Effect of Thyromimetic GC-1 Selective Signaling on Reproductive and Lactational Performance in the Hypothyroid Rat

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Abstract

Introduction: The potential of the thyroid hormone receptor β (TRβ1) selective analog GC-1 has been widely proven in animal models and humans. However, its effect on the reproductive stage of the female rat has not been evaluated. ***Methods:*** The effect of the administration of GC-1 or equimolar doses of triiodothyronine (T3) was evaluated on the reproductive performance of the hypothyroid female rat and the indirect effect on pup thyroid status, weight, and survival. ***Results:*** Hypothyroidism reduced the number of embryos implanted in the uterus, whereas T3 and GC-1 treatment in hypothyroid females reestablished the number of implanted embryos to normal. Initiation of labor was delayed by hypothyroidism, and T3 replacement treatment reinstated the normal timing of parturition. The administration of GC-1 alone to the lactating mother did not affect pup survival, weight, or thyroidal status. ***Conclusions:*** Our findings show the differential effect of thyroid hormone selective signaling during gestation and the indirect exposure of the pups; we also emphasize the plausible use of GC-1 for treatment of hypothyroid mothers during the lactation period.

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Introduction

Thyroid diseases cause menstrual disturbances, reduced fertility, recurrent pregnancy loss, perinatal mortality, and lactational deficit [1, 2]. We have demonstrated that hypothyroidism delays the onset of parturition, reduces litter size, impairs pup weight, alters hormonal profiles during gestation and lactation, and modifies liver and mammary lipid metabolism in rats [3, 4].

The genomic actions of thyroid hormones (THs) are mediated by 2 main thyroid receptor (TR) isoforms, TRα and TRβ, encoded by THRA and THRB genes. The isoforms TRα1, TRα2, and TRβ1 are expressed in reproductive tissues, such as the human ovary, placenta, uterus, and mammary gland, and TRβ2 is restricted to the hypothalamus and pituitary [5–9].

Our previous findings indicate that a deficit of THs alters corpus luteum (CL) function, delaying the onset of parturition in the rat [10, 11]. It has adverse effects on PRL signaling in the mammary gland, leading to lower milk production and early involution [12–14, 31].

The effects of TR isoforms in reproductive tissues have been indirectly demonstrated in animals bearing TRα1

and TRβ1 mutations. Mice with negative dominant mutations in TRα1 are not viable, whereas those with heterozygous mutations present a moderate alteration of their thyroid status and have high mortality, fertility impairment, and fewer pups per litter [15]. Females lacking both receptors rarely become pregnant and, if they do so, are unable to sustain lactation [16]. Mice exhibiting mutation on TRβ are seemingly normal in terms of their reproduction [17].

Altogether, these findings suggest that TR isoforms are differentially expressed in reproductive tissues of humans and rodents and fluctuate depending on the physiological state. Hence, TH signaling outcome seems to depend on selective subtype binding and further activation.

Another approach to identify the selective action of TR in vivo is using thyromimetics. The first compound described with potential use as a selective TRβ1 thyromimetic was GC-1, now called sobetirome. This compound prevents the undesired TRα1-mediated TH effects, such as an increase in heart rate and muscle or bone catabolism. While GC-1 action has been demonstrated in vitro and in vivo, its effect on the reproductive process has not yet been evaluated in animal models or in humans [18–22]. We hypothesized that GC-1 exerts different actions than T3 due to its selective signaling on the reproductive tissues of hypothyroid rats. Hence, this study aims to demonstrate TH-selective signaling effects on reproductive and lactational performance in hypothyroid rats. In addition, we propose a potential safe usage of GC-1 during gestation and lactation in the rat.

Materials and Methods

Animals and Experimental Design
Adult female Wistar rats bred in our laboratory, 3–4 months old, weighing 200–230 g and with regular 4-day estrus cycles, were used. They were kept in a light (lights on 06:00–22:00 h) and temperature (22–24°C) controlled room. Gestational day (G) 1 of pregnancy was defined by the detection of spermatozoa in the vaginal smears the morning after caging with a fertile male the night of proestrus.

Experiments were carried out using 6 groups of 6–8 rats: (i) euthyroid control (C), (ii) hypothyroid (H), (iii) hypothyroid treated daily with 0.6 µg T3/100 g body weight (BW) (H + T3), (iv) hypothyroid treated daily with 0.3 µg GC-1/100 g BW (H + GC-1), (v) euthyroid rats treated daily with 0.6 µg T3/100 g BW (T3), and (vi) euthyroid rats treated daily with 0.3 µg GC-1/100 g BW (GC-1). Hypothyroidism was induced by administration of 6-propyl-2-thiouracil (PTU) at concentrations of 0.05 and 0.1 g/L in the drinking water. T3 and GC-1 doses were equimolar and equivalent to twice the physiological dose of T3 (2×). Additional groups were treated with double or quadruple T3 dosages (1.2 or 2.4 µg T3/100 g BW per day, designated as 4× and 8×, respectively), or with double or quadruple GC-1 dosages (0.6 or 1.2 µg GC-1/100 g BW per day, designated as 4× and 8×, respectively) [23]. All treatments started 8 days prior to mating and continued during gestation and lactation (online suppl. Fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000516432).

Blood was collected from the tail vein of the mothers on day 7 (G7) or 21 (G21) of gestation or day 14 (L14) of lactation, between 10:00 and 12:00 h. On day 21 of lactation (L21), mothers and pups were sacrificed by decapitation and trunk blood collected. Serum was separated by centrifugation and stored at −20°C until used for hormone determinations.

Reproductive Performance
Date and time of delivery, number of total/live newborns per litter, pup weight gain, and survival during lactation were registered. A group of dams was sacrificed on G7, and the total number of uterine implantation sites was recorded.

Hormone Determinations
TSH was measured as previously described [24]. T3 and T4 concentrations were measured by RIA using commercial kits for total hormones [25]. GC-1 did not crossreact with T3 or T4 RIA (online suppl. Fig. 3). For further details, see online suppl. information.

Western Blot Analysis
Western blot analysis of TR proteins was performed as described in the supplemental information (online suppl. Fig. 2).

Statistical Analysis
Statistical analysis was performed using one-way ANOVA followed by the Tukey post hoc test to compare between groups and the nonparametric Kruskal Wallis test followed by the Dunn post hoc test to compare groups. The Brown-Forsythe test was performed to determine Gaussian data distribution. The survival curves were compared using the log-rank (Mantel-Cox) test considering a 95% CI of ratio. All statistical analyses were performed using the software Graph Pad Prism 7 (GraphPad Software, San Diego, CA, USA).

Results

Effect of GC-1 and T3 Treatment on T3 and TSH Serum Concentrations in Hypothyroid and Control Rats during Gestation and Lactation
As expected, hypothyroidism increased TSH and reduced T3 levels during the entire gestation and lactation, confirming the thyroid status of the H group (shown in Fig. 1). In the H + T3 group, T3 and TSH were reduced at the beginning of gestation, indicating that T3 treatment compensates the effect of PTU. It is noteworthy that daily administration of T3 was performed after blood sampling. Hormone levels thus reflected the regulation of the
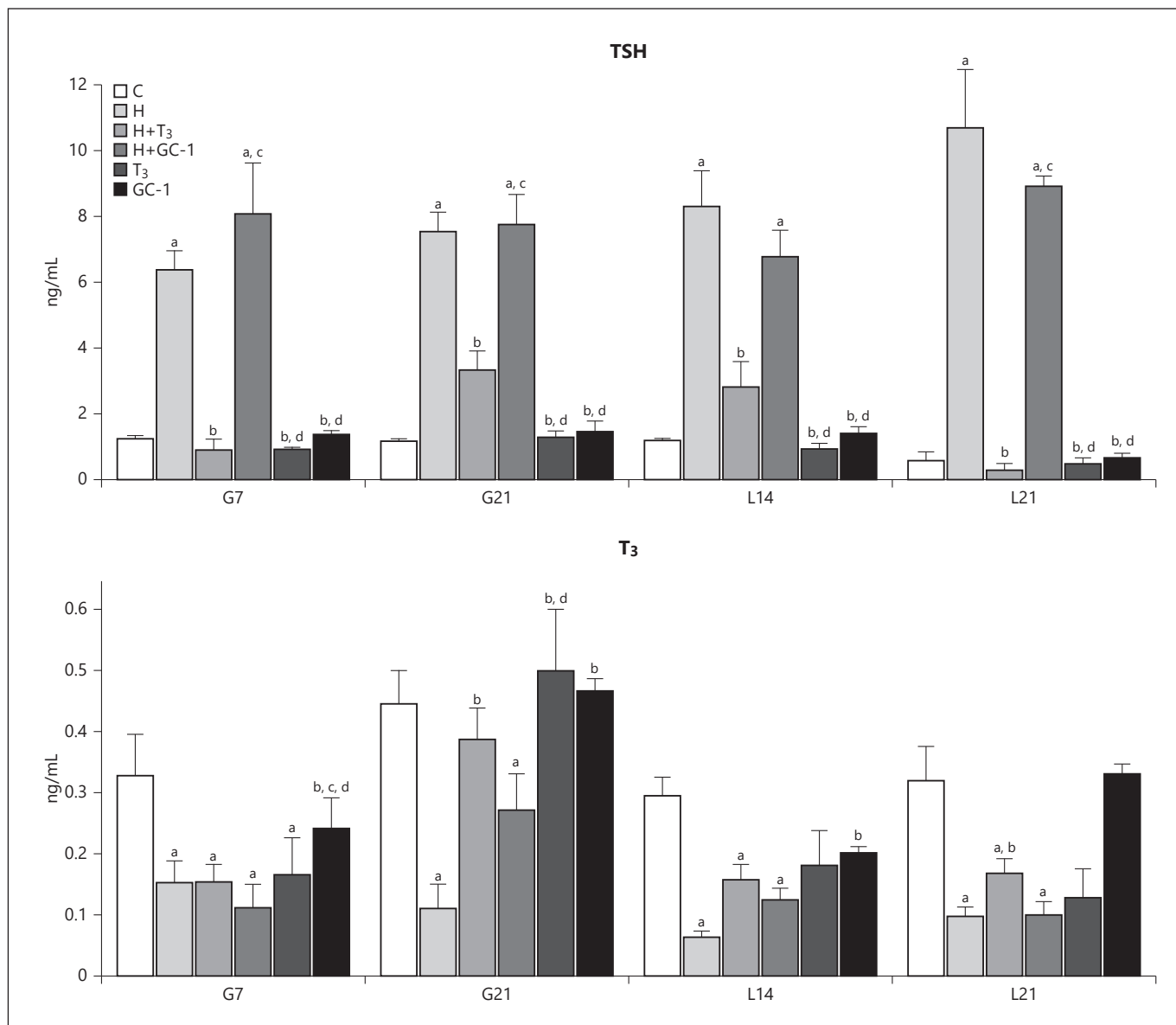
Fig. 1. TSH and T3 serum concentrations in rats during gestation and lactation treated with thyromimetic GC-1 or T3. Euthyroid (C), hypothyroid (H), hypothyroid T3-treated (H + T3), hypothyroid GC-1-treated (H + GC-1), euthyroid T3-treated (T3), and euthyroid GC-1-treated (GC-1) rats. Hormonal profile was determined on G7 and G21 of gestation and on L14 and L21 of lactation. Hypothyroidism was induced by administering propylthiouracil in the drinking water at a concentration of 0.1 g/L. The values represent means ± SEM ($n = 6–8$ rats per group). Kruskal-Wallis and Dunn post hoc tests were used to compare TSH levels and one-way ANOVA and Tukey post hoc tests to compare T3 levels between groups. G7 $F_{(5, 25)} = 18.08$, G21 $F_{(5, 53)} = 12.63$, L14 $F_{(5, 46)} = 9.958$, and L21 $F_{(5, 24)} = 8.983$. Statistical significance ($p < 0.05$) is indicated as follows: a versus C, b versus H, c versus H + T3, and d versus H + GC-1.

axis due to T3 administration 24 h earlier. T3 increased in the H + T3 group at the end of gestation, reaching control levels. All groups had lower T3 levels than controls on L14. At the end of lactation, only T3 remained at control levels in the GC-1 group.

Reproductive Performance

Hypothyroidism reduced the number of implantation sites and of pups per litter (shown in Fig. 2). T3 or GC-1 administration overcame the effect of hypothyroidism. Parturition was delayed in H and H + GC-1

groups com pared to control, but T3 treatment reestablished the normal onset of labor. Lactational performance was evaluated in terms of pup body weight on L14. It was lower in PTU-treated groups (H, H + GC-1, and H + T3).

TR Expression

Different patterns of TRα1 and TRβ1 expression were simultaneously found in CL, placenta, uterus, and mammary glands of the same animal at the end of gestation (shown in Fig. 3).
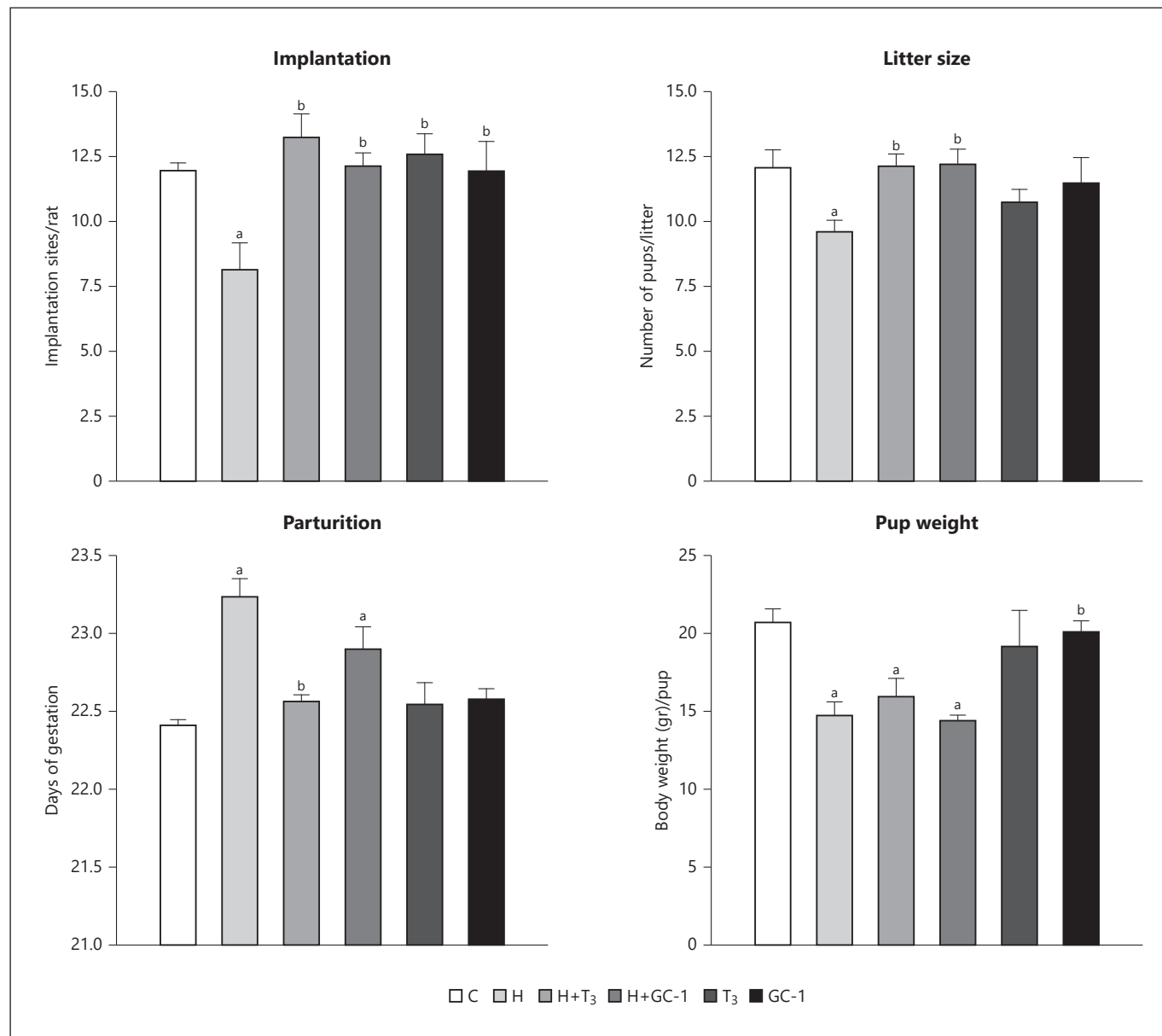

Fig. 2. Effects of T3 or GC-1 on reproductive performance of hypothyroid and euthyroid female rats. The number of uterine implantation sites on G7, date and time of parturition, number of total newborns per litter, and pup weight on L14 were registered for euthyroid (C), hypothyroid (H), hypothyroid T3-treated (H + T3), hypothyroid GC-1-treated (H + GC-1), euthyroid T3-treated (T3), and euthyroid GC-1-treated (GC-1) rats. Hypothyroidism was induced by administering propylthiouracil in the drinking water at a concentration of 0.1 g/L. The values represent means ± SEM (n = 6–8 rats per group). Kruskal-Wallis and Dunn post hoc tests were used to compare parturition, and one-way ANOVA and Tukey post hoc tests were used to compare implantation, litter size, and pup weight between groups. Implantation $F(5, 41) = 5.628$, litter size, $F(5, 62) = 4.332$, and pup weight $F(5, 46) = 9.958$. Statistical significance ($p < 0.05$) is indicated as follows: a versus C, b versus H, and d versus H + GC-1.

Effect of Maternal GC-1 and T3 Administration on the Regulation of Thyroid Axis, Pup Weight, and Survival

Maternal hypothyroidism affected the survival and weight of pups ($p > 0.001$), regardless if mothers were treated or not with GC-1 or T3 (shown in Fig. 4). T3 treatment slightly altered pup survival ($p = 0.0156$). Administration of GC-1 alone did not affect pup weight or survival. Maternal PTU treatment rendered pups hypothyroid, as demonstrated by T4. T3 was not affected in pups of the H group, but it was reduced in those of the H + T3 and H + GC-1 groups. TSH was higher in the pups of the H and H + GC-1 groups and lower in those of the H + T3 group than in the H group. Maternal treatment with either GC-1 or T3 alone did not affect TSH levels in pups.
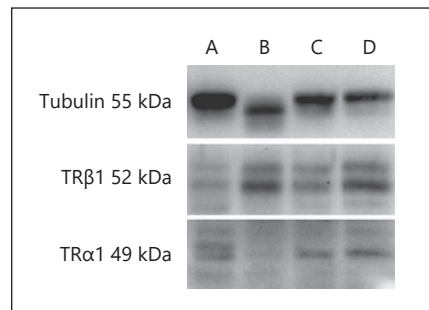

Fig. 3. Thyroid hormone receptor protein expression in reproductive tissues. (A) CL, (B) uterus, (C) placenta, and (D) mammary gland total proteins from a euthyroid rat were isolated and analyzed via Western blotting. Representative blots of TRα1, TRβ1, and tubulin protein expression are shown from the same animal on day G19 of gestation. CL, corpus luteum.
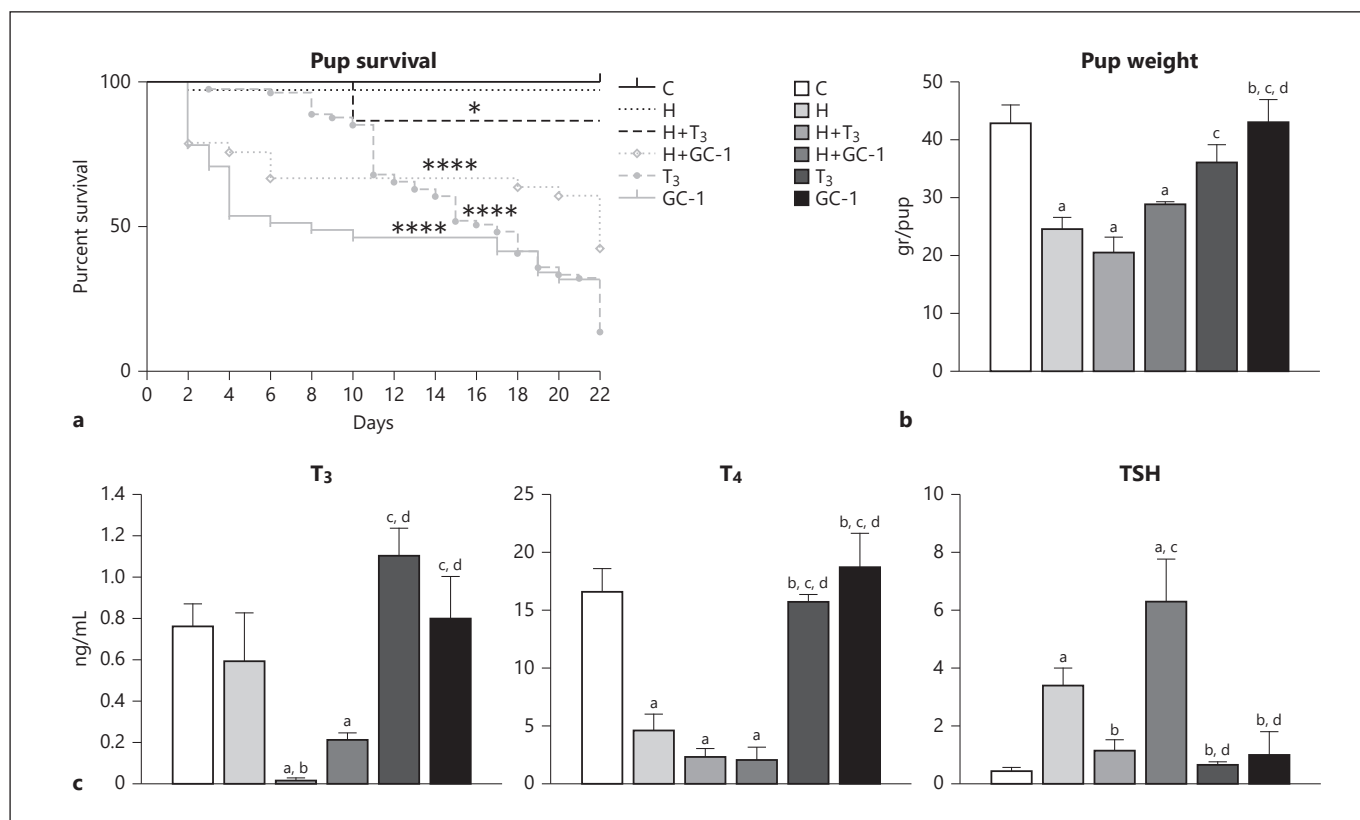

Fig. 4. Effect of maternal GC-1 and T3 administration on the regulation of thyroid axis, weight, and survival of pups. Pup survival and weight during lactation and T3, T4, and TSH serum concentrations were determined for euthyroid (C), hypothyroid (H), hypothyroid T3-treated (H + T3), hypothyroid GC-1-treated (H + GC-1), euthyroid T3-treated (T3), and euthyroid GC-1-treated (GC1) rats. **a** Kaplan-Meier survival curves of pups during lactation. Statistical differences between groups were evaluated using the log-rank (Mantel-Cox) test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$ with respect to the control group. **b** Pup weight at the end of lactation (L21). **c** T3, T4, and TSH serum concentrations in pups. Hypothyroidism was induced by administering propylthiouracil in the drinking water at a concentration of 0.1 g/L. The values represent means ± SEM ($n = 6$–8 rats per group). One-way ANOVA and Tukey post hoc tests were used to compare pup weight and T3, T4, and TSH levels between groups. Pup weight $F_{(5, 20)} = 13.01$, T3 $F_{(5, 18)} = 8.96$, T4 $F_{(5, 16)} = 17.48$, and TSH $F_{(5, 20)} = 9.101$. Statistical significance ($p < 0.05$) is indicated as follows: a versus C, b versus H, c versus H + T3, and d versus H + GC-1.
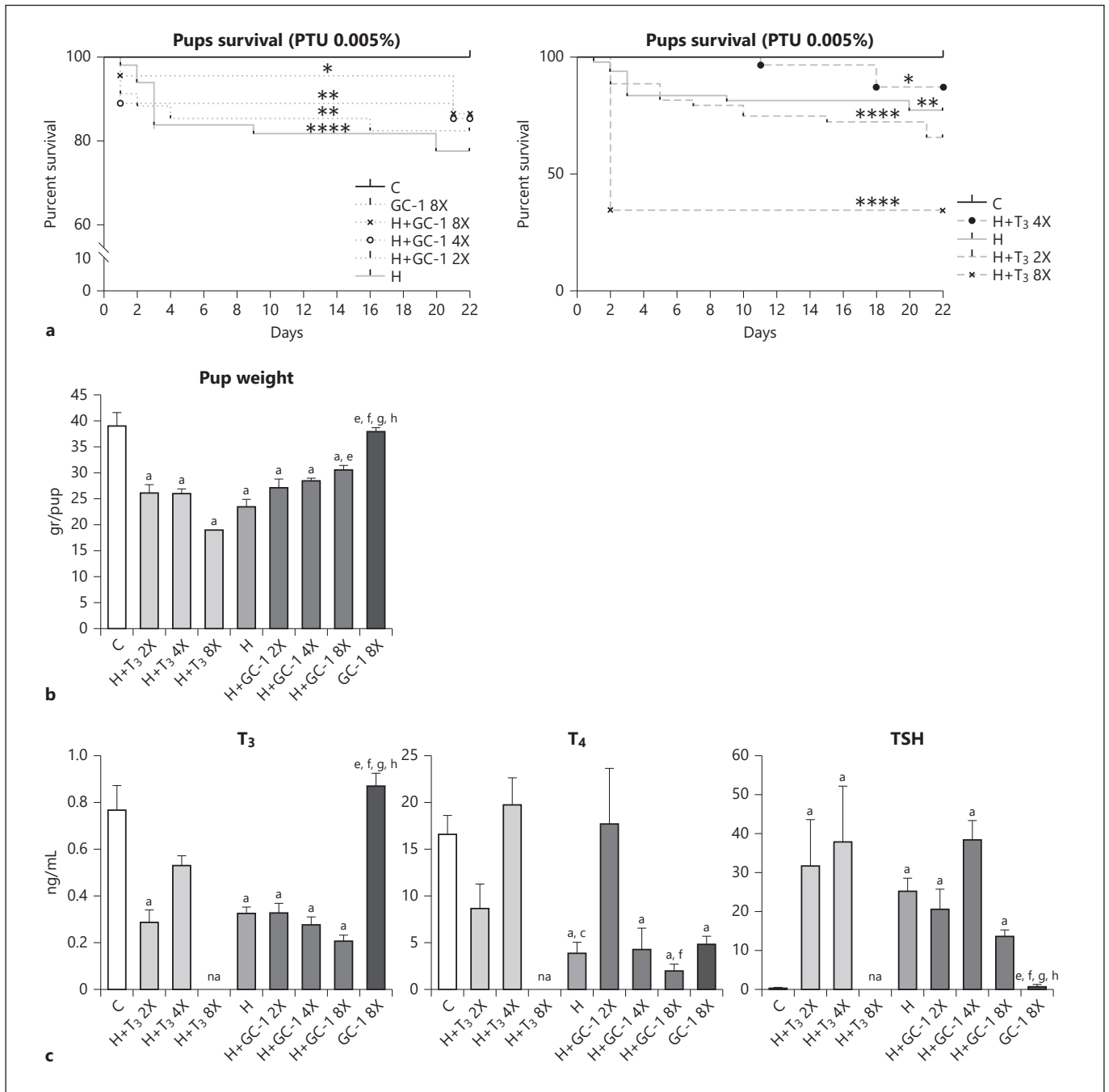
Fig. 5. Effect of maternal GC-1 and T3 administration on the regulation of thyroid axis, weight, and survival of pups. Pup survival and weight during lactation and T3, T4, and TSH serum concentrations were determined for euthyroid (C), hypothyroid (H), hypothyroid T3-treated (H + T3 2×, H + T3 4×, H + T3 8×), hypothyroid GC-1-treated (H + GC-1 2×, H + GC-1 4×, and H + GC-1 8×), and euthyroid GC-1 treated (GC-1 8×) rats. **a** Kaplan-Meier survival curves of pups during lactation. Statistical differences between groups were evaluated using the log-rank (Mantel-Cox) test. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$ with respect to the control group. **b** Pup weight at the end of lactation (L21).

c T3, T4, and TSH serum concentration in pups. Hypothyroidism was induced by the administration of propylthiouracil in the drinking water at a concentration of 0.05 g/L. The values represent means ± SEM ($n = 6$–8 rats per group). To analyze pup weight, T3, T4, and TSH, after log transformation, one-way ANOVA and Tukey post hoc tests were used to compare between groups. Pup weight $F_{(7,29)} = 16.26$, T3 $F_{(7,26)} = 9.95$, T4 $F_{(7, 24)} = 5.22$, and TSH $F_{(7,23)} = 31.43$. Statistical significance ($p < 0.05$) is indicated as follows: a versus C, e versus H, f versus H + GC-1 2×, g versus H + GC-1 4×, and h versus H + GC-1 8×. f, g, and h were compared within the groups treated with GC-1.

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GC-1 maternal administration did not interfere with the normal regulation of the thyroid axis of pups.

Dose-Dependent Effect of Maternal GC-1 and T3 Administration on Thyroid Axis, Pup Weight, and Survival

The effect of maternal administration of dose-dependent GC-1, T3, and 0.05 g/L PTU was evaluated in pups (shown in Fig. 5). A maximal maternal dose of GC-1 (GC-1 8×) during lactation was also tested. All groups treated with PTU showed reduced pup weight and survival compared to controls but to a lesser extent than those treated with a double dose of PTU (online suppl. Fig. 4). T3 treatment did not revert PTU effects. The H + T3 8× group was the most affected, showing significantly higher perinatal mortality than H and C groups. However, GC-1 administration in the H + GC-1 8× group led to a more rapid pup weight gain than H. Administration of GC-1 8× did not affect pup weight. Thyroidal axis was also influenced. TSH increased and T3 diminished in all groups treated with PTU, evidencing the hypothyroid state of pups. Administration of GC-1 8× to lactating mothers reduced T4 levels but did not affect TSH or T3 levels in pups.

Discussion/Conclusion

The coordinated function of the CL, placenta, and uterus in rats allows the maintenance of gestation and initiation of labor. After birth, the mammary gland is essential for the fulfillment of lactation. These tissues differentially express TR subtypes depending on the physiological state, but it is challenging to demonstrate in vivo how TH signaling promotes a certain outcome [10, 25–27]. GC-1 allowed us to differentiate TR selective actions in vivo, from initiation of gestation until newborns are capable of living on their own at weaning.

During pregnancy, the maternal thyroid axis is regulated depending on the demands of fetuses, increase in plasma volume, and TH synthesis and binding proteins. Euthyroid mothers adjust to these changes by modifying their TH metabolism, iodine uptake, and thyroidal axis [2]. PTU administration increased TSH as expected, and its effect was compensated by T3 treatment. GC-1 at equimolar doses did not reestablish TSH levels. This was predictable, as the regulation of TSH by THs at the pituitary is mediated exclusively by the TRβ2 selective signaling [28]. GC-1 alone (36 nmoL/kg/day) did not disturb the normal regulation of TSH, confirming the specificity of the thyroid analog. Other authors obtained comparable results when administering GC-1 at doses above 154 nmoL/kg/day [20]. T3 was most affected during lactation, reflecting the increased demands to produce milk. The administration of GC-1 did not disturb the TH axis during gestation and lactation when administered alone.

We further investigated the action of GC-1 on reproductive performance. Hypothyroidism diminished the number of embryos implanted in the uterus. T3 or GC-1 treatment of hypothyroid rats reestablished the number of implantation sites, suggesting that TRβ1 signaling plays a key role in the first stages of maternal and embryo interaction. Litter size reflected comparable results, revealing that the main reason for the lower number of pups was an alteration in the implantation process. We had previously reported that hypothyroidism diminishes litter size without interfering in ovulation, thus confirming that it impacts on the implantation process [3, 24]. The present results evidence that THs are essential during this stage and that TRβ1 selective signaling is involved.

The exact mechanism associated with the initiation of labor in rats is not fully understood. However, it is known that the increase of estradiol levels at the end of gestation stimulates the synthesis and release of uterine prostaglandins, which then trigger uterine contraction. This, in turn, instigates the release of oxytocin by the posterior pituitary and the endometrium. In cooperation with relaxin produced by the CL, oxytocin then provokes parturition [29]. We confirmed that hypothyroidism delays parturition and T3 treatment reestablishes the normal length of pregnancy. In contrast, treatment with GC-1 did not hinder the delay in labor induction, suggesting that TH signaling mediated by TRα1 is critical at the end of gestation. This assumption is further supported by our result that TRα1 is simultaneously present at the end of gestation in the CL, placenta, and uterus. Likewise, the lack of THs at the end of gestation delays parturition due to a postponement of luteal regression and a surge of prostaglandins [11].

Lactation is another critical stage regulated by THs. Postnatal exposure to antithyroid drugs and hormones through the maternal feeding has important consequences for pups. Thus, we examined the indirect outcome of maternal hypothyroidism and T3 or GC-1 treatment on pup weight, survival, and thyroid status. Maternal PTU treatment adversely affected pup weight, which was reflected in lower pup weight at the time of weaning. Treatment with active TH T3 or its TRβ selective analog could not defeat the antithyroid effects. These results evidence that PTU is responsible for those outcomes. Although it

has been reported that PTU usage during lactation is preferred to other antithyroid drugs due to its lower passage into breast milk, it has also been demonstrated that it may be hepatotoxic [1, 30]. As expected, the passage of PTU through milk induced hypothyroidism. Maternal treatment with supraphysiological doses of T3 also had a detrimental effect on pup survival and weight. Meanwhile, maternal administration of GC-1 at equimolar doses resulted innocuous, as it did not affect pup survival, weight gain, or thyroidal status.

PTU reduction increased pup survival, confirming the presumption that PTU negatively affects the health of the newborns. Despite the negative effect of hypothyroidism induced by PTU, treatment with GC-1 improved the well-being of the pups in terms of survival and weight gain in a dose-dependent manner. It thus may be assumed that TRβ1 selective signaling plays a significant role in mammary gland activity, pup weight gain, and survival during lactation.

The relevance of our findings for humans might be limited, given that the mechanism triggering parturition in the rat is not fully understood. However, the physiology of the uterus and cervix during parturition, as well as that of the mammary gland, is similar in rats and humans. The biological concepts developed based on the rat model may thus be enlightening for human reproduction and lactation.

In conclusion, this work demonstrates that THs play a vital role in the reproductive process. It also suggests that TRα selective signaling is critical at the end of gestation and leads to a normal onset of parturition. The use of the selective TRβ agonist GC-1 may be useful for the treatment of hypothyroidism during lactation, as it prevents the detrimental effects of TRα signaling during this period. As far as we know, this is the first time that evidence is provided about the effect of the maternal TRβ selective agonist GC-1 on the reproductive process and its indirect impact on the pups.

Statement of Ethics

Animal maintenance and handling was performed according to the Guiding Principles in the Care and Use of Laboratory Animals of the US National Institutes of Health (NIH Publication No. 86-23, revised 1985 and 1991) and the United Kingdom's requirements for ethics of animal experimentation (Animals Scientific Procedures, Act 1986). All procedures were approved by the Institutional Animal Care and Use Committee of the School of Medical Science, National University of Cuyo, Mendoza, Argentina (Protocol Approval No. 17/2012 and 67/2015).

Conflict of Interest Statement

The authors have no conflicts of interest to disclose.

Author Contributions

E.R., F.C.A., M.B.H., and C.G.L. performed the in vivo studies, hormonal determinations, and Western blots, analyzed the data, and prepared the figures. M.E. discussed and analyzed the data. M.B.H. and C.G.L. supervised the study and analyzed and discussed the data. M.B.H. wrote the manuscript. All authors discussed and edited the manuscript.

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