Evidence that decreased heart rate in thyroid hormone receptor-α1-deficient mice is an intrinsic defect

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Johansson, Catarina, Björn Vennström, and Peter Thorén. Evidence that decreased heart rate in thyroid hormone receptor-α1-deficient mice is an intrinsic defect. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R640–R646, 1998.—Using a telemetry system with implantable transmitters, we recorded heart rate, electrocardiogram (ECG), body temperature, and locomotor activity continuously in awake, freely moving mice deficient in the thyroid hormone receptor-α1 (TRα1). We have previously reported that the TRα1-deficient mice have a 20% lower mean heart rate and a 0.5°C lower body temperature compared with wild-type control animals. In this study we found that when 3,5,3'-triiodothyronine (T3) was given once a day, there was a parallel increase in heart rate (occurring 1 day later in the TRα1-deficient mice than in controls) and body temperature. Analysis of single-lead ECG revealed a prolonged QRS and Q-T time (from Q to end of the T wave), which was shortened after T3 treatment. Monophasic action potential durations, measured in hearts from anesthetized mice at 90% of repolarization, were significantly prolonged in TRα1-deficient mice. Air-jet stress and a single injection of an anticholinergic agent induced a parallel increase, and a β-adrenergic receptor blocker induced a decrease in heart rate in both groups. There was no difference in β-adrenergic receptor density. The results indicate that the TRα1-deficient mice have a specific defect in intrinsic heart rate regulation.

electrocardiogram; monophasic action potentials; body temperature; blood pressure

THYROID HORMONE (TH) plays a major role in development, metabolism, and cardiovascular function. It is well known that hypothyroidism in childhood leads to short stature and mental retardation. Adults have decreased body metabolism and decreased heart rate. TH exerts its effect through specific nuclear receptors inducing synthesis of new proteins (19). The receptors belong to the superfamily of intranuclear receptors that also includes receptors for glucocorticoids, estrogen, retinoic acids, and vitamin D3. Four different mammalian TH receptors, encoded by two different genes, have been characterized to date (20, 25): TRα1, TRα2, TRβ1, and TRβ2.

Three important mechanisms (as reviewed in Ref. 5) mediate the TH-induced effects on the heart: 1) a direct effect (nuclear or extranuclear) on cardiac cells, 2) the influence of an altered thyroid status on the sympathoadrenal system, and 3) indirect effects mediated by increased peripheral oxygen consumption and hemodynamic changes generated in the periphery. The direct effect results from interaction between 3,5,3'-triiodothyronine (T3) and specific intranuclear TH receptors. TRα1 and TRβ3 act as T3-dependent transcription factors. However, T3 has also been described to have rapid effects, indicating extranuclear mechanisms of action, although these are poorly understood (4). Many studies have indicated that TH increases β-adrenergic receptor density (23, 24). Different thyroid states are also known to affect the duration of the ventricular action potential in the heart (7). Some patients with hypothyroidism have a prolonged Q-T interval in the electrocardiogram (ECG) (17). The mean arterial blood pressure is usually unchanged (as reviewed in Ref. 16).

The TRα1-deficient mice used in this study were generated by homologous recombination in embryonic stem cells. A targeting vector was constructed so that the TRα1-specific coding sequence was replaced with that of TRα2, as described in our recently published paper (26). The embryonic stem cells were used for generation of TRα1-deficient and matching wild-type mice (26).

We have previously reported (26) that the TRα1-deficient mice are normal with respect to gross anatomy and reproduction. Measurement of hypophysial thyroid-stimulating hormone and serum L-thyroxine (T4) in male mice showed that the levels were slightly lower in the TRα1-deficient mice than in wild-type mice, indicating a mild hypothyroidism (female mice have normal hormone levels). However, T3 levels were normal. Furthermore, the TRα1-deficient mice have a 20% lower heart rate than the control mice, a prolonged QRS and Q-T time (from Q to end of the T wave), and a 0.5°C lower body temperature. The aim of the present study was to further investigate the mechanisms and time course of these disturbances in heart rate, ECG, and body temperature before and after T3 treatment in TRα1-deficient mice. We also investigated the possible role of the autonomic nervous system to determine whether the bradycardia is an extrinsic neurogenic effect or an intrinsic effect of TRα1 deficiency.

METHODS

Animals. Twenty-four male TRα1-deficient mice and 23 male wild-type control animals, all between 9 and 13 wk of age and weighing 23–36 g, were used. The mice represent a cross between the SV-129 and BALB/c strains. Experiments were also performed in six mice of the strain SV-129 (origin of the embryonic stem cells used for developing the TRα1-deficient mice). The animals were kept in a climate-controlled room on a 12:12-h light-dark cycle. Standard diet (Beekay Feeds; B&K Universal, Stockholm, Sweden) and water were...
provided ad libitum. The experimental procedures were approved by the North Animal Ethical Committee of Stockholm.

The telemetry system. As described earlier (15), the telemetry system (Data Sciences, St. Paul, MN) consists of implantable transmitters (TA10 ETA F-20), telemetry receivers (RA1010), and a consolidation matrix (BCM 100). Eight universal adapters (UA 10 PC), receiving calibration factors specific for each transmitter, were coupled to the consolidation matrix. The receiver detects the modified signal sent out from the transmitter, and the UA 10 adapter reconstructs the calibrated body temperature and ECG as analog signals. The gross locomotor activity was detected by the receiver. The data acquisition system consists of a Data translation (DT 2801) AD converter in a Pentium computer. The computer program PC-LAB v. 5.0 (1) sampled calibrated values of body temperature and ECG and noncalibrated locomotor activity counts repeatedly during the course of the experiments. The calculation of data has been described earlier (15).

Operation procedure. The operation procedure was performed as described earlier (15). In brief, the animals were anesthetized with 7 ml/kg ip of a mixture of 0.315 mg/kg fentanyl and 10 mg/ml fluanisone (Hypnorm, janssen), 5 mg/ml midazolam (Dormicum, Roche), and sterilized water in a 1:1:2 ratio. The transmitter was implanted in the peritoneal cavity of each mouse at least 7 days before the experiments. The electrodes were seated subcutaneously; the negative lead was positioned and sutured subcutaneously at the right shoulder, and the positive lead was sutured toward the lower left chest.

Experimental protocol. Each experiment was performed on age-matched homozygote TRα1-deficient mice and wild-type control animals. After baseline registration for 48 h, the animals received T3 (Sigma Chemical) at 1 mg/kg. The hormone was first dissolved in a small volume (20 µl) of 0.5 M NaOH and diluted with saline and was given subcutaneously at 1:00 PM daily for 4 days. On the fifth day the animals were killed by cervical dislocation. In another series of experiments, the mice were given a single dose of a cholinergic blocker (methylscopolamine, 0.1 mg/kg, Sigma) subcutaneously and after another 20 min, a single dose of an unspecific β-adrenergic receptor blocker (timolol, 1 mg/kg, Sigma) was given before T3 treatment.

Air-stress experiment. After 30 min of baseline registration, a jet of air was blown through plastic tubing into the cage for 15 min, inducing acute mental stress, without physically harming the animals. We have previously described similar methods used in rats (18).

Monophasic action potentials. Four TRα1-deficient and four control mice were anesthetized and put on a heating pad. Rectal temperature was monitored and kept between 36 and 37°C by use of an infrared heating lamp. After tracheotomy, the animals were mechanically ventilated by a pressure-controlled respirator (built at Astra Hässle, Göteborg, Sweden). In pilot experiments, a tidal volume of 0.2 ml and a respiratory frequency of 90 breaths/min were found to be optimal for keeping blood gases and pH within the physiological range. First, needle electrodes were inserted into the limbs for recording of the lead II electrocardiogram on a Grass polygraph (model 7), and then the heart was exposed by dividing the sternum and transecting two ribs on the left side of the sternum. Left ventricular epicardial monophasic action potentials (MAPs) were recorded by using two electrodes consisting of Teflon-coated platinum wires (0.1 mm thick) held 1–2 mm apart. One wire was covered with a saline-filled sponge, whereas the other was desinulated for 1 mm and pressed into the myocardium. The signal was sent through a custom-built MAP amplifier and transcribed on the Grass polygraph at a speed of 100 mm/s. The action potential duration was measured at 90% of the repolarization. The mean duration of the action potentials was calculated from 5–10 consecutive beats.

β-Adrenergic receptor density measurement. Nine hearts from the TRα1-deficient mice and seven hearts from wild-type controls, each heart weighing 135 ± 8.2 mg, were used. All the following procedures were carried out at 0°C. Each heart was thawed and cut into small pieces and homogenized in 2 ml of ice-cold 0.1 M potassium phosphate buffer (pH 7.4) with a polytron (Ultraturrax) at high speed three times for 10 s. The mixture was then centrifuged (Heraeus Instruments, Hanau, Germany) at 15,000 rpm (20,124 g) for 20 min at 4°C. Each pellet was then resuspended in 5 ml of ice-cold buffer and homogenized with a glass homogenizer. Duplicate test tubes containing 100 µl membrane suspension, 100 µl β-adrenergic receptor ligand (−)-(−)-[5,7−H]CGP-12177 (44 Ci/mmol, 22.7 µM; New England Nuclear, Boston, MA) (final concentration 50 nM), or (−)-propranolol (final concentration 25 µM) were prepared. After 60 min of incubation, the suspensions were drawn through a filter by the use of a Skatrol cell harvester (Packard Instrument Co., USA), and the filters were then placed in scintillation vials with 5 ml of scintillation fluid (Ready Safe, Beckman) and incubated for 24 h. Radioactivity was then counted in a β-counter (Packard liquid scintillator). Specific binding was defined as the difference in binding determined in the absence and presence of (−)-propranolol. Complete binding assay data were analyzed by a nonlinear least-squares curve-fitting procedure (Origin v. 3.5).

Blood pressure measurement. Six TRα1-deficient mice and five wild-type controls were used. Animals were anesthetized with Ketalar (25 mg/ml) and Narcoxyl (5 mg/ml) mixed 2:1, of which 6 ml/kg was given intraperitoneally. The carotid artery was then cannulated with a PE-10 catheter for continuous blood pressure monitoring. The catheter was exteriorized at the neck of the mouse and connected to a swivel system. The animals were then allowed to wake up and recover for 24 h before recording started. The pressure line was connected to a transducer and an online computer system that calculated mean arterial blood pressure two times each minute. The recordings were performed for 2–3 h the following afternoon (1:00–4:00 PM).

Statistics. All parameters are expressed as means ± SE. Student’s t-test was used for the comparison of two means. A pairwise multiple-comparison ANOVA for repeated measurements followed by post hoc tests (Student-Newman-Keuls) were used to evaluate significance. Statistical significance was defined by P < 0.05.

RESULTS

Heart rate. We have previously reported (26) that the TRα1-deficient mice have a lower mean heart rate than wild-type controls (515 beats/min compared with 632 beats/min). In this study, we found that the SV-129 strain of mice (n = 5) had a mean heart rate of 568 ± 13.6 beats/min.

In the previous study the circadian variability of heart rate was studied in detail. When T3 was given once a day after 2 days of baseline recording, a parallel increase in mean heart rate was observed in TRα1-deficient mice and wild-type mice (Fig. 1). However, T3 treatment seemed to have a different effect in the TRα1-deficient mice compared with the controls. Thus before T3 treatment the difference between day and night heart rate was 76 ± 14 beats/min in the TRα1-
TR$_{a1}$-deficient mice and controls showed that there was no correlation between the heart rate and the Q-T$_{end}$ time either in baseline values or after T$_3$ treatment (average slopes were 0.001 ± 0.004 and r = 0.19 ± 0.03). This is in agreement with data obtained previously in C57 mice (18).

Table 1 shows that after 3 days of T$_3$ administration there was a significant decrease in QRS and Q-T$_{end}$ time in both TR$_{a1}$-deficient and wild-type mice. There was a slight but nonsignificant increase in PQ time (from start of P wave to Q) in the homozygote mice in the first experiments performed in five TR$_{a1}$-deficient and seven control mice. However, in the whole material (n = 13 and n = 15, respectively) the difference was significant: 33.2 ± 0.8 ms in the TR$_{a1}$-deficient mice and 30.0 ± 0.4 ms in the controls. The ECG values of the SV-129 strain of mice (n = 5) shown in Table 1 were similar to those of the wild-type control mice.

MAP measurement. The T wave is not separated from the QRS complex in ECG of mice. To elucidate whether we actually measure the repolarization phase, we measured bipolar surface ECG and MAP duration. Measurement of bipolar surface ECG revealed that in anesthetized TR$_{a1}$-deficient mice the Q-T$_{end}$ time was prolonged (37.5 ± 1.4 ms, n = 4) compared with that in controls (30.0 ± 0.0 ms, n = 4). The MAP duration was also prolonged: 60 ± 4.1 ms in TR$_{a1}$-deficient mice compared with 42.5 ± 1.4 ms in the controls (Fig. 4).

Air stress. We elicited an activation of the sympathetic nervous system by blowing a jet of air on the mice. The air stress resulted in a significant parallel increase in heart rate; the TR$_{a1}$-deficient mice reached a mean maximum heart rate of 604 ± 26 beats/min compared with the wild-type animals, which reached 679 ± 14 beats/min (Fig. 5). Mean values were calculated from 15 min of stress. Similar differences were observed in T3-treated mice. Thus the T3-treated TR$_{a1}$-
deficient mice reached a mean maximum heart rate of 717 ± 30 beats/min and the control mice 840 ± 21 beats/min.

Cholinergic- and β-adrenergic receptor blockade. A functional heart denervation was performed by pharmacological blockage of the sympathetic and parasympathetic nervous system. After 2 days of baseline registration, an anticholinergic agent (methylscopolamine, 0.1 mg/kg) was injected and a parallel increase in heart rate was seen in both groups (Fig. 6). An unselective β-adrenergic receptor blocker (timolol, 1 mg/kg) was then administered and a parallel decrease in heart rate was observed. Thus the difference in basal cardiac rhythm remained.

β-Adrenergic receptor density. Using the hydrophobic ligand [5,7-3H]CGP-12177, a β-adrenergic receptor antagonist that selectively binds to cell surface β-receptors (22), we determined the number of [5,7-3H]CGP-12177 binding sites expressed on intact cells. We found no difference in β-adrenergic receptor density between the TRα1-deficient mice [maximal binding capacity (Bmax) = 32.1 fmol/mg] and the control mice (Bmax = 31.8 fmol/mg).

Body temperature, locomotor activity, and blood pressure measurement. The TRα1-deficient mice have a 0.5°C lower body temperature than the wild-type controls. A parallel increase in body temperature was seen in both groups after T3 treatment (Fig. 1B), and the

Table 1. Different time intervals in ECG complex and body temperature in TRα1-deficient mice mice compared with wild-type control mice before and after 3 days of injection of T3

<table>
<thead>
<tr>
<th>Time interval, ms</th>
<th>Baseline</th>
<th>After T3 Treatment</th>
<th>Baseline</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>WT</td>
<td>TRα1 deficient</td>
<td></td>
</tr>
<tr>
<td>PQ</td>
<td>30.2 ± 0.7</td>
<td>31.0 ± 0.7</td>
<td>29.1 ± 0.8</td>
</tr>
<tr>
<td>QRS</td>
<td>9.8 ± 0.3</td>
<td>11.4 ± 0.2†</td>
<td>9.1 ± 0.4†</td>
</tr>
<tr>
<td>QToend</td>
<td>18.8 ± 1.4</td>
<td>27.2 ± 1.4‡</td>
<td>15.6 ± 0.7‡</td>
</tr>
<tr>
<td>Body temperature, °C</td>
<td>36.5 ± 0.1</td>
<td>36.0 ± 0.05†</td>
<td>37.0 ± 0.1§</td>
</tr>
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Values from baseline recording of strain SV-129 are also shown; 24-h mean values are presented as means ± SE. ECG, electrocardiogram. Significant difference between wild-type (WT) and TRα1-deficient mice: *P < 0.05, †P < 0.01. Significant difference between untreated and 3,5,3'-triiodothyronine (T3)-treated mice: ‡P < 0.05, §P < 0.01.
increase occurred after 2 days. The SV-129 strain of mice \( n = 5 \) had a slightly higher average of body temperature of 36.8 ± 0.1°C. There was no significant difference between the groups in locomotor activity either before or after T3 treatment. The arterial blood pressure of the TRα1-deficient mice \( n = 6 \) did not significantly differ from the controls \( n = 5 \): 114 ± 6.4 mmHg compared with 125 ± 6.6 mmHg.

**DISCUSSION**

A number of studies have shown tissue-specific expression of TH receptors, and it has been suggested that the TRα and TRβ proteins have distinct functions (8–10). In this study, we have used mice with a specific deficiency of the TRα1 subtype to study the role of this receptor in (metabolism and) cardiac function.

We have previously reported that TRα1-deficient mice have a 20% lower heart rate, a prolongation of the QRS and Q-Tend time in ECG, and moderately lower body temperature than the control mice. We also showed that male TRα1-deficient mice had normal T3 levels but slightly subnormal levels of T4 (26). Female mice have normal T3 and T4 levels. Data from experiments performed in female animals show the same values as those from male mice (data not shown), indicating that the bradycardia seen is not due to low T4 levels.

Heart rate and body temperature in rodents show circadian and ultradian (27) variability. In the control situation, both the TRα1-deficient mice and the control mice show similar circadian and ultradian patterns. However, on T3 treatment the circadian variability was decreased in the controls but was unaffected in the homozygote mice. Despite the fact that the basal heart rate was markedly lower in the TRα1-deficient mice, the increase in heart rate was similar after 4 days of T3 treatment in the TRα1-deficient mice and the controls. However, scrutinization of the data revealed that the time course of the response was different. Thus in the TRα1-deficient mice we observed no effect until after 2 days of treatment (Fig. 2) at a time when the heart rate increase was almost maximal in the control mice (Fig. 2). This suggests that different mechanisms mediate the increase in heart rate seen after T3 treatment in TRα1-deficient mice and in controls.

The decreased heart rate in the control situation in the TRα1-deficient mice might be explained by several mechanisms: elevated neurogenic activity, a downregulation of the cardiac β-adrenoreceptors, or an intrinsic effect, e.g., decreased excitability in the sinus node pacemaker. In the present study we therefore compared the heart rate response to stress (Fig. 5) and to autonomic blockade (Fig. 6) in TRα1-deficient and control mice. The results showed a similar response to stress in TRα1-deficient and control mice. If the heart rate disturbance was due to attenuated expression of cardiac β-adrenergic receptors in TRα1-deficient mice, we would most likely have seen a smaller response to a β-adrenergic receptor blocker. However, this was not the case. Furthermore, we performed classic β-adrenergic receptor binding studies on hearts from TRα1-

![Fig. 4. Typical example of recording of monophasic action potential duration in heart of control mouse (A) and TRα1-deficient mouse (B). Note that recordings are drawn with a standard Grass unit recorder, which generates curvilinear printouts.](http://ajpregu.physiology.org/)

![Fig. 5. Air-jet experiment. A: mean heart rate from 1 h to 30 min before air was blown on TRα1-deficient mice (dotted line; \( n = 5 \)) and controls (solid line; \( n = 6 \)). Thereafter, 30 min is left out (break in lines) before air-jet was applied (marked with a black bar) and the subsequent resting period. B: mean changes in heart rate from 30 min of recording 1 h before air-jet stress and from 15 min of recording during time air was blown on TRα1-deficient (open bars; \( n = 5 \)) and wild-type (filled bars; \( n = 6 \)) mice. Parallel increase in heart rate is seen. *Significant difference between wild-type and TRα1-deficient mice (\( P < 0.05 \)). †Significant difference in 24-h mean values within same group (\( P < 0.05 \)).](http://ajpregu.physiology.org/)
The conclusion is then that the bradycardia is due to an intrinsic effect and not due to an altered neurogenic activity or a change in β-adrenergic receptor density.

We also show in this study that the QRS and QT-end times in ECG are prolonged in the TRα1-deficient mice. In developing the TRα1-deficient mice, embryonic stem cells from the strain SV-129 were used. Some reports (12) suggested that the phenotypic findings observed in gene-manipulated mice might derive from the embryonic stem cells used. However, mice of the strain SV-129 did not show any such difference compared with controls, as seen in Table 1. Thus the prolongation of the QT-end time in ECG is presumably due to lack of the TRα1 and not due to the SV-129 strain background.

It is difficult to measure ECG in small rodents. With our computerized telemetry system, we have managed to obtain data that can be quantified. However, in contrast to, e.g., the human ECG, the T wave in the mouse is not separated from the QRS complex. Therefore, we used the MAP duration recording technique, which is the preferred method for evaluation of the repolarization phase (14). The MAP recordings reproduce more faithfully the repolarization phase of intracellular action potentials of the myocardial cells (11). Earlier reports of ECG recordings in normal mice of different strains demonstrated QRS and QT-end times similar to those found in our study (3). However, in a recent study the QT-end time in mice was reported to be much longer (105 ms) (2). We therefore measured the MAP and compared it with the bipolar surface ECG in parallel in anesthetized animals. Our results showed that the MAP duration was significantly longer in the TRα1-deficient mice compared with the controls (60 ± 4 ms vs. 42 ± 2 ms). The relatively longer duration of the action potential in the MAP recording than in the telemetry system is not surprising because the animals were anesthetized. The thorax was opened, and the temperature of the heart surface was not controlled directly.

What is then the mechanism for the disturbance in cardiac action potentials and heart rate control? The most likely mechanisms are a change in activities of different ion channels and pumps, i.e., Na+ (8), Ca2+, and K+ channels and/or in the control of pacemaker activity [the hyperpolarization-activated "pacemaker current" and L-type Ca2+ current (13), possibly also T-type Ca2+ current]. However, our results cannot explain, at the cellular level, the changes in the TRα1-deficient mice. Further studies with voltage-clamp techniques are needed.

In the present study, we observed an interesting phenotype in mice deficient in TRα1. Thus the animals have a prolongation of the QRS and QT-end time in ECG combined with the bradycardia. The present study suggests that the ECG changes are due to an intrinsic defect in the cardiac pacemaker and ventricular currents in the TRα1-deficient mice. We also conclude that the bradycardia is due to an intrinsic effect and not to an altered neurogenic activity or altered expression of β-adrenergic receptors.

Perspectives

TH is involved in several physiological and developmental processes. TH plays a major role in the cardiovascular system, such as regulation of heart rate, cardiac output, and blood lipid status. TH acts through nuclear hormone receptors, which are ligand transcription factors encoded by two different genes. The proteins from the TRα1-gene bind TH and regulate gene expression. Today, no specific agonists or antagonists are developed with an effect on TRα1.

Today, the standard class I and class III antiarrhythmics are accompanied by severe proarrhythmic side effects. Amidarone is an antiarrhythmic compound acting as an unspecific antagonist on the intranuclear TRs. The compound is widely used to treat arrhythmias in humans despite unfavorable kinetics and bioavailability. In the future, new compounds with specific effects on subtypes of intranuclear TH receptors will be very important. The present experiments using mice deficient in TRα1 are therefore of great importance because the data will allow us the study the cardiovacular selectivity of different TRs.

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