Sensitivity of Hyperthyroid Cat Myocardium to Decreased Extracellular Calcium (36871)

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The mechanism of the observed alteration in the contractile state of the heart observed in clinical hyperthyroidism has remained a difficult mechanism to delineate. Formerly it was thought that increased sensitivity of the sympathetic nervous system mediated by thyroxine could explain the responses (1, 2). However, this hormone also has a direct myocardial (cellular) effect which could be responsible for the altered contractility (3, 4, 5, 6). Indeed, both of these mechanisms may operate *in vivo* and account for the altered chronotropic and inotropic responses of hyperthyroid hearts. Since *in vitro* experiments have revealed increased contractility of hyperthyroid cat myocardium, the purpose of the present experiment was to examine the responses of hyperthyroid cat myocardium to calcium, the key element in the excitation–contraction coupling mechanism.

In work previously done in our laboratory, we have demonstrated that ouabain is capable of producing negative inotropic responses in isolated cat papillary muscle exposed to extracellular calcium concentrations less than 0.6 m*M* (7). The responses to ouabain at low extracellular calcium concentrations of hyperthyroid cat myocardium, however, appeared to be more pronounced than controls, *i.e.*, greater depression of contractility.

The purpose of the present experiments was to examine the sensitivity of two different cellular processes of hyperthyriod myocardium to extracellular calcium concentrations. The developed isometric tension and its first derivative and the transmembrane action potential were the measured responses.

Materials and Methods. Right ventricular papillary muscles were removed from 56 control cats and from 30 cats which had received l-thyroxine, 0.75 mg/kg/day for 21 days. One end of the muscle was held in a fixed position and the other end attached to a force displacement transducer for tension measurement. Isometric tension was measured at L_{max}, and the first derivative of the tension tracing (dT/dt) was monitored on-line by means of an active differentiator with a time constant of 1 msec. A horizontal, 25 ml lucite flow-through bath was perfused with Krebs solution at 25° and equilibrated with 95% O_2-5% CO_2. Muscles were stimulated to contract isometrically at 12/min by means of fine platinum electrodes placed in close proximity on either side of the muscle base at a voltage 5% to 10% above threshold. Transmembrane action potentials (TAP) were recorded by means of standard 3*M* KCl-filled glass micro-electrodes mounted in a right-angle assembly with signal amplification by a Bak standard electrometer and a Tektronix model 502 dual beam oscilloscope. Total duration of the TAP as well as times for 50% repolarization were the characteristics examined. Tension, dT/dt and transmembrane action potentials were recorded on a direct writing oscillograph as well as on a multichannel Hewlett-Packard 3955 tape system.

Since Ca^{++} concentration was critical in these experiments, great care was taken in the preparation of the low Ca^{++} solutions. Solutions were prepared in plastic containers previously rinsed in distilled and deionized water using a stock Krebs solution containing no Ca^{++}. Ca^{++} was then added in amounts needed to attain the various concentrations.

Following equilibration, control studies were obtained at the normal Ca^{++} concentration of 2.5 m*M*. The perfusing solution was then changed to one that had a lower Ca^{++} concentration, but was otherwise identical in

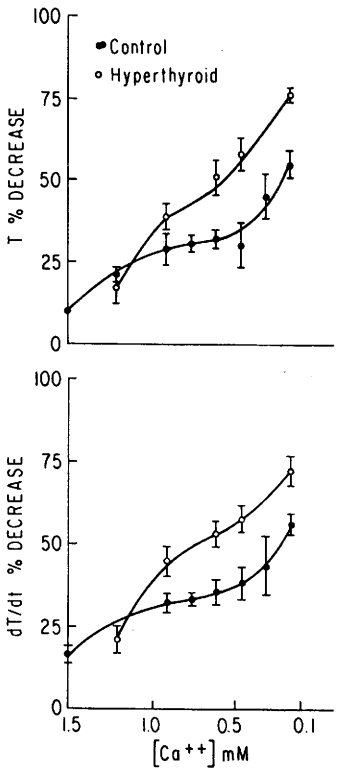

FIG. 1. Percent decrease in tension and dT/dt as a function of extracellular calcium. Control $[(Ca^{++})_e]$ was 2.5 mM.

composition to the control solution. After 15 min equilibration, records were obtained. Each muscle was exposed to only one low Ca^{++} concentration.

Statistical methods. Tension and dT/dt were expressed as percent change from baseline. Where necessary, group means were compared by Student's t test and the statistical significance of the difference was expressed as p values.

Results. In experiments performed at Ca^{++} concentrations varying from 0.15 mM to 2.5 mM, it was found that both tension and dT/dt decreased with decreasing Ca^{++} concentrations. Exposure to Ca^{++} concentrations less than 0.85 mM resulted in a significantly greater decrease in both responses in the hyperthyroid muscles as compared to control (Fig. 1). Since control tension and dT/dt of the hyperthyroid group were higher, the absolute decrease in these responses was even greater, and the resultant absolute tension at the low Ca^{++} concentrations was less than the control group.

As has been well documented, the effect of decreasing the extra-cellular Ca^{++} concentrations $[(Ca^{++})_e]$ is a lengthening of phase 2 of the action potential. In Fig. 2 it is seen that lowering the Ca^{++} concentration from 2.5 mM (normal) to 0.15 mM results in a prolongation of the 50% repolarization time secondary to a lengthening of phase 2. The data in Table I represent the change in the duration of action potential in control and hyperthyroid muscles at normal Ca^{++} and at the lowest concentration used 0.15 mM. The data indicate that the change in action potentials to low Ca^{++} of hyperthyroid muscles is no different from that of control muscles. Although the control action-potential duration was somewhat shorter in the hyperthy-

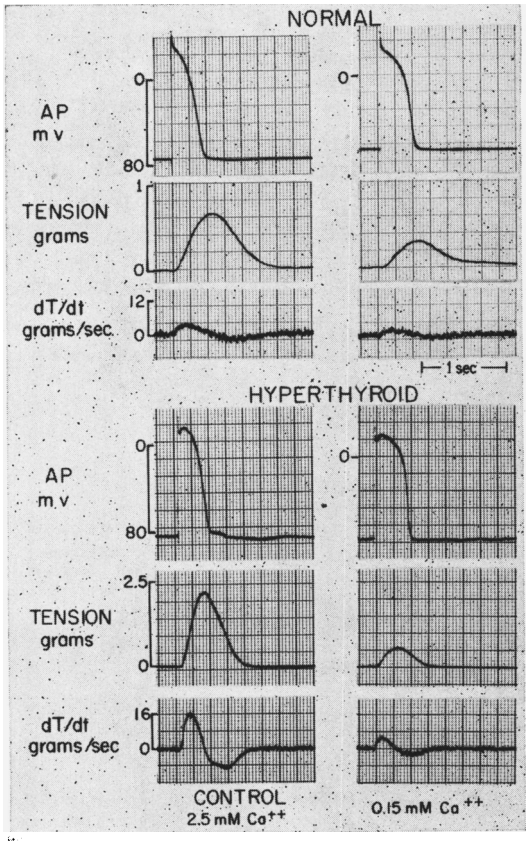

FIG. 2. Transmembrane action potential, tension and dT/dt in papillary muscles from normal and hyperthyroid cats at control and low $[(Ca^{++})]_e$.

TABLE I. Action Potential Duration.

	Total time (msec)		% Δ	Time to 50% repolarization (msec)		% Δ
$[(Ca^{++})_e]$	2.5	0.15		2.5	0.15	
Control	64	72	13 ± 4[a]	39	57	46 ± 6
			$p > .1$			$p > .1$
Hyperthyroid	38	45	18 ± 6	24	35	46 ± 4

[a] Average % change ± SEM.

roid muscles (a fact now under study), the percent change after exposure to low Ca^{++} was the same as that in the controls.

In previous experiments from this laboratory designed to show the role of calcium in the inotropic effect of ouabain, a negative inotropic effect is produced by ouabain in extracellular Ca^{++} concentrations less than 0.6 m*M*. Figure 3 shows that this negative inotropic effect of ouabain in low Ca^{++} is quite apparent in muscles excised from the hearts of hyperthyroid animals.

Discussion. These data demonstrate that there is a fundamental cellular alteration that occurs in ventricular myocardium from hyperthyroid animals. Specifically, the myocardial contractile apparatus is more sensitive to decreased $(Ca^{++})_e$ than is the myocardium from normal animals. However, the effects of $(Ca^{++})_e$ on the membrane electrical characteristics are otherwise similar in hyperthyroid and normal myocardium.

The early literature generally attributed alterations in the cardiovascular system in hyperthyroid states either to an actual increase in the functional level of the sympathetic nervous system or to an increase in the sensitivity of the postganglionic sympathetic neurons to catecholamines (8, 9). More recent evidence suggests that there are no differences in either the physiological responses to catecholamines (10, 11) or changes in the adenyl cyclae system in myocardium from hyperthyroid animals (12). Levey examines these points in detail in his excellent review (13), and notes that the most plausible explanation for the increased adrenergic effects on the heart in hyperthyroidism is that offered by Wurtman, Kopin, and Axelrod (14). Their experiments suggest an increase in concentration of catecholamines at the myocardial receptor sites, rather than an al-

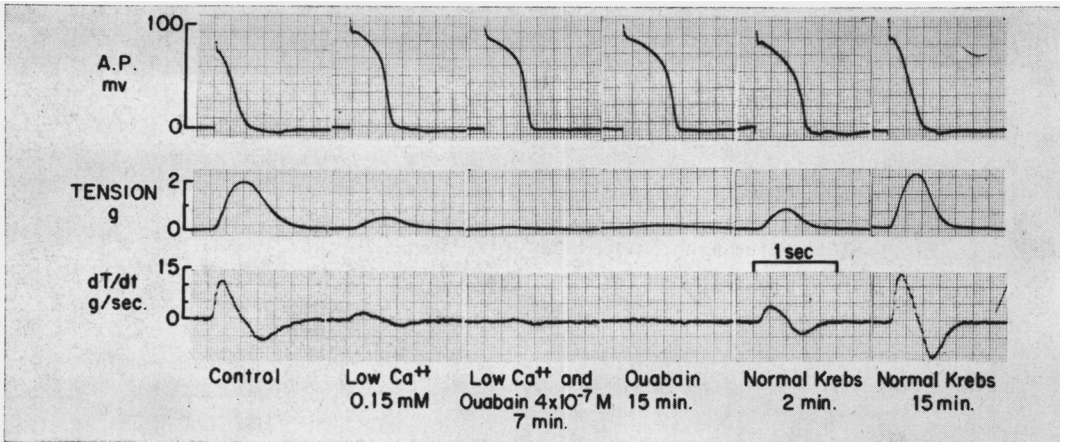

FIG. 3. The effect of ouabain on the transmembrane action potential, tension and dT/dt in hyperthyroid myocardium. The negative inotropism of ouabain at low calcium is exaggerated but the effect on the AP is similar to normal myocardium (see text).

teration in sensitivity to them.

Murayama and Goodkind (15) have examined force–frequency relationships in guinea pig atria from hyperthyroid animals, and in this preparation they noted a similar percent decrease in tension of both control and hyperthyroid atria when $(Ca^{++})_e$ was lowered. The resultant tension of the hyperthyroid guinea pig atria was higher than the controls at all Ca^{++} concentrations. This appears to be either a fundamental difference between atrial and ventricular myocardium, or a species difference.

The exaggeration of the negative inotropic effect of ouabain (example in Fig. 3 and unpublished observations) at low $(Ca^{++})_e$ in the hyperthyroid group suggests that ouabain and thyroxine share a common pathway in mediating the effects on contractility.

Since the effect of $(Ca^{++})_e$ on the action potential is unchanged in the hyperthyroid muscles, the sensitivity to $(Ca^{++})_e$ appears to be a specific effect on the contractile apparatus. The $(Ca^{++})_e$ sensitivity of the contractile apparatus of hyperthyroid myocardium may provide a basis for the observed increase in contractility independent of the autonomic nervous system.

Summary. Tension, dT/dt, and transmembrane action potentials were recorded in papillary muscles excised from hearts of control and hyperthyroid cats. The effect of $(Ca^{++})_e$ on the action potential was unchanged in hyperthyroid muscles, but the contractility was more sensitive to decreased $(Ca^{++})_e$ than the controls. This may provide a clue as to the basis of the increased contractile state documented in hyperthyroid myocardium.

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