Atrial Electrophysiology in Experimental Hyperthyroidism in Rabbits

By Morton F. Arnsdorf, M.D., and Roderick W. Childers, M.D.

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 26% 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 1835 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 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.

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
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; CaCl₂ 1.8 mM; MgCl₂ 1.05 mM; glucose 2 g/liter; and buffered with Na 16 mM, HCO₃⁻ 12.5 mM, H₂PO₄ 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 (S₁) and test stimuli (S₂) 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 554 oscilloscope with a type 3A3 dual trace differential amplifier. A stimulus programmer, accurate to 1/25,000 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 (S₁), a late diastolic test pulse (S₂) 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 S₂ when the S₁-S₂ interval was progressively shortened. The effective refractory period at a given basic driving frequency was defined as the minimum S₁-S₂ interval which permitted a successful response when the amplitude of S₂ 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 S₂ to produce 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 S₂ to determine the zone in which multiple responses could be elicited.

In 10 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 nontreated 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

![Figure 1](http://circres.ahajournals.org/lookup/suppl/doi:10.1161/01.RES.26.1.576/-/DC1/fig1)

Strength-interval curves on rabbit atria in control and thyroid-treated groups. The driving frequency (S₁-S₁) was 240 msec. The current requirement of the test pulse (S₂) is expressed in multiples of the late diastolic threshold value.
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Stimulus Strength (mA)

10
8
6
4
2
0

10-09
8-06
6-03
4-01
2-00
0-00

60 80 100 120 140 160 180 200 S₁-S₂ INTERVAL (msec)

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 S₂ strengths of two to seven times threshold.

FIGURE 2

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.

Threshold in late diastole

<table>
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<th>CURRENT IN mAmp</th>
<th>CONTROL</th>
<th>THYROID TREATED</th>
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<tr>
<td>0.28</td>
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<td>0.26</td>
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<td>0.04</td>
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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.

In four experiments employing epinephrine perfusion, the mean spontaneous control cycle length was 519 msec (450 to 580 msec), and...
the minimum cycle length after epinephrine perfusion was 337 msec (320 to 370 msec). At
a basic driving cycle length (Si-Si) 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 (Si-
Si) 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 epineph-
rine 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^Si of 240 msec) was 38*.

Discussion

The present study demonstrates that treat-
ment 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). Simi-
larly, the response in heart rate to
sympathetic stimulation does not differ signifi-
cantly 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) sug-
gest 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 susceptibil-
ity 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 dem-
onstrated 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 exercised (hence
denervated), isolated, and perfused. Most
importantly, they were not significantly in-
fluenced 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 demon-
strated 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 (22).

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.

References
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