Atrial Electrophysiology in Experimental Hyperthyroidism in Rabbits

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ABSTRACT

Male rabbits received L-thyroxine (250 μg/kg) subcutaneously for 7 days and exhibited marked tachycardia, elevation of protein-bound iodine levels and a 25% mean weight loss. Their hearts were isolated and perfused by the Langendorff technique. In 8 thyroid-treated rabbits the mean heart rate before they were killed and also after 30 minutes of perfusion was much higher than that of 10 controls; threshold in late diastole was lower, and the mean effective refractory period was shorter: 109 msec (95 to 116) as compared with 147 msec (130 to 170). The atrial multiple-response threshold was strikingly lower, and strength-interval curves were shifted to the left without overlap or change in contour. Atropine, 7 μg/liter, did not significantly affect these changes.

Acute perfusion of four hearts with L-thyroxine (500 μg/liter) produced similar, but less consistent, results. Perfusion with either epinephrine or norepinephrine, regardless of concentration, could not reproduce the changes seen with thyroid treatment. It is concluded that thyroxine profoundly alters electrophysiologic variables of atrial tissue independently of the autonomic nervous system and that these changes are responsible for many cardiac effects of hyperthyroidism, including the enhanced susceptibility to atrial fibrillation in thyrotoxicosis.

ADDITIONAL KEY WORDS hyperthyroidism atrial electrophysiology atrial fibrillation thyroxine refractory period

Since 1825 when Parry (1) described tachycardia and irregular pulse in thyrotoxicosis, the frequency of atrial fibrillation in this condition has been repeatedly stressed (2, 3). It is present in some 15% of patients when the diagnosis is first made (2). Reversion to sinus rhythm is common following treatment of hyperthyroidism and virtually uniform when there is no preexisting heart disease (2, 3).

The relationship between excess thyroid hormone and the heart has been the subject of both clinical and laboratory investigations. Since the alterations in cardiac function in hyperthyroidism resemble sympathetic overactivity, the major focus in previous reports has been on the role of catecholamines and on the influence of thyroxine in sensitizing the heart to the effects of the sympathetic nervous system. Although these previous studies may in part provide information as to the nature of sinus tachycardia, they reveal little which could explain the increased susceptibility of the heart to atrial fibrillation.

We have reexamined certain electrophysiologic variables of atrial tissue and compared the electrophysiologic properties of the atria in euthyroid and experimentally induced hyperthyroid rabbits.

Methods

Adult male white rabbits weighing 1.82 to 2.60 kg were randomly assigned to a control or thyroid-treated group. In the latter, hyperthyroidism was induced by a daily subcutaneous injection of 250 μg/kg L-thyroxine for 7 days. The injection was prepared by dissolving sodium L-thyroxine (Sigma Chemical Company) in a solution made alkaline by 0.1M NaOH and adjusted to a pH of 8.0 by adding 0.1M HCl. The control group received an equivalent volume of saline subcutaneously. Animal care and feeding were identical in the two groups. The thyroid state was evaluated in vivo by

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protein bound iodine, weight loss, and heart rate. Before the rabbits were killed, they were placed in a dark box until heart rate was stable, and the rate was then recorded by surface electrocardiography.

The rabbit hearts were perfused by the method of Langendorff with a standard Tyrode solution (NaCl 137 mM; KCl 4.0 mM; CaCl$_2$ 1.8 mM; MgCl$_2$ 1.05 mM; glucose 2 g/liter; and buffered with Na$_2$HPO$_4$ 12.5 mM, NaHCO$_3$ 2.4 mM). The temperature of the perfusate was maintained at 35.5° ± 0.2° C by a thermostatically controlled water bath. A stopcock permitted the change of solutions without altering the temperatures of the perfusate. Bipolar steel, platinum, or Ag-AgCl electrodes were attached to the right and left atrial appendages for recording and stimulating.

Electrophysiological Studies—Driving ($S_1$) and test stimuli ($S_2$) originated from independent constant-current generators. The basic driving stimulus was a square wave with a pulse width of 6 msec delivered with a current strength of twice threshold. The test stimulus was a square wave of 6 msec duration delivered through a separate but closely located bipolar electrode. Current strength was measured by the voltage drop method on a Tektronix RM 584 oscilloscope with a type 3A3 dual trace differential amplifier. A stimulus programmer, accurate to 1/23,000 msec, triggered the timed driving and test stimuli.

Electrograms were recorded either on the Tektronix storage oscilloscope by means of a Polaroid camera, or on a Grass Polygraph. Threshold was determined by delivering, after 16 driving pulses ($S_1$), a late diastolic test pulse ($S_2$) and was defined as the minimum current in milliamperes required to produce a response to each test stimulus. At a given basic driving frequency, strength-interval curves were determined by measuring the threshold requirements of $S_2$ when the $S_1$-$S_2$ interval was progressively shortened. The effective refractory period at a given basic driving frequency was defined as the minimum $S_1$-$S_2$ interval which permitted a successful response when the amplitude of $S_2$ was one and a half times threshold. Atrial multiple-response threshold was defined as the minimum current in milliamperes, or alternatively, the multiple of threshold strength required for $S_2$ to produce either three or more spontaneous atrial beats or, more commonly, transient atrial fibrillation. At a given basic driving frequency, the cardiac cycle was scanned with the test stimulus $S_2$ to determine the zone in which multiple responses could be elicited.

In 16 experiments, atropine sulfate was used in a concentration of 7 µg/liter. The spontaneous heart rate accelerated by 10 to 20% in 14 of these experiments.

In four experiments previously untreated rabbit hearts were perfused with 500 µg/liter L-thyroxine in standard Tyrode's solution, and the spontaneous rate, strength-interval curves, effective refractory period, and intra-atrial conduction time were determined. The last was recorded during atrial drive by measuring the interelectrode time from right and left atrial electrograms.

The effects of epinephrine and norepinephrine on perfused rabbit hearts not treated with thyroid were evaluated in four experiments. The spontaneous rate and the effective refractory period at a driven cycle length of 240 msec were determined before and after perfusion with standard Tyrode's solution containing either epinephrine or norepinephrine. A wide range of concentrations were employed up to and including that which was sufficient to produce maximum heart rate.

Results

Evaluation of the thyroid state was determined by the PBI, weight loss, and heart rate. Seven control rabbits had a mean PBI of 8.4
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Stimulus Strength (mA)

-0.1 0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1

INTERVAL (msec)

200 180 160 140 120 100 80 60

representative strength-interval curve (solid circles) in a thyroid-treated rabbit at a driving cycle length of 200 msec. Multiple atrial responses (or fibrillation) occurred at points X, between S1-S2 intervals of 110 and 130 msec and at S1 strengths of two to seven times threshold.

The atrial multiple-response threshold of control and thyroid-treated animals expressed as a multiple of threshold (as previously determined in late diastole). Several animals had no obtainable atrial multiple-response threshold, and one thyroid-treated rabbit had spontaneous transient atrial fibrillation.

- The spontaneous cycle length of the Langendorff preparation was determined after 30 minutes of perfusion. Ten control rabbits had a mean spontaneous cycle length of 490 msec (440 to 560 msec), eight thyroid-treated rabbits showed an average cycle length of 265 msec (236 to 310 msec). No overlap between the two groups was observed.

- At a given basic driving cycle length (S1-S2) of either 200 or 240 msec, the strength-interval curves of the thyroid-treated rabbits were displaced to the left as compared to the control group. Figure 1 shows the strength-interval curves of five control and four thyroid-treated animals at a driving cycle length of 240 msec. The shift to the left of the curve was not accompanied by a change in contour or overlap between the two groups.

- The mean effective refractory period was uniformly shorter in the thyroid-treated than in the control group. At a driving cycle length (S1-S2) of 240 msec, ten control rabbits had a mean effective refractory period of 147 msec (130 to 170 msec) while five thyroid-treated animals had a mean effective refractory period of 100 msec (95 to 118 msec). At a driving cycle length (S1-S2) of 200 msec, the mean effective refractory period in four control

Control

Thyroid Treated

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Transient Atrial Fibrillation

- μg/100 ml while eight thyroid-treated animals had values greater than 50 μg/100 ml. The control rabbits maintained a stable weight. Eight thyroid-treated rabbits had a mean initial weight of 2.14 kg; all animals lost weight, the mean figure after treatment being 1.56 kg, an average loss of 26%. Ten control rabbits had a mean cycle length of 346 msec (270 to 390 msec) as compared to 177 msec (155 to 210 msec) in eight thyroid-treated rabbits.

- The spontaneous cycle length of the Langendorff preparation was determined after 30 minutes of perfusion. Ten control rabbits had a mean spontaneous cycle length of 490 msec (440 to 560 msec), eight thyroid-treated rabbits showed an average cycle length of 265 msec (236 to 310 msec). No overlap between the two groups was observed.

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Threshold in late diastole in control and thyroid-treated rabbits.

A representative strength-interval curve with the zone in which atrial multiple responses were elicited during the cardiac cycle is shown in Figure 2. Atrial multiple responses were found in 5 of 10 controls and in 6 of 8 thyroid-treated animals. Of greater significance is that all six of the thyroid-treated animals had an atrial multiple-response threshold below twice the late diastolic threshold. One thyroid-treated rabbit which had not been subjected to further study had frequent spontaneous transient episodes of atrial fibrillation during perfusion. Only 3 of 10 controls had an atrial multiple-response threshold of less than five times threshold; the remaining two required very high multiples of threshold. Data is summarized in Figure 3. Atropine had no consistent or predictable effect on these results.

Threshold determined by a test pulse delivered late in the basic driven cardiac cycle showed a mean value of 0.17 ma (0.11-0.28 ma) in ten control rabbits and 0.12 ma (0.06-0.19 ma) in eight thyroid-treated rabbits. The Student t-test indicates significance at a level of P = 0.05. This was the only variable in which no clear separation existed between the control and the thyroid-treated groups (Fig. 4). Atropine had no significant effect on the threshold.

Four normal rabbit hearts using the Langendorff preparation were perfused with 500 μg/liter thyroxine in standard Tyrode’s solution. No significant difference was noted in the spontaneous rate before and after thyroxine perfusion. Changes in both the strength-interval curves and the effective refractory period were qualitatively the same as in the experiments on the chronically thyroid-treated group, but the results were less consistent and predictable. No significant alteration in the intra-atrial conduction time was noted in experiments lasting up to 4 hours.

In four experiments employing epinephrine perfusion, the mean spontaneous control cycle length was 519 msec (450 to 590 msec), and
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the minimum cycle length after epinephrine perfusion was 337 msec (320 to 370 msec). At a basic driving cycle length (S_i-S_i) of 240 msec, the mean effective refractory period in the control was 150 msec (140 to 160 msec) and after epinephrine was 147 msec (142 to 155 msec). In four experiments employing norepinephrine, the mean spontaneous control cycle length was 522 msec (480 to 550 msec), and following norepinephrine perfusion, the minimum cycle length was 331 msec (320 to 340 msec). At a basic driving cycle length (S_i-S_i) of 240 msec, the mean effective refractory period of the control was 151 msec (146 to 158 msec) and after norepinephrine perfusion was 138 msec (132 to 140 msec). Thus epinephrine in doses sufficient to produce maximum heart rate did not significantly alter the effective refractory period, but norepinephrine reduced it 12%. The difference between the mean effective refractory periods obtained in control and thyroid-treated rabbit hearts (at an S_i-S_i of 240 msec) was 38%.

Discussion

The present study demonstrates that treatment of the rabbit atrium with thyroxine induces a sinus tachycardia, a shift to the left of the strength-interval curves, a shortening of the effective refractory period, a lowering of the atrial multiple-response threshold, and a probable decrease of the threshold in late diastole. These changes appear to be a direct effect of thyroxine treatment on the atrium—that is, they are not mediated by either catecholamines or acetylcholine.

Although others have claimed a role for the sympathetic nervous system in the tachycardia of hyperthyroidism (4-7), recent studies deny this relationship. Catecholamine infusion does not inappropriately potentiate the heart rate and blood pressure in the thyroid-treated cat (8), dog (9), or hyperthyroid human (10). Similarly, the response in heart rate to sympathetic stimulation does not differ significantly in the thyroid-treated dog as compared to the control, except that the baseline rate is higher in the former (11).

Although Herndon and Wenger (12) suggest that atrial fibrillation may be due to an increased sensitivity to catecholamines, the evidence of this and other studies (13) is that epinephrine and norepinephrine produce little alteration in the electrophysiologic properties of atrial tissue (14, 15) and cannot mimic the changes induced by thyroxine.

Acetylcholine has long been known to produce atrial fibrillation in the experimental animal, and mecholyl has been shown to produce atrial fibrillation in humans (16). Vagal stimulation produces a shortening of the action potential and hence the refractory period. Alessi et al. have shown that this effect is not uniform in its distribution and suggest that the resulting electrical inhomogeneity makes conditions ideal for reentrant excitation and atrial fibrillation (17).

Leveque observed an increased susceptibility to atrial fibrillation in thyroid-treated dogs infused with acetylcholine; 81% had atrial fibrillation compared to 31% of the controls (18). Hoffman et al., however, stated that the thyrotoxic heart is less sensitive than normal to vagal stimulation (19).

The shortening of the refractory period and increased tendency to atrial fibrillation demonstrated in the thyroid-treated hearts of our study are certainly reminiscent of the typical changes induced by vagal stimulation or by the administration of acetylcholine. However, in our experiments these effects remained long after the heart had been excised (hence denervated), isolated, and perfused. Most importantly, they were not significantly influenced by atropine.

The tachycardia of the thyroid-treated preparations in our study has been noted by other investigators (20). It can be presumed that such preparations, particularly after 30 minutes have elapsed, are under greatly diminished autonomic influence. In similar vein, Markowitz and Yater as early as 1932 demonstrated that thyroid hormone increased the heart rate in 2-day-old chick embryos, an age before neutral elements develop (21). The independence of these rate changes of the autonomic nervous system has been demonstrated clinically, using propranolol. The latter either does not significantly alter the resting...
heart rate in thyroid-treated normal humans (22), or reduces the rate comparably in patients with and without hyperthyroidism (23).

Although the changes in atrial electrophysiology noted in this study are attributed to a direct action of thyroxine, it is clear that the clinical picture of thyrotoxicosis can be additionally affected by the autonomic nervous system. For example, the actual initiation of fibrillation could occur during a sudden increase in vagal effect acting on an already "primed" atrial myocardium.

Since the cellular electrical properties of spontaneous rate and refractoriness are mediated by the cell membrane, the action of thyroxine on atrial muscle must involve the membrane directly or indirectly. Acetylcholine produces changes secondary to an increase in the potassium permeability of the membrane. In the present experiments, an acetylcholine effect is presumably ruled out by unresponsiveness of our preparation to atropine. The induction of a similar change in potassium permeability by thyroxine seems unlikely, in view of its reduction of end-diastolic threshold. Acceleration of repolarization can also be induced by any membrane change that increases outward membrane current during the action potential, such as more rapid inactivation of inward sodium current. There is no reason to deny that thyroxine has a direct influence on active or passive ionic transport across cardiac cellular membranes. Thyroid hormone has been found to affect ionic movement in several tissues. In a series of papers, Matty and Green found that active transport of sodium in the toad bladder was inhibited by thyroid hormone (24-26). Timiras and Woodbury noted decreased extracellular and increased intracellular sodium in the brain of thyroxine-treated rats (27). Liu and Overman found an accumulation of sodium and calcium within skeletal muscle cells of thyroxine-treated rats and concluded that active transport was inhibited (28). The interrelationship between sodium pump activity, or indeed any metabolic activity, and membrane events in excitable tissue needs to be better understood before this mode of action of thyroxine can be evaluated.

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