Pregnancy-induced increased heart rate is independent of thyroid hormones

Valérie Long, MSc,*† Sophie Mathieu, PhD,*† Céline Fiset, PhD*†

From the *Research Center, Montreal Heart Institute, Montreal, Canada, and †Faculty of Pharmacy, Université de Montréal, Montreal, Canada.

BACKGROUND The heart rate increases by 10–20 beats per minute (bpm) throughout pregnancy in women, reaching maximum heart rate in the third trimester. During pregnancy, important changes in thyroid hormones also occur, with increases of up to 50% in the levels of triiodothyronine (T3), the biological active thyroid hormone. In addition, T3 has been shown to regulate cardiac electrophysiology.

OBJECTIVE Thus, in the present study the potential contribution of T3 in pregnancy-induced increased heart rate was explored.

METHODS We compared the heart rate between nonpregnant and pregnant mice under control conditions and after altering thyroid hormone levels with T3 and propylthiouracil (PTU, an antithyroid drug) treatments.

RESULTS Consistent with the clinical data, we found a 58% rise in T3 levels during pregnancy in mice. Although pregnant mice had a higher baseline heart rate (607 ± 8 bpm, P = .004) and higher T3 levels (1.9 ± 0.4 nM, P = .0005) than nonpregnant mice (heart rate: 546 ± 16 bpm; T3 levels: 1.2 ± 0.1 nM), their heart rate responded similarly to T3 treatment as nonpregnant mice (nonpregnant: Δ130 ± 22 bpm; pregnant: Δ126 ± 17 bpm, P = .858). Additionally, the heart rate remained significantly elevated (607 ± 11 bpm, P = .038) and comparable to untreated pregnant mice, after the use of the antithyroid drug PTU, although T3 levels (1.3 ± 0.2 nM, P = .559) returned to nonpregnant values.

CONCLUSION Based on these results, it is unlikely that T3 contributes significantly to the pregnancy-induced increased heart rate.

KEYWORDS Heart rate; Mouse; Pregnancy; Propylthiouracil; Triiodothyronine

Introduction

Pregnancy is associated with several physiological changes, including a significant increase in the resting heart rate,1,2 which creates an arrhythmogenic environment.1,3 Pregnancy also induces complex changes in several circulating hormones necessary for the maintenance of pregnancy and for the healthy development of the fetus.4,5 Hence, hormonal changes related to pregnancy are likely to be involved in the faster heart rate and increased incidence of arrhythmias reported in pregnancy.6,7 It is indeed conceivable that the significant increase in their concentrations might affect the electrical properties of the sinoatrial node (SAN), the pacemaker of the heart, leading to greater vulnerability to sinus tachyarrhythmias.8

Besides estrogens and progesterone, which are the main pregnancy hormones, triiodothyronine (T3), the biological active thyroid hormone, is also elevated during pregnancy and has been reported to alter the electrical activity of the heart.9,10 The concentration of T3 gradually increases up to 50% at mid-gestation,9,11,12 which is maintained until delivery.12,13 T3 levels found during pregnancy are required to maintain pregnancy and prepare for lactation.14 Although necessary, abnormal levels of thyroid hormone may result in many complications for both the mother and the fetus.4,15

Fluctuations in levels of T3 have been shown to affect the function and expression of ion channels16,17 and calcium handling protein18,19 involved in the electrical activity of the heart. Particularly, increased T3 levels have been reported to accelerate the heart rate9,10,16,20 and lead to sinus tachycardia,9,21 by a direct effect on the automaticity of the SAN.20

We previously showed that, as in women, pregnancy in mice is also associated with an increased resting heart rate and greater vulnerability to supraventricular tachycardia.22,23 We also identified some of the mechanisms involved in the increased cardiac automaticity of pregnant mice. Importantly, we demonstrated that the increase in heart rate is not secondary to changes in autonomic tone, blood pressure, or circulating catecholamine levels. Instead, we have identified major changes in the pacemaker current (I<sub>p</sub>) and Ca<sup>2+</sup> homeostasis in the SAN cells. We have also provided strong evidence highlighting a novel role for estrogen in regulating heart rate.

Considering that both heart rate and T3 levels are increased during pregnancy and that T3 has been shown to...
affect cardiac ion channel function, the objective of this study was to establish the contribution of T₃ on the increased heart rate associated with pregnancy in mice.

Methods

Animals

All experiments were performed on adult female CD1 mice (2–3 months old) obtained from Charles River (St-Constant, QC, Canada). Nonpregnant (NP) and late pregnant (P, 18–19 gestation days) mice were used. All experiments were realized in accordance with the guidelines published by the Canadian Council Animal Care (Ottawa, Canada) and approved by the Montreal Heart Institute Animal Care Committee (reference number: 2012-80-02).

Triiodothyronine treatment

Nonpregnant and pregnant mice were treated with daily subcutaneous injection of T₃ (0.2 µg/g body weight) in saline solution with NaOH for 5–6 days, as previously reported.²⁴ T₃ was first dissolved in NaOH and then in saline solution (0.9% NaCl). Control mice received subcutaneous injections of the vehicle only. T₃ and vehicle treatments were randomized but not blinded.

5-Propyl-2-Thiouracil treatment

Lowering of T₃ levels was obtained by treating pregnant mice with daily subcutaneous injection of 20 µg/g of an antithyroid drug, 5-propyl-2-thiouracil (PTU), for 5–6 days, as previously reported.²⁴ PTU was dissolved in NaOH and then in saline solution (0.9% NaCl). Control mice were treated with vehicle only. PTU and vehicle treatments were randomized but not blinded.

Experimental groups

Experiments were done on 5 groups of animals: (1) nonpregnant mice; (2) nonpregnant mice treated with T₃ (Sigma Chemical, St. Louis, MO); (3) pregnant mice; (4) pregnant mice treated with T₃; (5) pregnant mice treated with PTU (Sigma Chemical, St. Louis, MO).

The same vehicle was used for both T₃ and PTU treatment. One group (n = 5) of nonpregnant and pregnant mice received the vehicle only and electrocardiogram (ECG) recordings indicated that the vehicle had no effect on heart rate.

KEY FINDINGS

- Triiodothyronine (T₃) similarly increases heart rate in nonpregnant and pregnant mice.
- The increased heart rate observed in pregnant mice persisted after the use of propylthiouracil, although T₃ levels have returned to values found in nonpregnant mice.
- Despite the important role of T₃ in pregnancy, results presented here indicate that T₃ is not involved in the faster heart rate during pregnancy.

Results

Heart rate and T₃ levels are increased in pregnant mice

To explore a potential influence of thyroid hormones in the pregnancy-induced increased heart rate, we first measured heart rate and T₃ levels in nonpregnant and pregnant mice under control conditions. More precisely, heart rate and T₃ levels were compared between control and saline-treated nonpregnant and pregnant mice. Typical surface ECG recordings obtained from nonpregnant and pregnant anesthetized mice are illustrated in Figure 1A. As previously reported,²²,²³ the resting heart rate was faster in pregnant mice (607 ± 8 bpm, n = 10, P = .004) in comparison to nonpregnant mice (546 ± 16 bpm, n = 10) (Figure 1B). In agreement with the clinical data,⁴,¹¹,¹² we found a 58% increase in plasma T₃ levels in late pregnancy in mice (Figure 1C). Moreover, Figure 1 shows that heart rate and T₃ levels are similar between control and saline-treated mice in both the nonpregnant and pregnant group, indicating that the vehicle (saline) had no effect on heart rate and T₃ levels. Thus, for the following series of experiments, only the saline-treated nonpregnant and pregnant mice were

Measurements of T₃ plasma levels

Thyroid status was confirmed by collecting plasma from all groups of mice and measuring T₃ levels using radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX) following the manufacturer’s instructions, which was carried out by the Clinical Laboratory of the Montreal Heart Institute, Montreal, Quebec, Canada.

Surface ECG

Surface ECGs were performed as previously described.²²,²³ Briefly, mice were anesthetized by inhalation of 2% isoflurane. Body temperature was maintained at 37°C using a heating pad. Surface ECGs were recorded in lead I configuration with Biopac System MP100 (EMKA Technologies, Paris, France) at a rate of 2 kHz. Signal was low-pass (100 Hz) and notch (60 kHz) filtered. Surface ECG acquisition was blinded. R-R interval was automatically detected with a library created for the study from signal averaged ECG recordings (500–1000 cardiac cycles) using ECGauto v2.8.1.18 Software (EMKA Technologies). The library was used to analyze all samples. The analysis was not blinded.

Statistical analysis

Data are presented as mean ± SEM, where n represents the number of mice. Statistical analyses were performed using GraphPad Prism 8 software. Unpaired Student t test was used. P value less than .05 was considered statistically significant.
used for comparison with other experimental groups. Since both heart rate and T3 levels are augmented during pregnancy, we then explored the contribution of T3 to the pregnancy-related increased heart rate.

**T3 treatment similarly increases heart rate of nonpregnant and pregnant mice**

To determine whether the higher T3 levels found in pregnant mice was responsible for the increase in heart rate, T3 (0.2 ug/g body weight) was administered daily for 5–6 days to nonpregnant and pregnant mice. Change from baseline after T3 administration resulted in a comparable increase in heart rate between nonpregnant (Δ130 ± 22 bpm, n = 5) and pregnant mice (Δ126 ± 17 bpm, n = 5, P = .858) (Figure 2B) in comparison to their respective controls, although the T3 levels increased much more in pregnant (95%) than in nonpregnant (67%) mice (Figure 2C). These results indicate that despite the fact that pregnant mice had a higher baseline heart rate and higher T3 levels than nonpregnant mice, their heart rate responded similarly to T3 treatment as nonpregnant mice. Additionally, untreated pregnant mice had mean T3 levels similar to those of treated nonpregnant mice (P = .834), but the heart rate of pregnant mice (598 ± 10 bpm, n = 5) was lower than in T3-treated nonpregnant mice (686 ± 22 bpm, n = 5, P = .003), as shown in Figure 2A. Thus, by reproducing the same T3 levels, we could not observe the same effect on the heart rate.

**PTU reduces T3 levels without affecting heart rate in pregnant mice**

To further examine the contribution of thyroid status to the increased heart rate observed in pregnancy, in the next series of experiments, we treated pregnant mice with PTU to reduce their levels of thyroid hormones. Pregnant mice were treated daily with PTU (20 ug/g body weight) or the vehicle for 5–6 days and their heart rate was then compared. The results reported in Figure 3B show that treatment with PTU reduces the T3 levels of pregnant mice to values similar to those observed in nonpregnant mice (P = .599). Figure 3A shows that the heart rate in PTU-treated pregnant mice remained significantly elevated (607 ± 11 bpm, n = 5, P = .038) compared to nonpregnant mice (556 ± 20 bpm, n = 5), although the T3 levels return to control values. Thus, these results indicate that PTU treatment does not revert the increased heart rate observed during pregnancy.

**Discussion**

In women, the heart rate increases by 10–20 bpm throughout pregnancy, peaks in the last trimester, and then returns to nonpregnant values within 10 days after delivery. During pregnancy, there are also well-documented changes in thyroid hormones, with increases of up to 50% in T3 levels, which return to normal in the postpartum period. In this study, to explore a potential contribution of thyroid hormones in the pregnancy-induced increased heart rate, we compared
the heart rate between nonpregnant and pregnant mice under control conditions and after altering thyroid hormone levels with T3 and PTU treatments. By doing so, we provided strong evidence that the increase in heart rate during pregnancy is independent of thyroid hormones. T3 administration resulted in a substantial increase in heart rate in nonpregnant and pregnant mice; however, the heart rate was still significantly higher in the pregnant mice, suggesting that a pregnancy-mediated effect persisted and had an additive effect with T3 on heart rate. The PTU studies also provided critical information. In fact, under hyperthyroid conditions, antithyroid drug therapy, such as PTU or methimazole, is usually given to reduce T3 plasma levels and thereby reduce the heart rate. However, here we showed that the increased heart rate observed in pregnant mice remains after the use of PTU, although T3 levels have returned to values found in nonpregnant mice. Based on these findings, it is unlikely that thyroid hormones would contribute significantly to the pregnancy-induced increased heart rate.

A number of studies have shown that thyroid hormones have a significant effect on expression and function of various cardiac ion channels. However, the majority of these studies focus on ventricular or atrial tissue, while very little work has been done on the electrophysiology of the SAN. To our knowledge, this is the first study to examine the contribution of T3 levels to the increased heart rate associated with pregnancy, although previous studies have examined the effects of hypo- and hyperthyroidism on heart rate. Specifically, episodes of tachycardia have been described under hyperthyroid conditions, while hypothyroidism has often been associated with bradycardia. At the cellular level, the density of the hyperpolarization-activated current or pacemaker current (If), which is one of the major ionic conductances modulating pacemaker activity of the heart, has been shown to be increased by hyperthyroidism in rabbit SAN. Other studies under hyperthyroid conditions have also reported an increase in rat ventricular mRNA expression of HCN2, one of the isoforms contributing to If, as well as an increase in the current generated by the sodium-calcium exchanger (I_{NCX}) in neonatal rat atrial myocytes. These latter studies were conducted in ventricular and atrial tissue and not in the SAN. Therefore, although these genes are also expressed and functional in the SAN, it remains to be determined
whether T3 could also affect these ionic conductances in the SAN and, in so doing, regulate cardiac automaticity and heart rate.

All the electrophysiological changes reported previously were observed using models of hypo- and/or hyperthyroidism. However, during a normal pregnancy, the T3 concentrations are still within the euthyroid range, which means that the T3 pregnancy levels, while high, are not considered hyperthyroid levels. Therefore, it is likely that even if T3 can modulate heart rate, in the presence of an already elevated heart rate as seen in pregnant mice, T3 levels measured in pregnancy would not have a noticeable effect on the heart rate. The results reported here strongly support this notion.

In the present study, we showed that T3 levels measured in euthyroid mice are similar to those observed in women and that during pregnancy T3 levels are increased to a similar extent in mice and humans. Indeed, consistent with the clinical data, we found a 58% increase in T3 levels during pregnancy in mice. T3 levels measured in pregnancy would not have a noticeable effect on the heart rate. The results reported here strongly support this notion.

In the present study, we showed that T3 levels measured in euthyroid mice are similar to those observed in women and that during pregnancy T3 levels are increased to a similar extent in mice and humans. Indeed, consistent with the clinical data, we found a 58% increase in T3 levels during pregnancy in mice. However, despite the important role of T3 in pregnancy, results presented here indicate that T3 is not involved in the faster heart rate during pregnancy. The hormonal profile of women undergoes major changes during pregnancy. Many hormones besides T3 increase throughout pregnancy, including estrogens, progesterone, relaxin, and prolactin. We previously demonstrated a major role for 17β-estradiol in the increased cardiac automaticity and accelerated heart rate associated with pregnancy. It is likely that other hormones are also involved in the chronotropic effects of pregnancy. Therefore, additional studies are needed to further investigate the influence of these pregnancy hormones on the heart rate, as some of them, such as progesterone and relaxin, have been shown to influence cardiac electrophysiology.

Limitations
An alpha error is always possible and cannot be excluded. However, data obtained with T3 and PTU treatments strongly suggest that change in thyroid status is unlikely to contribute significantly to the increase in heart rate observed in pregnancy.

The main limitation of the mouse heart to study cardiac automaticity is the fact that the cycle length of the murine SAN is much shorter than in larger mammalian species. However, the functional properties and molecular basis of the ionic currents in mouse SAN have been well defined. Moreover, from a hormonal standpoint mice have an estrus cycle and express sex steroid hormone receptors in the heart, and similar hormonal changes occur in pregnant women and mice, notably in E2 and T3 levels. Equally important, pregnant mice reproduce the increased heart rate and increased vulnerability to arrhythmias observed during pregnancy in humans, thus reinforcing the validity of the mouse as an animal model to study the influence of pregnancy on cardiac automaticity.

Conclusion
In conclusion, by altering T3 levels using in vivo T3 and PTU treatments in nonpregnant and pregnant mice and ECG recordings, we provided evidence that, although T3 can accelerate heart rate, under pregnant conditions T3 does not participate in the pregnancy-induced increased heart rate.

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Disclosures
The authors have no conflicts to disclose.

Authorship
All authors attest they meet the current ICMJE criteria for authorship.
Ethics Statement
All experiments were realized in accordance with the guidelines published by the Canadian Council Animal Care (Ottawa, Canada) and approved by the Montreal Heart Institute Animal Care Committee (reference number: 2012-80-02).

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