Comparison of heat and cold stress to assess thermoregulatory dysfunction in hypothyroid rats

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Gordon, Christopher J., Peggy Becker, and Beth Padnos. Comparison of heat and cold stress to assess thermoregulatory dysfunction in hypothyroid rats. Am J Physiol Regulatory Integrative Comp Physiol 279: R2066–R2071, 2000.—How borderline impairment of thyroid function can affect thermoregulation is an important issue because of the antithyroidal properties of a number of environmental toxicants. This study compared the efficacy of heat and cold stress to identify thermoregulatory deficits in rats subjected to borderline and overt hypothyroidism via subchronic exposure to propylthiouracil (PTU). After 3 wk of exposure to PTU in the drinking water (0, 2.5, 5, 10, and 25 mg/l), rats were subjected to a heat stress challenge (34°C for 2.5 h). After one more week of PTU treatment, the same rats were subjected to a cold stress challenge (7°C for 2.5 h). Core temperature ($T_c$) was monitored by radiotelemetry. Baseline $T_c$ during the light phase was reduced by treatment with 25 mg/l PTU. The rate of rise in $T_c$ during the cold stress challenge was similar in all PTU treatment groups. There was a dose-related decrease in serum thyroxine ($T_4$) at PTU doses $\geq$5 mg/l. Serum triiodothyronine ($T_3$) was reduced at PTU doses of 5 and 25 mg/l. Serum thyroid-stimulating hormone (TSH) was marginally elevated by PTU treatment. Overall, heat stress was more effective than cold stress for detecting a hypothyroid-induced thermoregulatory deficit. Cold stress resulted in a 1.0°C increase in $T_c$ regardless of PTU treatment. The rate of rise in $T_c$ during the cold stress challenge was similar in all PTU treatment groups. There was a dose-related decrease in serum thyroxine ($T_4$) at PTU doses $\geq$5 mg/l. Serum triiodothyronine ($T_3$) was reduced at PTU doses of 5 and 25 mg/l. Serum thyroid-stimulating hormone (TSH) was marginally elevated by PTU treatment. Overall, heat stress was more effective than cold stress for detecting a thermoregulatory deficit in borderline (i.e., 10 mg/l PTU) and overtly hypothyroid rats (i.e., 25 mg/l PTU). A significant thermoregulatory deficit is manifested with a 78% decrease in serum $T_4$. A thermoregulatory deficit is more correlated with a reduction in serum $T_4$ compared with $T_3$. Serum levels of TSH are unrelated to thermoregulatory response to heat and cold stress.

body temperature; thyroxine; triiodothyronine; thyroid-stimulating hormone

THE HYPOTHALAMIC-PITUITARY-THYROID (HPT) axis is critical for the regulation of body temperature, maintenance of metabolic rate, and a myriad of other physiological processes. Thyroidectomy or placing rats on antithyroidal drugs such as propylthiouracil (PTU) results in a thermoregulatory dysfunction characterized by a reduced baseline core temperature, decreased metabolic rate, and impaired ability to thermoregulate when subjected to cold stress (2, 8, 15).

There is a considerable data base on the pathological effects of acute thyroid dysfunction such as occurs after thyroidectomy or treatment with antithyroidal chemicals that reduce circulating levels of thyroxine ($T_4$) to minute levels. However, there is relatively little known on how borderline impairment of thyroid function can affect thermoregulation. This is an important question to address in pathophysiological studies of the thyroid. Another important issue is the toxicology of dioxins, polychlorinated biphenyls (PCBs), and other environmental toxicants that have antithyroidal properties (3). Animals and humans are exposed to PCBs and dioxins because they bioaccumulate and are very resistant to degradation. Thyroid dysfunction is a likely cause of many of the pathophysiological effects of these toxicants (3).

The pathophysiological consequences of moderate reductions in circulating levels of $T_4$, triiodothyronine ($T_3$), and/or thyroid-stimulating hormone (TSH) are not well understood. Radiotelemetric monitoring of core temperature provides an ideal means of monitoring the effects of a borderline thyroid deficit, because core temperature can be monitored without artifacts associated with handling stress. Cold stress has conventionally been used to detect thermoregulatory deficits in hypothyroid animals (2, 8, 15, 16). However, largely overlooked is evidence that the response to heat stress may be a more sensitive than cold stress for assessing a hypothyroid-induced thermoregulatory deficit (4, 6, 15). Hence, the purpose of this study was to assess the relationship between serum levels of $T_3$, $T_4$, and TSH and the ability of the rat to regulate core temperature when subjected to heat stress and cold stress.

MATERIALS AND METHODS

Animals used in this study were male rats of the Long-Evans strain obtained from Charles River Laboratories (Raleigh, NC) at 60 days of age. The rats were housed individually in acrylic cages with wood shaving bedding at an ambient temperature ($T_a$) of 22°C, relative humidity of 50%, and a 12:12-h photoperiod.

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Surgery was performed at ~70 days of age to implant radio transmitters (model TA10TA-F40; Data Sciences, St. Paul, MN) into the abdominal cavity to monitor core temperature and motor activity (15). Following surgery rats were administered a penicillin antibiotic (30,000 U im) and analgesic (buprenorphine; 0.03 mg/kg sc). The rats were allowed to recover from surgery for at least 10 days prior to testing.

PTU (Sigma, St. Louis, MO) was diluted in tap water to concentrations of 0, 2.5, 5.0, 10, and 25 mg/l and administered to the rats as their sole source of liquid for 5 wk. Water consumption and body weight were measured at weekly intervals. Fresh PTU solutions were prepared weekly. Experiments were performed in 4 cohorts of 12 animals. In the first cohort, PTU doses of 0, 10, and 25 were performed (n = 3 per group). One group of three rats was administered 50 mg/l PTU and tested as described below. Although this dose was extremely effective, it also caused substantial weight loss and was not used in subsequent studies. The next cohort evaluated PTU doses of 0, 2.5, and 5 mg/l (n = 4 per group); the final cohort evaluated doses of 0, 5, 10, and 25 mg/l (n = 3 per group).

Thermal challenge. After 21 days of PTU treatment, the rats were subjected to a heat stress challenge. One group of four rats was housed individually and placed in an environmental chamber maintained at a T_a of 22°C with a 12:12-h photoperiod (lights on at 0600). The rats were provided with food and water or PTU treatment throughout the thermal challenge. Since wood shaving bedding can alter heat loss and thermoregulatory capacity (9), the rats were housed in acrylic cages with wire-screen floors for the thermal challenge experiments. Core temperature and motor activity were recorded at 5-min intervals with an automated recording system (Data Sciences, St. Paul, MN). Because core temperature of rats remains elevated for several hours following placement in a novel environment (7), the rats were allowed to acclimate to the environmental chamber overnight so that their core temperature would be at basal levels prior to the heat and cold stress challenge. At 0930 the following day, T_a of the chamber was increased from 22 to 34°C over a period of 30 min. T_a was maintained at 34 ± 0.5°C for 120 min and then raised back to 22°C over the next 30 min. The rats were returned to the animal facility and maintained on their PTU treatment.

Each cohort of 12 rats was tested in the environmental chamber over a 3-day period. Seven days after the heat challenge, the rats were placed in the environmental chamber as described above and then subjected to a cold-stress challenge the following day. During the cold stress, T_a was reduced from 22 to 7°C over a 30-min period starting at 0930. A T_a value of 7 ± 0.5°C was maintained for 120 min and then was raised back to 22°C over the next 30 min. Each cohort of rats was subjected to cold stress over a 3-day period in a similar manner as for the heat stress challenge. Following the cold stress, the rats were returned to the animal facility and maintained on PTU for another 5 days. The rats were then killed by CO_2 asphyxiation, and blood was taken by cardiac puncture. Serum was separated and frozen at −22°C for later analysis.

T_a, T_m, and TSH analyses. Total T_3 and T_4 (free and bound) were measured usingRIA detection kits ( Coat-A-Count; Diagnostic Products, Los Angeles, CA). Detection limits are as low as 2.5 ng/ml for T_4 and 0.07 ng/ml for T_3. Serum TSH was analyzed in duplicates with a double antibody RIA method of Zoeller and Rudeen (16). Rat TSH RIA kits (including rat TSH antiserum NIDDK-anti-rat TSH-RIA-6; rat TSH reference preparation NIDDK-rTSH-RP3) were provided free by the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases. 125I-labeled TSH was purchased from Covance Laboratories (Vienna, VA).

Data analysis. The 5-min telemetry data were averaged into 20-min bins. These averaged data were then subjected to a repeated measures ANOVA to detect effects of PTU treatment on the time course of core temperature and motor activity during the heat and cold stress challenges. The slope of the change in core temperature during the heat/cold challenge was calculated with linear regression. A one-way ANOVA followed with a Dunnett’s multiple comparison test was used to assess effects of PTU treatment on the slopes as well as the baseline core temperatures, motor activity prior to thermal challenge, and serum levels of T_3, T_4, and TSH.

RESULTS

Thermoregulatory response. There was a significant reduction in nocturnal and diurnal baseline core temperature after 21 days of treatment (i.e., prior to heat stress challenge) with 25 mg/l PTU (Fig. 1). Nocturnal baseline core temperature was significantly reduced at 10 and 25 mg/l when the rats were retested after another 7 days of PTU treatment. Baseline core temperature during the day of PTU testing was reduced in rats treated with 25 mg/l (Fig. 1). Baseline motor activity was unaffected by PTU treatment (data not shown).

Core temperature and motor activity decreased gradually from 0600 to the onset of the heat stress challenge (Fig. 2). During the heat stress challenge, core temperature was stable as chamber T_a gradually increased then rose abruptly as T_a approached 34°C. Core temperature recovered rapidly as chamber T_a was reduced to 22°C.

There was a significant effect of PTU on the overall elevation and rate of rise in core temperature during heat stress. Rats treated with 10 and 25 mg/l PTU had a significantly reduced rate of elevation in the core temperature during heat stress (Fig. 3). The control group reached a steady-state core temperature of 38.7°C after 1 h of heat stress. Rats dosed with 2.5 and 5.0 mg/l displayed a similar pattern of rise in core temperature. The elevation in core temperature exceeded 1.5°C in the control and the 2.5 and 5.0 mg/l PTU groups. Rats treated with 10 mg/l PTU had a peak core temperature of 38.0°C, whereas rats treated with 25 mg/l had a peak temperature of 37.6°C. The increase in core temperature was ~1.0°C in the rats treated with 10 and 25 mg/l PTU. Baseline motor activity prior to and during heat stress was unaffected by PTU treatment. There was a significant treatment-time interaction of motor activity. This was attributed to the increase in activity of the 25 mg/l group during heat stress (Fig. 2).

The time course of core temperature and activity prior to cold challenge was similar to that of the heat challenge. At the onset of cold stress, core temperature and motor activity increased abruptly in all treatment groups (Fig. 4). The peak core temperature during cold stress was significantly reduced at PTU doses of 10 and 25 mg/l. On the other hand, the rate of elevation in core temperature during cold stress was unaffected by PTU treatment (Fig. 3). Core temperature reached a maxi-
mum elevation ~80 min after cold stress and then declined gradually despite the continued exposure to cold stress. Motor activity also increased with cold stress, a response that was unaffected by PTU. PTU led to significant reductions in growth over a 21-day period (Table 1). Rats treated with PTU doses of 10 and 25 mg/l exhibited 40 and 50% of the weight gained by controls, respectively. Total fluid intake was unaffected by PTU up to a dose of 10 mg/l. However, fluid intake was reduced from 3.6 to 3.0 ml·kg⁻¹·day⁻¹ in the rats treated with 25 mg/l PTU. Although the
A difference in fluid consumption in the rats treated with 25 mg/l PTU was not statistically significant, it is likely that depression in fluid consumption would have been a factor contributing to the reduced body weight in this treatment group. The rats appeared healthy despite the reduced body weight.

**Blood chemistry.** Serum levels of T4 were reduced in a dose-related fashion with increasing dose of PTU (Fig. 5). The lowest dose of 2.5 mg/l led to a slight but insignificant lowering of T4 by 20%. PTU at 5.0 and 10 mg/l significantly reduced serum T4 by 35 and 78%, respectively. PTU (25 mg/l) led to greater than 90% reduction in T4. Serum levels of T3 did not show as a predictable decrease with increasing dose of PTU as was found with T4 (Fig. 5). PTU doses of 2.5 and 10 mg/l caused a significant 25% decrease in T3; however, the 5.0 mg/kg dose of PTU had no effect. PTU at 25 mg/l led to 65% reduction in T3. There was no overall treatment effect of PTU on TSH (Fig. 5). However, there was a trend for higher levels of TSH in all PTU groups. Rats administered 10 mg/l PTU had a 60% increase in TSH that was significantly higher than the control group.

**Table 1. Effect of dose of PTU on fluid intake, PTU intake, and change in body weight before and after 36 days of PTU treatment**

<table>
<thead>
<tr>
<th>PTU Dose, mg/l</th>
<th>Fluid Intake, ml·kg⁻¹·day⁻¹</th>
<th>PTU Intake, ml·kg⁻¹·day⁻¹</th>
<th>Initial Body Wt, g</th>
<th>Δ Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.66 ± 0.03</td>
<td>389 ± 7</td>
<td>80.5 ± 7</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>3.71 ± 0.29</td>
<td>295 ± 0.03</td>
<td>383 ± 7</td>
<td>99 ± 10</td>
</tr>
<tr>
<td>5.0</td>
<td>3.23 ± 0.04</td>
<td>48 ± 0.01</td>
<td>380 ± 5</td>
<td>89 ± 7</td>
</tr>
<tr>
<td>10</td>
<td>3.05 ± 0.27</td>
<td>30 ± 0.06</td>
<td>401 ± 15</td>
<td>48 ± 12+</td>
</tr>
<tr>
<td>25</td>
<td>3.05 ± 0.2</td>
<td>2.1 ± 0.11</td>
<td>407 ± 20</td>
<td>39 ± 9†</td>
</tr>
</tbody>
</table>

Values are means ± SE. PTU, propylthiouracil. *P < 0.05 and †P < 0.01 compared with controls.
controls (t = 2.5, P = 0.02). The lack of a significant effect when all dose groups were analyzed by ANOVA is attributed to the variability of the 5 mg/l group.

DISCUSSION

There are several important observations in this study that should improve the understanding of the role the thyroid gland in thermoregulation. 1) A heat stress challenge is a more sensitive test than cold stress to acquire a rapid assessment of borderline thermoregulatory dysfunction in the hypothyroid rat. An impaired ability to increase core temperature during a heat challenge was detected in rats on a PTU treatment that had no effect on their baseline core temperature. Cold stress challenge was not as effective for detecting a thermoregulatory dysfunction. 2) Thermoregulatory deficits to heat challenge appear to be related to a reduction in serum levels of T4. On the other hand, changes in serum levels of T3 and TSH have less predictable effects on thermoregulatory function.

Because the HPT axis is activated during cold exposure and suppressed with heat exposure, tests to evaluate thermoregulatory function of thyroid-impaired animals have focused on their inability to thermoregulate during cold exposure, especially in the young and aged (5, 13). Other endpoints to evaluate thyroid dysfunction have centered on brown adipose tissue thermogenesis (10) and response to thermogenic drugs such as norepinephrine (1). It would seem intuitive to study thyroid function and a homeotherm’s ability to thermoregulate in the cold. However, the peculiarities of the rat’s response to heat stress should lead one to reconsider heat stress as a better measure of a thyroid-related dysfunction in the whole animal. Thyroidectomized (4) and PTU-induced hypothyroid rats (6) displayed an attenuated hyperthermic response during exposure to heat stress. The attenuated hyperthermic response was shown to be a result of a reduced metabolic rate when the hypothyroid rat was exposed to heat stress. Control rats placed in a heated chamber (e.g., Ta = 35–40°C) become hypermetabolic and undergo a precipitous elevation in core temperature compared with hypothyroid animals (7). A hypermetabolic response is counter to homeostatic mechanisms to regulate core temperature during heat stress. The hypothyroid state clearly dampens the metabolic response and provides the rat with marked heat tolerance. Our laboratory found that PTU-induced hypothyroid rats exposed to heat stress have a slower rate of heating, lose less body weight, and do not groom their fur as much as control animals. All together, these observations suggest that the hypothyroid rat is very tolerant to heat stress (15).

A problem with using cold stress to assess thermoregulatory function is that the rat’s thermogenic response during the onset of cold exposure results in an elevation in core temperature. It is important to note that the rats were not handled or disturbed during the thermal challenge. Hence, the rise in core temperature during cold stress represents an artifact-free thermoregulatory response to cold stress. The hyperthermic response is thought to reflect an overcompensation of autonomic effectors to raise heat production and reduce heat loss (7). The thermogenic response to cold stress was unaffected by PTU treatment. Indeed, the rate of rise in core temperature of rats treated with 25 mg/l PTU and exposed to cold stress was slightly greater than controls. This suggests that the hypothyroid rat possesses a robust thermogenic response to short bouts of cold exposure. Of course, prolonged exposure to acute cold stress might allow one to detect an effect of thyroid dysfunction; however, because the rats are well adapted to resist a decrease in core temperature during cold exposure, a cold-stress challenge would have to be severe and last for a relatively long period of time before an effect is detected. Mild heat stress allows one to more easily detect a hypothyroid deficit because rats are poorly adapted to thermoregulate in the heat (7).

PTU treatment had marked effects on serum levels of T4 compared with T3 and TSH. Serum levels of T3 do not decrease as much as T4 in rats administered PTU (11–13). Although PTU blocks the synthesis of T4, there are alternative mechanisms to maintain serum levels of T3 when T4 is depleted by PTU treatment (see Ref. 11) Fifteen-day intraperitoneal administration of PTU at 1.0 mg·kg−1·day−1 leads to a greater than twofold increase in TSH (12). In our study, a PTU treatment of 10 mg/l PTU, which resulted in a dose of ~1 mg·kg−1·day−1, led to a 60% increase in TSH.

How do the changes in serum levels of thyroid hormones correspond to thermoregulatory deficits? No significant thermoregulatory effects of PTU were observed at 5.0 mg/l PTU, a treatment associated with a 35% reduction in serum levels of T4. However, there were borderline effects at this treatment, and we suspect that significant thermoregulatory dysfunction would develop when the reduction in T4 exceeded 35%. We can conclude that thermoregulatory dysfunction is manifested in a reduced hyperthermic response to heat stress at T4 reductions of 78% (i.e., 10 mg PTU/l). On the other hand, it is not clear whether there is a relationship between serum levels of T3 and/or TSH and thermoregulatory deficits. Reduction in the level of T3 was not a good indicator of a thermoregulatory deficit. TSH levels were also a poor indicator of a thermoregulatory deficit.

Perspectives

Radiotelemetric monitoring of body temperature allows one to detect subtle effects of hypothyroidism when animals are subjected to heat stress. A normal thyroid gland is of utmost importance in the regulation of body temperature during cold exposure; however, the hypothyroid rat is capable of mounting a substantial thermogenic response when challenged with cold stress. The thermogenic response and resulting increase in core temperature makes it difficult to assess a thyroid impairment during brief exposures to cold stress. On the other hand, heat stress allows one to
detect a thyroid-induced thermoregulatory deficit in the rat. This is probably a result of the suppression of the hypermetabolic response in the hypothyroid rat when exposed to heat stress. A heat stress test could also be used to assess deficits in animals exposed to antithyroidal environmental contaminants such as di-xins and PCBs.

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This report has been reviewed by the National Health and Environmental Effects Research Laboratory, US Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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