Effect of Thyroid Hormone on the Frequency-Force Relationship of Atrial Myocardium from the Guinea Pig

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ABSTRACT

Measurements of the frequency-force relationship were made in left atrial muscle from euthyroid and hyperthyroid guinea pigs and the effects of calcium and norepinephrine on this relationship examined. Isometric contractile force was measured with the muscle suspended in a bath of Krebs-Henseleit solution oxygenated by a gas mixture of 95% O₂-5% CO₂ at 28°C. Frequency of stimulation was varied from 0.5 to 200/min at suprathreshold voltage delivered by plate electrodes. Low frequency stimulation caused less of a negative inotropic effect in hyperthyroid than in normal controls. The maximum negative and positive inotropic effects in the thyroxine-treated guinea pigs occurred at lower frequencies than in normal controls. These changes altered the shape of the myocardial frequency-force curve of hyperthyroid animals from that of normal controls. Norepinephrine and calcium in the dose levels examined produced a smaller than normal increment in the developed tension of hyperthyroid animals, but the maximum developed tension was similar in the two groups of animals. The lack of effect of reserpine on the frequency-force curve of left atrial muscle from hyperthyroid animals suggests that endogenous norepinephrine stores are not necessary for the production of a change in the curve. The alteration in the frequency-force relationship of atrial muscle from hyperthyroid guinea pigs is further evidence of a direct effect of thyroid hormone on the myocardial contractile mechanism.

ADDITIONAL KEY WORDS calcium norepinephrine reserpine myocardial contractility interval-strength relation

The frequency of myocardial contraction is a major determinant of its contractile force (the frequency-force relationship), and within a physiological range of frequencies, contractile force in vitro is increased as the frequency increases (1). Changes in heart rate have a similar though smaller effect on contractile force in vivo (2, 3). Both increased myocardial contractility and tachycardia are present in the intact hyperthyroid guinea pig (4). The increased contractility of hyperthyroidism does not appear to be caused by increased sympathetic nervous system activity or by hypersensitivity of the myocardium to catecholamines (4-11), but by a direct effect of the thyroid hormone on the heart.

The studies reported in this paper were designed to investigate in vitro the influence of frequency of contraction on myocardial contractility (developed tension) and on the response to inotropic agents in hearts from hyperthyroid animals. We postulated that a change in the frequency-force relationship...
could contribute to the previously reported increased contractile response to aortic constriction and might explain the decreased myocardial responsiveness to isotropic agents in hyperthyroid guinea pigs (11).

Methods

One hundred and thirty-five male guinea pigs of the Hartley strain weighing 300 to 400 g were used. All received a standard diet of Purina guinea-pig feed and water ad libitum. The guinea pigs were randomly divided into two groups. One group was made hyperthyroid by intraperitoneal administration of 100 mg/animal/day of sodium l-thyroxine for a period of 8 to 10 days. The second group was given daily injections of a volume of saline equal to that of the thyroxine injection. Some of the hyperthyroid guinea pigs were given two reserpine injections, 100 mg, ip, 24 and 12 hours before they were killed. This dosage regimen was previously found to reduce atrial myocardial catecholamines to undetectable levels.

Animals were killed by a blow on the head, and the heart was rapidly removed. The left atrium was dissected free from the remainder of the heart while immersed in a bath of Krebs-Henseleit solution (NaCl, 118 mM; CaCl2, 2.5 mM; KCl, 4.8 mM; KH2PO4, 1.0 mM; MgSO4, 1.2 mM; NaHCO3, 27.2 mM; glucose, 200 mg/100 ml; with a pH of 7.4) oxygenated with a gas mixture of 95% O2 and 5% CO2. One end of the left atrial muscle was attached to the hook of a muscle holder with capillary silk thread and the other end attached by a second silk thread to a Statham G105 strain gauge. Ligatures were placed at similar sites on each atrial muscle to avoid the effect of different orientations of the muscle fibers. The muscle holder was suspended in a glass chamber filled with Krebs-Henseleit solution; 95% O2 and 5% CO2 was bubbled through the solution via a sintered glass filter. The chamber was then placed in a water bath at a temperature of 38 ± 0.5°C by a circulating pump. The Krebs-Henseleit solution in the chamber was replaced with fresh solution every 30 minutes during the experiment. After contractility studies were completed, the muscle between the two ligatures was blotted and weighed.

An Electronics for Medicine, Inc., DR-8 oscillograph amplified and recorded signals from the strain gauge. Field stimulation of the muscle was achieved by two parallel platinum plate electrodes connected to an American Electronics Laboratories 104A Stimulator which delivered square-wave dc impulses of a voltage approximately 1.5 to 2.0 times greater than threshold at a stimulus duration of 2 msec. Threshold, at a frequency of 30/min in normal Krebs-Henseleit solution, was similar in 31 euthyroid (3.7 ± 0.33 v) and 22 hyperthyroid guinea pigs (4.0 ± 0.14 v) (P > 0.05). The muscle was stimulated at one or more of the following frequencies: 0.5, 2, 4, 6, 10, 20, 30, 35, 40, 65, 100, 120, 150, 170, 185, or 200/min. In all experiments resting tension was maintained at 0.5 g; developed tension during stimulation at this resting tension was stable for periods up to at least 3 hours. Before the initial experiment the atrial muscle was stimulated isometrically at a frequency of 10 to 30/min for 1 hour to permit the muscle to reach a state of equilibrium. No consistent change in contractile force was observed during this period. The developed isometric contractile tension was measured after stabilization at each frequency, and the results were expressed in absolute terms of grams of force.2 Developed tension was found to be unrelated to the weight of the muscle within the range of atrial tissue weights observed in these experiments.

External calcium concentrations of 0.625, 1.25, 2.50, 3.75, 5.00, 8.25, and 12.00 mM were obtained by cumulative addition of calcium chloride to the Krebs-Henseleit solution without adjusting the concentrations of other components of the solution. Norepinephrine bitartrate was administered in a concentration of 1 /Ag/ml of bath solution (calculated as the salt) for the frequency-force curves; for dose-response curves, cumulative additions of the drug were made to the bath solution to reach each new concentration. Dose response curves for calcium and norepinephrine were obtained at frequencies of 30 and 120/min. Dose responses to high calcium (greater than 2.5 mM) and norepinephrine (greater than 1 /Ag/mlem of bath solution) were not obtained at a frequency of 30/min, since spontaneous contractions frequently occurred at these elevated drug levels when stimulation frequencies equal to or slightly greater than 30/min were applied. Throughout this study atrial preparations that demonstrated persistent spontaneous contractions were discarded.

Statistical analysis of the group means was done with Student's t-test for unpaired data and mean differences with a P value less than or equal to

1 Voltages are expressed as direct readings from the dial of the stimulator.
2 Throughout this report contractile strength is measured during isometric contractions and the tension force and tension are used interchangeably. "Developed tension" equals the total tension minus the resting tension which in these studies was always 0.5 g.

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The frequency-force curve in euthyroid guinea pigs was triphasic (Fig. 1). As the frequency of contraction increased from 0.5/min, the developed tension initially decreased to a level corresponding to a maximum negative inotropic effect, then increased to a peak (the maximum positive inotropic effect), and finally decreased again. A similar frequency-force curve was observed in atrial myocardium from hyperthyroid guinea pigs (Fig. 1). However, the average frequency for the maximum negative inotropic effect was lower in hyperthyroid (20 ± 3.4 beats/min) than in euthyroid (27 ± 3.2 beats/min) guinea pigs ($P < 0.01$), and similarly, the average frequency for the maximum positive inotropic effect was lower in hyperthyroid (150 ± 5.4 beats/min) than in euthyroid (171 ± 4.8 beats/min) guinea pigs ($P < 0.05$). At frequencies below 100/min, developed tension was greater in atrial muscle from hyperthyroid than in that from euthyroid animals, but was not different at frequencies greater than 100/min in the two groups. The developed tension of the "rest contraction," i.e., that observed in myocardium after a 5-minute period without contractions, was the same for euthyroid and hyperthyroid guinea pigs in Krebs-Henseleit solution containing 2.5 mM of calcium chloride.

Effect of Calcium

The effect of low (0.625 mM) and high (5.00 mM) calcium concentrations is shown.

These data were evaluated by use of the Kolmogorov-Smirnov test for two samples (12).
in Figure 2. Low calcium concentration reduced developed tension in both euthyroid and hyperthyroid guinea pigs, but it was still greater at all frequencies in muscle from hyperthyroid than in those from euthyroid animals. The average frequency for the development of the maximum negative inotropic effect was lower in hyperthyroid than in euthyroid guinea pigs (20 ± 6.3 beats/min) than in euthyroid guinea pigs (35 ± 5.4 beats/min) (P < 0.001), but the average frequency for the maximum positive inotropic effect was similar in the two groups at a calcium concentration of 0.625 mM (150 ± 5.6 beats/min for euthyroid vs. 150 ± 6.7 beats/min for hyperthyroid).

In the high concentration of calcium (Fig. 3), the maximum negative inotropic effect in myocardium from euthyroid animals occurred at a lower frequency (10.0 ± 5.3 beats/min) than in a calcium concentration of 2.5 mM (P < 0.001). Muscle from hyperthyroid animals did not demonstrate a negative inotropic effect in the high calcium concentration. A maximum positive inotropic effect occurred at the same average frequency in myocardium of euthyroid (120 ± 3.9 beats/min) and hyperthyroid (120 ± 5.4 beats/min) animals in a calcium concentration of 5.0 mM. In hyperthyroid guinea pigs, the developed tension exceeded the normal at frequencies of 5 to 30/min with the high concentration of calcium, and above 30/min no difference was observed in the force of contraction of the two groups of muscles.

Figure 3 illustrates the changes in developed tension observed with varied concentrations of calcium at stimulation frequencies of 30 and 120/min. Developed tension of atrial myocardium from hyperthyroid guinea pigs was greater than that from euthyroid controls at calcium concentrations of 0.625 and 1.25 mm at a frequency of 120/min, and at all concentrations examined (up to 5.00 mM) at a frequency of 30/min. Muscle from both groups appeared to reach a plateau of developed tension in a calcium concentration of 5.00 mM and a frequency of 150/min.
Effect of variations in calcium concentration [Ca\(^{2+}\)] on the developed tension of left atria at frequencies of 30 and 120 beats/min. Symbols as in Figure 1.

Effect of norepinephrine

Norepinephrine (1 \(\mu g/ml\)) always prevented the negative inotropic effect observed at low frequencies of stimulation (Fig. 4), and it increased developed tension at all frequencies, except at 0.5 and 1/min in atrial muscle from hyperthyroid guinea pigs.

Figure 5 illustrates dose-response curves to norepinephrine at frequencies of 30 and 120/ min. The increment in tension of the myocardium from euthyroid animals to norepinephrine was greater at concentrations of \(10^{-1}\ \mu g/ml\) and above than that observed in hyperthyroid guinea pigs. However, the maximum developed tension achieved with norepinephrine (data not shown) was similar in the two groups of animals at both stimulation frequencies.

The response to norepinephrine (1 \(\mu g/ml\)) was greater in 2.5 mM of calcium than in low or high concentrations of calcium in both euthyroid and hyperthyroid guinea pigs (Fig. 6). Atrial muscle from euthyroid animals developed a greater increment in contractile force with norepinephrine than did that of hyperthyroid animals in calcium solutions of both 2.5 and 5.0 mM. The maximum response was the same in the two groups, except at the calcium concentration of 5.0 mM where the myocardial contractile force of euthyroid animals exceeded that of hyperthyroid guinea pigs. At a calcium concentration of 5.0 mM, the myocardial response of the hyperthyroid animal to the cumulative effects of norepinephrine and calcium was less than its response in a concentration of 2.5 mM. Developed tension of these atria was also less than the response of atria from euthyroid animals to 2.5 mM of calcium and norepinephrine. In contrast, the additive effects of norepinephrine and calcium were similar in euthyroid guinea pigs at 2.5 and 5.0 mM.
Effect of varied concentrations of norepinephrine bitartrate on the increment (A) in developed tension at 30 and 120 beats/min of left atria from ten euthyroid and ten hyperthyroid guinea pigs. Symbols as in Figure 1.

Effect of Reserpine

Frequency-force curves were measured in atrial preparations from six hyperthyroid guinea pigs pretreated with reserpine, and the results were compared with data obtained from the normal and hyperthyroid animals already described. Developed tension was the same at all frequencies in myocardium from hyperthyroid and reserpine-treated hyperthyroid animals, and in both groups developed tension (0.8 ± 0.06 and 0.7 ± 0.07 g, respectively) was greater at low frequencies (30 beats/min) than in euthyroid controls (0.5 ± 0.05 g) (P < 0.001). The "rest contraction" for atrial myocardium from reserpine-treated hyperthyroid guinea pigs (0.6 ± 0.06 g) achieved the same developed tension as that of hyperthyroid guinea pigs (0.8 ± 0.06 g). Reserpine-treated hyperthyroid guinea pigs had the same response to norepinephrine at all frequencies as did hyperthyroid animals. At a frequency of 30/min, the maximum contractile response to norepinephrine did not differ in the three groups of animals, though the control values before drug administration were lower in euthyroid controls than in the other two groups (0.3 ± 0.05 g vs. 0.8 ± 0.10 and 0.6 ± 0.06 g) (P < 0.001). Pretreatment of hyperthyroid animals with reserpine also did not alter the contractile response to increasing external concentrations of calcium (Fig. 3).

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Discussion

The frequency-force relationship of left atrial myocardium from hyperthyroid guinea pigs demonstrated a smaller negative inotropic effect, and therefore a greater developed tension, with low frequencies of stimulation than was observed in euthyroid animals. In addition, the maximum negative and positive inotropic effects occurred at lower frequencies than those of normal controls. These differences resulted in both an upward shift and a shift to the left of normal of the frequency-force curve of atrial myocardium from thyroxine-treated animals.

Norepinephrine and calcium produce an increment in left ventricular myocardial contractile response to aortic constriction in hyperthyroid guinea pigs in vivo which is less than normal (11). At frequencies below approximately 120 beats/min, the increments in developed tension in vitro following administration of norepinephrine or calcium chloride are less in atrial muscle of hyperthyroid animals than in that of normal controls. To examine this response, schematized frequency-force curves are shown in Figure 7 which demonstrate the observed shift in the curve of developed tension for left atrial myocardium of hyperthyroid from that of normal animals, and compare these to the maximum curves for 1 \( \mu \)g/ml of norepinephrine and 5.0 mM of calcium chloride. The shift may explain the observed increase in myocardial contractility in response to aortic constriction and decreased myocardial response following the addition of inotropic agents in hyperthyroid guinea pigs in vivo. Two assumptions must be made: (1) the difference in the positive inotropic effect of increasing frequency in atrial myocardium between euthyroid and hyperthyroid guinea pigs also exists in ventricular myocardium; (2) data obtained in vitro are applicable to cardiac function in vivo. In vitro, if the heart rate of the hyperthyroid animal is greater than normal but below that which leads to a maximum positive inotropic effect, e.g., 100/min, and that of the euthyroid animal is slower, e.g., 75/min, the myocardial contractile tension in hyperthyroid animals will be greater than normal (Fig. 7). In addition, the increment in tension at these respective frequencies would be greater in normal (increment A-B) than in hyperthyroid guinea pigs (increment C-D) for norepinephrine and increased calcium concentration. Thus, though the tachycardia of hyperthyroidism is not the only determinant of the increase in myocardial contractility and decreased response to inotropic agents, it is a contributing factor.

A modification by thyroxine administration of the frequency-force relationship implies that thyroxine alters the contractile state.
of the myocardium. The nature of this change is not evident, especially as the physiological basis for the triphasic frequency-force curve of atrial muscle has not been satisfactorily explained at the present time. Blinks and Koch-Weser (13) consider two opposing forces, one to decrease and the other to increase the strength of subsequent contractions, which may result from alterations in frequency of contraction. The contractile tension observed at any given frequency would then be determined by the algebraic summation of these two forces. Their explanation is descriptive and does not define the basic mechanism of the frequency-force relationship.

The positive inotropic effect of increasing frequency appears to be related to alterations in membrane transport of calcium. The data of Winegrad and Shanes (14), Grossman and Furchgott (15), and Langer (16) indicate that an increased calcium influx into mammalian cardiac muscle occurs and may be responsible for the associated increase in contractility with increased frequency of contraction.

An altered frequency-force relationship in hyperthyroidism might be attributable to a proportionately greater increase in calcium uptake for any given frequency by the myocardium of thyroxine-treated guinea pigs. A higher than normal calcium uptake at low frequencies in the myocardium from hyperthyroid animals would then result in a greater than normal tension development at these frequencies. Calcium uptake by myocardium is also directly related to the external calcium concentration (14). A low external calcium concentration may accentuate differences in the calcium transport mechanism which would be obscured by high calcium concentration. The greater contractile response at low external calcium concentrations of myocardium from hyperthyroid than from euthyroid animals supports the concept that thyroid hormone may stimulate calcium uptake. Lack of significant difference in response to high external calcium at high frequencies implies that maximum potential contractility is similar in both groups of animals.

The altered cardiovascular function in hyperthyroidism has been thought to be mediated by the sympathetic nervous system and catecholamines (17). However, recent reports have demonstrated that thyroid hormone does not increase the sensitivity of adrenergic receptor sites (5-11) and β-receptor blockade with drugs does not alter the contractile response to aortic constriction in the hyperthyroid guinea pig (4). Since the frequency-force curve observed in muscle from reserpine-treated hyperthyroid animals did not differ from hyperthyroid animals, the role of endogenous norepinephrine appears insignificant in the production of an altered frequency-force curve in atrial muscle from hyperthyroid animals. The lack of effect of reserpine on the myocardial contractile response of hyperthyroid animals at varied calcium concentrations also supports the concept that endogenous norepinephrine does not play a primary role in the myocardial contractile response to inotropic agents in hyperthyroidism. The alteration in the frequency-force relationship suggests that thyroid hormone may have a direct effect on the myocardium which ultimately produces the observed changes in contractility.

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