Enhanced Contractile Response and Protein Kinase Activation to Threshold Levels of β-Adrenergic Stimulation in Hyperthyroid Rat Heart

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A B S T R A C T   The contractile response measured as maximum rate of force development to a near threshold concentration of isoproterenol (1 nM) was enhanced in perfused interventricular septa from hyperthyroid (128±4% control) compared with euthyroid rats (105 ±2%, $P < 0.01$). This enhanced contractile response was accompanied by a significant activation of cyclic (c)AMP-dependent protein kinase (protein kinase activity ratio increased from 0.159±0.008 to 0.218 ±0.019, $P < 0.005$, although no significant changes from base line occurred in euthyroid septa, 0.152 ±0.007–0.179±0.012). No difference between hyperthyroid and euthyroid hearts was observed in the contractile response to 0.1 mM dibutyryl cAMP (126.5 ±2.5% and 122.0±9.2% in hyperthyroid and euthyroid, respectively), and the magnitude of the response to dibutyryl cAMP was comparable with that observed in the hyperthyroid group with 1 nM isoproterenol. These results suggest that the mechanism for enhanced protein kinase activation and contractile response to low concentrations of isoproterenol in the hyperthyroid heart is at or proximal to cAMP generation. The maximum contractile response to isoproterenol (0.5 μM), however, was decreased in hyperthyroid myocardium (192±13%) compared with euthyroid (291±37%, $P < 0.05$). Both protein kinase activity ratio (0.356 ±0.017 and 0.344±0.013) and the maximum contractile response to Ca++ (335±15 and 340±12% control in hyperthyroid and euthyroid, respectively) were similar, suggesting that the mechanism of the diminished maximum response was distal to protein kinase activation but not a function of an altered Ca++-troponin interaction. The diminished maximum rate of force development response in the hyperthyroid hearts was accompanied by significantly less shortening of the contraction duration that was 85.6±2.1% control in hyperthyroid vs. 66±2.8% control in euthyroid, $P < 0.001$. Although the basal rate of Ca++ accumulation was greater in microsomes isolated from hyperthyroid than from euthyroid hearts, there was significantly less additional stimulation of Ca++ accumulation in response to exogenous cAMP and protein kinase in hyperthyroid compared with euthyroid hearts. This reduction may explain the diminished effect of isoproterenol on the shortening of contraction duration in hyperthyroid compared with the euthyroid myocardium, and may explain, at least in part, the diminished maximum contractile response to isoproterenol.

INTRODUCTION

The apparent increase in adrenergic activity observed in the presence of a normal level of circulating catecholamines in both humans and animals in the hyperthyroid state (1, 2) may result from enhanced responsiveness to basal or threshold concentrations of catecholamines as a result of the changes demonstrated in the β-receptors (3–6). Consistent with this hypothesis are the observations that the hyperthyroid heart preparations in vitro exhibited enhanced phosphorylase activation (7–9) and an enhanced chronotropic response to threshold doses of catecholamines (10, 11) compared with that in the euthyroid heart. However, neither enhanced contractile responsiveness nor enhanced stimulation of adenylate cyclase or elevation of cyclic (c)AMP, purported mediators of the contractile response, has been conclusively demonstrated in the hyperthyroid heart after β-adrenergic stimulation in the threshold range (9, 12–14).

Subsequent to β-receptor stimulation, cAMP-dependent protein kinase is activated, and the time- and dose-dependent characteristics of this activation are appro-

priate for this enzyme to mediate the contractile response to catecholamines (15, 16). Furthermore, in noncardiac tissue at threshold levels of stimulation, cAMP-dependent protein kinase activation has been detected in the absence of a measurable increase in cAMP (17, 18) and thus may serve as a more sensitive index of changes in cAMP action than the level of cAMP itself. The purpose of the present study was to measure the cAMP-dependent protein kinase activation and contractile response subsequent to threshold and maximal levels of β-stimulation with isoproterenol in the hyperthyroid and euthyroid heart. The protein kinase mediated phosphorylation of sarcoplasmic reticulum and subsequent enhancement of Ca^{++} transport (19–21) appears to be integral to the contractile response (22). Therefore, the cAMP-dependent protein kinase stimulation of Ca^{++} accumulation in sarcoplasmic reticulum-enriched microsomal preparations from euthyroid and hyperthyroid hearts was also measured.

METHODS

Adult virgin male Wistar rats, aged 9–11 mo, from the Gerontology Research Center's colony were used in these experiments. Rats were injected with thyroxine, 6.4 mg/kg i.m. daily for 7 d (4). On day 8, the animal was injected with heparin, 100 U i.p., and was sacrificed by a sharp blow to the base of the skull. The heart was immediately removed from the animal, the right ventricular free wall excised, and the septal artery cannulated with a perfusion catheter (polyethylene [PE] tubing 50). The catheter was secured in place and the free left ventricular wall and other non-perfused tissue were removed, leaving a triangular-shaped perfused interventricular septum (23). The apex of the septum was attached to a strain gauge (Statham Instruments, Inc., Oxnard, Calif.; FTA100) by a loop of surgical silk. The bottom corners were fixed to two metal clamps that also served as pacing electrodes.

The septa were perfused with a modified Krebs-Ringer bicarbonate solution equilibrated with 95% O_2 and 5% CO_2. The perfusate composition was (mM): NaCl 119.8, Na_2HCO_3 25.0, KH_2PO_4 1.2, Mg_2SO_4 1.2, KCl 6.0, and dextrose 10. The $[Ca^{++}]$ was varied by addition of $CaCl_2$ over the range of 0.3–2.5 mM. Perfusate temperature was controlled by a variable resistance coil proximal to the perfusion catheter. Tissue temperature was maintained at 29°C and measured by a tissue thermistor (Yellow Springs Instruments Co., Yellow Springs, Ohio) that was inserted into each septum. Perfusion rate was held constant at ~4 ml/min per g wet wt. The septa were paced (Grass Instrument Co., Quincy, Mass.; SD 9) regularly at a rate of 80/min at twice threshold voltage.

After equilibrating 20 min, the apex of each septum was stretched until developed force was maximal. Under these conditions septa maintained stable mechanical performance for several hours. Force and maximum rate of force development were recorded on a Hewlett-Packard 4578A multichannel recorder (Hewlett-Packard Co., Palo Alto, Calif.). In all septa the following indices of mechanical performance were determined: resting force, developed force (DF)[1], the

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[1] *Abbreviations used in this paper*: CD, contraction duration; DF, developed force; dF/dt, the maximum rate of force development; E, euthyroid group; H, hyperthyroid group; RT ½, half relaxation time; TPF, time to peak force.

maximum rate of force development (dF/dt), time to peak force (TPF), half relaxation time (RT ½), and contraction duration (CD), the sum of TPF and RT ½. Under the experimental conditions described, in this and in the isolated rat trabecular preparation (24), a perfusate $[Ca^{++}]$ of 1.0 mM results in ~90% maximal steady-state dF/dt, and a $[Ca^{++}]$ of 0.3 mM reduces performance to ~30–40% of its peak value, which is achieved as perfusate $[Ca^{++}]$ approaches 2 mM (Fig. 1). DF varies as a function of $[Ca^{++}]$ in a manner identical to dF/dt. Preliminary experiments indicated that at a perfusate $[Ca^{++}]$ of 2 mM at this temperature and stimulation frequency, neither paired stimulation nor catecholamines resulted in significant further enhancement of mechanical performance. After measuring contractile performance at a $[Ca^{++}]$ of 1.0 and 0.3 mM, the $[Ca^{++}]$ was maintained at 0.3 mM and each septum was then used in assessing responsiveness to isoproterenol or dibutyryl cAMP. When the mechanical response reached its peak, typically 3 min after isoproterenol and 15 min after dibutyryl cAMP, the septum was quick frozen in precooled metal clamps, immersed in liquid nitrogen, stored at −60°C, and subsequently analyzed for protein kinase activity and activity ratio as described below.

Protein kinase assay. Frozen septa were powdered as described by Neely et al. (25). Using a polycarbonate centrifuge tube with a custom fit Teflon pestle, 40–50 mg powder was homogenized in 1.0 ml of 10 mM potassium phosphate containing 10 mM EDTA and 0.5 ml 1-methyl-3-isobutylxanthine at pH 6.8. In studies of protein kinase activation, the homogenate was centrifuged at 2°C and the supernate used for assay. In subcellular distribution studies, 0.6 ml of the homogenate was similarly centrifuged, the pellet resuspended in 0.6 ml of homogenizing buffer, and centrifuged again. The resulting supernates were combined and the pellet resuspended in 1.2 ml of homogenizing buffer. To assure proportionality of activity to sample volume, the homogenate, supernatant, and membrane suspensions were diluted as
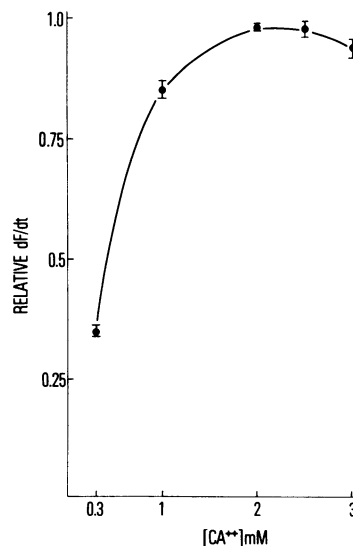

FIGURE 1   The contractile performance in six isolated perfused rat interventricular septa as a function of perfusate $[Ca^{++}]$. The preparation was stimulated to contract at 80/min at 29°C. Note that peak performance occurs at a $[Ca^{++}]$ of 2.0 mM. In other experiments it was demonstrated that at this $[Ca^{++}]$, inotropic interventions, such as paired pacing and isoproterenol, did not further potentiate contractile performance.

required in homogenizing buffer containing 0.5 mg/ml bovine serum albumin.

A 20-μl aliquot of extract, or tissue fraction diluted as described, was added to 80 μl of a solution containing 17 mM potassium phosphate at pH 6.8, 0.31 mM [γ-^{32}P]ATP (40–100 cpm/pmol), 7.5 mM magnesium acetate, 6.25 mg/ml histone (Sigma type II-A; Sigma Chemical Co., St. Louis, Mo.), and 0.31 mM 1-methyl-3-isobutylxanthine (15), with or without 2.5 μM cAMP. Incubations were at 30°C for 5–15 min and the reaction was stopped by the addition of 1.0 ml 10% TCA, 6 mM ATP, 5 mM sodium phosphate, and 100 μl of 0.63% bovine serum albumin. After centrifugation at 2,700 rpm for 10 min in a Beckman TJ-4 (Beckman Instruments Inc., Fullerton, Calif.), the supernate was removed and the precipitated protein dissolved in 1 N NaOH, reprecipitated with TCA, collected, and washed on glass fiber filters and counted.

The septal content of a protein inhibitor of cAMP-dependent protein kinase (26, 27) was also assayed in the present study. Aliquots of undiluted homogenates were heated for 5 min in boiling water, 0.1 vol 2% Triton X-100 (Rohmand Haas Co., Philadelphia, Pa.) was added, and particulate matter removed by centrifugation. Aliquots of the supernate were tested for inhibition of the catalytic subunit of beef heart cAMP-dependent protein kinase with histone f₂b as substrate at 1.0 mg/ml. 1 U is defined as the amount causing 50% inhibition of 44 pmol/min at 30°C.

cAMP protein kinase stimulation of Ca⁺⁺ accumulation in cardiac microsomes. Cardiac microsomes (28) from the left ventricle of hyperthyroid and euthyroid rats were prepared as previously reported (29). The protein content of the microsomal suspension was determined by the method of Lowry et al. (30) using bovine serum albumin as the standard. The studies were completed within an hour after isolation of the microsomes.

The microsomes were preincubated at 25°C in the presence of protein kinase alone, cAMP alone, in the presence of both, and in the absence of both. The preincubation reaction mixture contained 111 mM KCl, 44 mM histidine (pH 6.8), 27.7 μM [^{45}Ca] CaCl₂ (10¹³ cpm/mol), and 2.77 mM potassium oxalate. To each 1.7 ml of this mixture was added either 100 μl of distilled H₂O or 50 μl of H₂O plus 50 μl of either protein kinase (2 mg/ml) for the protein kinase control or 50 μl of cAMP (1 nM) for the cAMP control, or 50 μl of protein kinase and 50 μl of cAMP (1 nM–10 μM). After 10 min of preincubation, 0.2 ml of 50 mM Mg ATP (pH 7.0) was added and aliquots were removed at 0.5, 1.0, 1.5, 2.0, and 3.0 min for determination of Ca⁺⁺ uptake by the Millipore filtration method used previously (29). Optimal cAMP protein kinase-stimulated increase in Ca⁺⁺ accumulation rate in this preparation was observed with [Ca⁺⁺] of 0.56 μM, 2.5 mM oxalate, and 50 μl (2

mg/ml) protein kinase/10 μg of microsomal protein. In any given experiment, these were the final concentrations of each variable. Velocity of Ca⁺⁺ accumulation, estimated from the Ca⁺⁺ accumulated over the five sampling times, as determined by least mean squares, was linear over the 3-min period studied ($r = 0.977 \pm 0.002$, $n = 142$).

Materials. Histone II-A, histone f₂b, ATP, cAMP, dibutyryl cAMP, beef heart cAMP-dependent protein kinase, protein kinase catalytic subunit, and thyroxine were from Sigma Chemical Co. [γ-^{32}P]ATP and ^{45}Ca⁺⁺ were from New England Nuclear, Boston, Mass. Isoproterenol hydrochloride was obtained from Winthrop Laboratories, New York.

Statistical analysis. Data are expressed as the mean$\pm$SE. Means were compared when appropriate by (a) t test, (b) two-way analysis of variance, and (c) regression of analysis of variance (31).

RESULTS

Thyroxine injections significantly reduced body weight that was 477 ± 13 g in the hyperthyroid group (H), $n = 17$, compared with 560 ± 17 g, $n = 16$, in the euthyroid group (E), $P < 0.001$. Heart weight (left ventricle plus septum) increased 12% in H vs. E (1.22 ± 0.03 g in H, 1.08 ± 0.4 g in E, $P < 0.002$). The heart weight/body weight was 2.58 ± 0.06 g/kg in H vs. 1.94 ± 0.05 g/kg in E, $P < 0.001$. When heart weight is normalized to tibial length, a parameter of body size that is not affected by the hyperthyroid intervention, the extent of relative hypertrophy is 13% (0.28 ± 0.01 g/cm in H vs. 0.24 ± 0.01 g/cm in E, $P < 0.001$), which is similar to the increase in absolute heart weight.

Base-line contractile performance. The base-line contractile performance measured at two levels of perfusate [Ca⁺⁺] in septa isolated from H and E animals is presented in Table I. DF was not different, dF/dt significantly increased, and parameters of twitch duration (TPF, RT ½, CD) were substantially shortened in H when compared with E. RF was not different between groups at either perfusate [Ca⁺⁺] and was 22.0 ± 0.79 g in H and 23.1 ± 1.2 g in E. Base-line contractile performance in the septa in any protocol employing catecholamines was not significantly different from that indicated in Table I. The wet weight of the

TABLE I
Base-line Contractile Performance

	No.	DF	dF/dt	TPF	RT ½	CD
		g	g/s	ms	ms	ms
Perfusate [Ca] 1.0 mM						
Euthyroid	28	18.6±1.3	169.2±11.6	136.4±2.93	123.0±3.34	259.46±8.43
Hyperthyroid	28	18.5±1.2	220.6±14.0	88.4±2.23	73.6±3.06	162.0±4.99
P		NS	<0.005	<0.001	<0.001	<0.001
Perfusate [Ca] 0.3 mM						
Euthyroid	30	6.09±.67	58.3±5.5	139.6±3.1	117.4±3.1	257.0±5.4
Hyperthyroid	33	6.71±.54	78.2±6.1	84.5±2.5	71.2±2.0	155.6±3.99
P		NS	<0.02	<0.001	<0.001	<0.001

septa studied averaged 0.76±0.022 g in H and 0.75±0.022 g in E, and normalization of the contractile performance in each septum by its weight did not alter the results in Table I.

Dose response to isoproterenol. The isoproterenol dose-response curve for dF/dt in H and E is depicted in Fig. 2. The dose-response relationship compared by regression analysis is, as indicated in Fig. 2, significantly different in H compared with E. Specific differences in the responses in the two groups are: (*a*) at a near threshold-isoproterenol concentration (1 nM) a significant response is observed in H (125% control, $P < 0.001$), whereas E was not different from control. The significant difference is also observed when the results are compared as absolute change rather than percent control. Similar differences between the two groups were also observed in the response of DF; (*b*) the maximum response (0.5 μM in both groups) in H is significantly diminished compared with E (190% vs. 260% control, respectively, $P < 0.05$).

The dose-response relationship of CD was also different in H vs. E ($P < 0.005$ by regression analysis of variance). The maximum shortening of CD in response to isoproterenol occurred at 0.5 μM and was significantly less in H (CD was 82% of control in H vs. 72% control in E; $P < 0.001$). This difference is more marked when analyzed in absolute shortening of the twitch that decreased 67.5±4.6 ms in E and 28.02±2.2 ms in H septa ($P < 0.001$).

Near threshold response. In additional experiments, when 1 nM isoproterenol was perfused continuously, the peak response in DF and dF/dt in H was significantly greater than in E (Table II). These results are nearly identical to those at this concentration in the cumulative dose-response curve (Fig. 2). CD was not substantially shortened in either group. The time to achieve the peak response ranged from 120 to 160 s for both H and E. The protein kinase activity ratio in these septa perfused with 1 nM isoproterenol was unchanged from the control level in E (Table III) but significantly increased in H. The control protein kinase activity ratios, determined in perfused septa not exposed to isoproterenol, were not different in H and E (Table III). Thus, the enhanced contractile response at a near threshold concentration of isoproterenol in H is accompanied by enhanced protein kinase activation. In assessing whether a change in the activity or properties of protein kinase occurred in H, total protein kinase activity (plus 2 μM cAMP) was also measured in the homogenate, cytosol, and membrane preparations of control septa. No difference was observed between the groups (Table IV). Dose-response curves of activation of the soluble enzyme to increasing concentrations of cAMP (1 nM–1 μM) were identical in H and E (not shown). In addition, no significant reduction in the level of catalytic subunit inhibitor was observed (7.95±0.30 U/mg protein in E; 7.21±0.29 U/mg protein in H, NS; $n = 6$).

The results, taken together, suggest that the difference in contractile response between H and E to a near threshold concentration of isoproterenol may be explained by an alteration in the β-receptor-mediated response in a step that precedes protein kinase activation, perhaps at or proximal to cAMP generation. This prompted us to determine the change in contractile performance in response to dibutyryl cAMP, a cAMP analogue that acts independently of the β-receptor (32, 33). In preliminary experiments the effect of dibutyryl cAMP at several concentrations over the range of 0.1–1 mM was tested. The maximum response occurred at 1 mM and was equivalent to the maximum response in isoproterenol in E. The concentration of dibutyryl cAMP used to compare hyperthyroid and euthyroid septa (0.1 mM) was that which elicited an increase in dF/dt in the hyperthyroid septa that was of the same magnitude as that observed in response to 1 nM isoproterenol. Table II indicates that the contractile response of DF, dF/dt, and CD to dibutyryl cAMP was of a similar magnitude in both H and E.

Maximum response to isoproterenol. In other experiments, when 0.5 μM isoproterenol was continuously administered, the increase in DF and dF/dt and the shortening of CD were all significantly diminished in H compared with E (Fig. 3A). The difference in shortening of CD was the result of significantly greater shortening in both TPF and RT ½ in E compared with H. Similarly, significant differences between the groups in each of these parameters were present when the data were analyzed as absolute changes. The absolute shortening in milliseconds was nearly fourfold greater in E (93±11.9 ms) vs. H
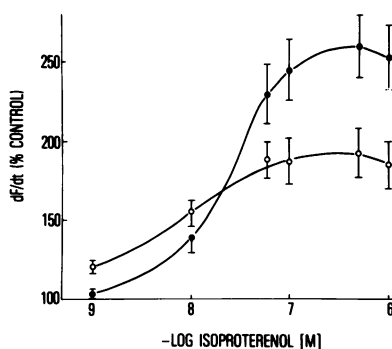

FIGURE 2 Response of dF/dt to cumulative doses of isoproterenol in euthyroid and hyperthyroid septa. The concentration of isoproterenol was increased to the next higher level when the contractile response to a given concentration reached a steady level. The statistical comparison of the curves given in the figure was a regression analysis of variance. Similar results were obtained when the dose response of DF was compared in H and E. ●, euthyroid, $n = 7$; ○, hyperthyroid, $n = 9$; variance ratio, $F_{1,72} = 12.07$; $P < 0.005$.

TABLE II
Peak Contractile Response to Continuous Infusion of Near Threshold Concentrations
of Isoproterenol or Dibutyryl cAMP in Perfused Interventricular
Septa from Euthyroid and Hyperthyroid Hearts

	No.	DF	dF/dt	CD
			% Control	
Isoproterenol (1 nM)				
Hyperthyroid	5	125.4±5.5	128.6±4.2	97.0±1.8
Euthyroid	5	105.7±2.0	105.1±1.6	97.8±0.7
P		<0.01	<0.01	NS
Dibutyryl cAMP (0.1 mM)				
Hyperthyroid	7	120.8±2.2	130.5±16.8	91.4±1.8
Euthyroid	8	115.5±12.8	122.0±9.2	90.2±2.2
P		NS	NS	NS

$(25.8±4.0 \text{ ms}, P < 0.006)$. A substantial increase in protein kinase activation occurred in H and E during 0.5 μM isoproterenol infusion, but the enhanced protein kinase activity ratio was nearly identical in the two groups $(0.344±0.013$ and $0.356±0.017$ in E and H, respectively).

On the basis of these results, the decrease in maximum contractile response to catecholamines in H compared with E appears to be the result of a difference in H and E that is distal to the step of protein kinase activation. One such possibility is that the maximum contractile performance achieved by increasing Ca^{++} delivery to the contractile protein is lower in H compared with E. The fact that this is not the case is indicated by Fig. 3B which compares the response with an increase in $[Ca^{++}]$ in the perfusion fluid to 1.0 mM. This was done in the same septa in Fig. 3A, before isoproterenol administration. As indicated, no difference in either dF/dt or DF between H and E is observed. Moreover, the peak level of DF and dF/dt in both H and E in response to an elevation of $[Ca^{++}]$ in the perfusate is significantly greater than that in response to isoproterenol. The diminished maximal response to isoproterenol in the hyperthyroid group, therefore, must be the result of some factor other than the ability of the contractile proteins to respond to enhanced Ca^{++} delivery. A notable difference in the response to

isoproterenol compared with increased $[Ca^{++}]$ is that after isoproterenol, CD is significantly shortened but remains essentially unchanged after an increase in Ca^{++} (Fig. 3).

It has been proposed that stimulation of Ca^{++} accumulation in sarcoplasmic reticulum mediates the shortening of twitch duration observed with catecholamines (19–21). In the present study, the high correlation between the extent of shortening of CD and protein kinase activation (Fig. 4) demonstrates that in a given heart there is a relationship between the isoproterenol-induced increase in protein kinase activation and shortening of CD. The slope of this relationship is significantly steeper in E (179.1 vs. 63.4 in H, $P < 0.001$). This is due, in large part, to the equal kinase activity ratio at 0.5 μM isoproterenol, but a significantly diminished response in shortening of CD in H compared with E (Fig. 3A). Thus, a possible explanation for the diminished shortening of the twitch duration (CD) in response to isoproterenol is that the protein kinase-stimulated increase in velocity of Ca^{++} accumulation by the sarcoplasmic reticulum is less in the hyperthyroid septa compared with the euthyroid. This was investigated directly by measuring the effect of cAMP and protein kinase on the stimulation of Ca^{++} accumulation in microsomal preparations isolated from H and E hearts. In the absence of exogenous protein

TABLE III
Protein Kinase Activity Ratio (PKAR) in Perfused Septa
from Euthyroid and Hyperthyroid Hearts

		Euthyroid		Hyperthyroid
	No.	PKAR (−cAMP/+cAMP)	No.	PKAR (−cAMP/+cAMP)
Control	6	0.152±0.007	6	0.159±0.008
Isoproterenol (1 nM)	5	0.179±0.012	5	0.218±0.014
P		NS		<0.005

TABLE IV
cAMP-dependent Protein Kinase Activity in Perfused Septa
from Euthyroid and Hyperthyroid Hearts

	No.	Homogenate	Cytosol	Membranes
		(pmol/min/mg wet wt)		
Euthyroid	6	21.1±1.1	19.2±1.2	3.2±0.33
Hyperthyroid	6	21.1±1.8	18.3±1.11	4.0±0.41

Protein kinase activity was measured with histone f_2b (1 mg/ml) in the presence of 2 μM cAMP.
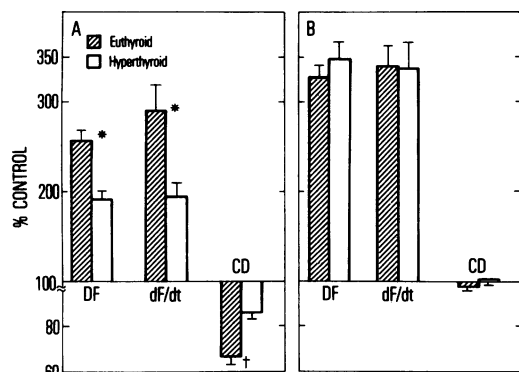
FIGURE 3 (A) The maximum contractile response to isoproterenol (0.5 μM) in hyperthyroid and euthyroid hearts. Differences between the groups were compared by t test for unpaired values. (B) The contractile response to an increase in perfusate [Ca^{++}], from 0.3 to 1.0 mM. No difference between the hyperthyroid and euthyroid septa was observed. *, $P < 0.05$; †, $P < 0.001$.

kinase or cAMP, the velocity of Ca^{++} accumulation in the microsomes from hyperthyroid hearts was nearly twice that of the euthyroid hearts and was 99.0±15.4 nmol/min in H ($n = 8$) and 48.7±4.2 nmol/min in E ($n = 8$), $P < 0.01$. The velocity of Ca^{++} accumulation with protein kinase alone (84.3±8.2 in H, 47.5±3.6 nmol/min in E) or with cAMP alone (92.6±11.1 in H, 47.6±1 in E) did not differ from the control value in either H or E. The effect of exogenous protein kinase
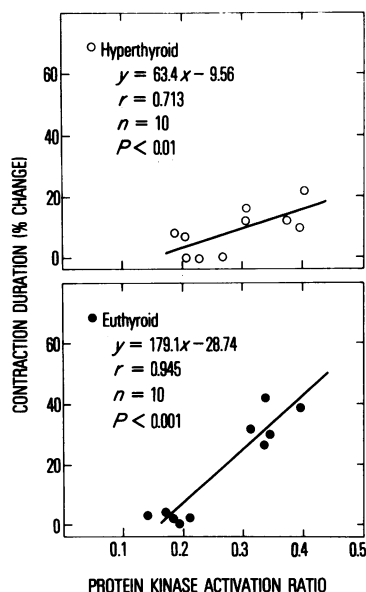

FIGURE 4 The relationship between the change in CD and protein kinase activity ratio in response to isoproterenol in hyperthyroid and euthyroid septa. Note that within each group a highly significant relationship between CD and protein kinase activity ratio was observed. The slope of this relationship in hyperthyroid septa differs from that in the euthyroid septa ($P < 0.001$).

and cAMP in varying concentrations on the velocity of Ca^{++} accumulation is given in Fig. 5. The difference in the curves of H and E is significant as indicated in Fig. 5, and this difference is the result of a greater response in E than H at the higher concentrations of cAMP. The mean maximal response independent of cAMP concentration at which it occurred was 36.4±9.1% in E vs. 14.1±3.6% control in H ($P < 0.03$).

DISCUSSION

The first major finding of this study is that the contractile response to near threshold (1 nM) concentration of isoproterenol (Fig. 2 and Table II) is greater in H than in E; this was accompanied by a significantly greater activation of cAMP-dependent protein kinase in H than in E. Furthermore, no difference in contractile response was observed when septa were perfused with dibutyryl cAMP at a concentration that produced effects in H similar to those obtained with 1 nM isoproterenol (Table II). These data suggested that the greater contractile response in H results from alterations at or proximal to cAMP generation, perhaps at the level of the β-receptor itself, which is altered in the hyperthyroid state (3–5). It is noteworthy that the enhanced dF/dt, DF, and protein kinase activity ratio in H to near threshold concentrations of isoproterenol is not accompanied by a greater shortening of twitch duration, which shortened only 2% in both H and E. Dissociation of the shortening of twitch duration from the enhanced rate of force production in response to catecholamines has been noted previously in the senescent heart (34) and under certain altered experimental conditions (35). A possible explanation for the dissociation in H in the present study is that the protein
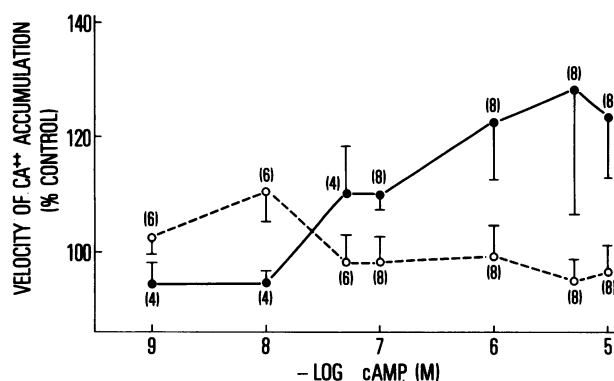

FIGURE 5 The effect of cAMP in the presence of protein kinase (0.05 mg/ml) on the velocity of Ca^{++} accumulation in microsomes isolated from euthyroid and hyperthyroid hearts. The number in parentheses accompanying each point is the number of preparations studied at that concentration. A greater response to cAMP was observed in the euthyroid microsomes. The difference between the dose-response relationships is significant when compared by a regression analysis of variance. ●, euthyroid; ○, hyperthyroid; variance ratio, $F_{1,60}$; $P < 0.025$.

kinase activation resulting from 1 nM isoproterenol is below the threshold needed to increase Ca^{++} accumulation by sarcoplasmic reticulum in H. Alternatively, the increase in protein kinase activation from 1 nM isoproterenol may have been compartmentalized or localized to the sarcolemma *in situ*. The increase in contractile response would then be explained by kinase-mediated enhancement of Ca^{++} flux into the cell, with no alteration in the Ca^{++} accumulation properties of sarcoplasmic reticulum.

The second major finding of this study is that the diminished maximal contractile response to β-adrenergic stimulation (12) cannot be attributed to a failure to activate protein kinase. Furthermore, our results demonstrate that after an increase in perfusate $[Ca^{++}]$, which by the design of these experiments elicited a response greater than that to catecholamines, no difference between H and E in the contractile response occurred. It appears likely, then, that neither the interaction of Ca^{++}-troponin nor the ability to mobilize the energy necessary to develop force is the factor limiting the maximum contractile response of dF/dt to isoproterenol in H. The failure of catecholamines to shorten CD in H to the same extent as in E appears to be a clue to the mechanism of the diminished contractile response. Shortening of the twitch duration by catecholamines has been attributed to an increase in the velocity of Ca^{++} accumulation by sarcoplasmic reticulum. This conclusion has been extrapolated to working muscle from observations in isolated sarcoplasmic reticulum (19, 20) or its functional equivalent in skinned fiber preparations (21). The correlation between isoproterenol-induced activation of protein kinase and shortening of contraction duration in a given heart as demonstrated by the present results (Fig. 4) suggests that the effect demonstrated in these cell fragments and isolated vesicles also pertains to the intact perfused myocardium. The observation that contraction duration was shortened to a significantly lesser extent in H in response to maximal concentrations of isoproterenol suggests that activated protein kinase is less able to effect a further increase in Ca^{++} accumulation velocity in the sarcoplasmic reticulum in the hyperthyroid heart. This hypothesis is further supported by the present demonstration that the maximum increase in Ca^{++} accumulation upon addition of cAMP and protein kinase was significantly less in sarcoplasmic reticulum isolated from hyperthyroid compared with euthyroid hearts. Failure of cAMP and protein kinase to enhance the Ca^{++} accumulation rate in H to the same extent as in E may be the result of the very high basal level of Ca^{++} accumulation in H microsomes. It seems reasonable to speculate that, in response to catecholamines, if less additional Ca^{++} is sequestered in H by this mechanism, less may be released upon subsequent excitation, resulting in less additional Ca^{++} delivered to the contractile protein.

This, then, is one factor that may contribute to the diminished maximum contractile response in DF and dF/dt to catecholamines in the hyperthyroid heart. Other β-adrenergic-mediated events, such as an increase in slow inward current or phosphorylation of contractile proteins, were not measured in the present study. Both of these could be altered in the hyperthyroid state, though the precise relationship between the latter mechanism and enhanced contractile performance remains controversial (36–38). Changes in these parameters as well as in rates of dephosphorylation of specific proteins may be factors involved in the diminished maximum response to isoproterenol in the hyperthyroid heart.

The present and previous studies (12, 39, 40) suggest that in H the shortened twitch duration may be related to the high basal rates of Ca^{++} accumulation by the sarcoplasmic reticulum. Alterations in twitch duration and directionally similar changes in steady-state Ca^{++} accumulation velocity in microsomes have also been demonstrated in the senescent heart (29), in the heart hypertrophied by pressure overload (41), and in different types of skeletal muscle (42). The cause of the enhanced basal level of Ca^{++} accumulation in microsomes isolated from H is not clear from the results. In the absence of exogenous catecholamines, septa not exposed to isoproterenol showed no difference in the base-line protein kinase activity between H and E in either homogenate, cytosol, or membrane preparations. In addition, the response of the soluble enzyme to cAMP and the level of the inhibitor of cAMP-dependent protein kinase were similar in H and E. Also, the addition of cAMP in the absence of exogenous protein kinase produced no significant change in Ca^{++} accumulation velocity in isolated microsomes from either H or E. Thus, we cannot attribute the enhanced basal level of Ca^{++} accumulation rate in microsomes isolated from H (39) to an increase in basal protein kinase activity.

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