized isoproterenol suggest that the inotropic response to soluble isoproterenol may also proceed via a propagated mechanism similar to that observed with isoproterenol-glass beads (7) and with copoly-Iso.

Does cyclic AMP play a role in response initiation? In our studies with cultured heart cells and, more recently, in the rat diaphram muscle (9), cyclic AMP can be shown to increase in cells in which drug-receptor interactions do occur. These findings indicate that cyclic AMP could be increasing in the cat papillary muscles, but in only a limited number of cells. This suggests that cyclic AMP could possibly play a role in response initiation; however, at present there is no evidence to support

this hypothesis. It is equally possible that cyclic AMP mediates only metabolic responses that are supportive of enhanced cardiac contraction (3), and that *beta* adrenergic receptor stimulation can increase cardiac contractility without the involvement of cyclic nucleotides.

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