Experimental Physiology

Hypothyroidism attenuates stress-induced prolactin and corticosterone release in septic rats

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We investigated the effects of sepsis, through the lipopolysaccharide (LPS)-induced inflammatory response, on plasma corticosterone and prolactin (PRL) levels during acute immobilization stress in normal and thyroidectomized rats. Thyroidectomized (TX) or sham-operated (N) rats were subjected to 120 min of immobilization stress. Rats were treated with an intraperitoneal injection of either LPS (250 μg (100 g body wt)$^{-1}$) or the same volume of vehicle (saline solution), 90 min before the induction of stress. Blood samples for hormone assays were collected before sepsis and stress induction for baseline measures (−90 min), and during sepsis and immobilization stress for the measurement of prolactin and corticosterone levels by radioimmunoassay. Our results show that the thyroid hormones are necessary for a proper response of PRL and corticosterone release during immobilization stress. Although sepsis enhanced PRL secretion, this was not true of corticosterone release in either group of rats. Low levels of thyroid hormones partially block the release of PRL, but do not block corticosterone secretion during sepsis. *Experimental Physiology* (2003) **88.6**, 755–760.

Interactions between the immune and neuroendocrine systems are mediated by several factors including neurotransmitters, cytokines, humoral mediators and hormones such as glucocorticoids, prolactin (PRL), growth hormone (GH) and thyroid hormones (Heninger, 1995; Besedovsky & del Rey, 1996). Stress induces changes in the secretion of several hormones, which affect immune function by either increasing or decreasing immune activity (Baldwin *et al.* 1997; Dhabhar, 1998; Davis, 1998; Sapolsky *et al.* 2000). It has also been suggested that acute immobilization stress and activation of the immune system by lipopolysaccharide (LPS), an endotoxin of Gram-negative bacteria, lead to the stimulation of common neuronal pathways (Dunn *et al.* 1999; Turnbull & Rivier, 1999).

The systemic response to LPS is mediated via the macrophage-derived pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) (Chaby, 1999; Karima *et al.* 1999). IL-1 has been shown to stimulate the hypothalamic-pituitary-adrenal (HPA) axis and increase plasma glucocorticoid levels (Gaillard, 1994; Besedovsky & del Rey, 1994, 1996). Elevated plasma levels of glucocorticoids feed back to inhibit the synthesis and release of pro-inflammatory cytokines and protect the organism from septic shock during inflammatory responses (Turnbull & Rivier, 1999). During inflammation, it is known that the factors IL-1, IL-6 and TNF-α inhibit the hypothalamic-pituitary-thyroid (HPT) axis (Kakucska *et al.* 1994; Kondo *et al.* 1997). This response may be important for survival as low thyroid hormone levels may help to reduce nitrogen losses and allow the organism to adapt better to the inflammatory reaction (Kakucska *et al.* 1994; Stathatos *et al.* 2001).

On the other hand, PRL is also a common mediator of the neuro-immuno-endocrine network. Prolactin plays a significant role in the regulation of the humoral and cellular immune responses (Davis, 1998). It appears that the immune responses *in vivo* are enhanced by PRL (Davis, 1998; Freeman *et al.* 2000). Moreover, PRL is required for mitogen-stimulated proliferation of lymphocytes (Freeman *et al.* 2000). Prolactin secretion is also increased by stress (Ramalho *et al.* 1992) and it has been proposed that this

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increase partially counteracts the negative or suppressive effects on the immune system of the stress-induced increase in glucocorticoid secretion (Davis, 1998).

The thyroid hormones, which are essential for the maintenance of secretion of circadian hormones and neurotransmitters associated with stress, have also a significant impact on the immune response. It has been shown that hypothyroidism is associated with thymic growth depression and with a decrease in circulating lymphocyte numbers (Dorshkind & Horseman, 2000). Thyroid hormones may also mediate immune effects by the modulation of various endocrine factors (Kruger, 1996). Studies have revealed that low levels of thyroid hormones reduce the synthesis of mRNA for corticotropin-releasing hormone (CRH) and pro-opiomelanocortin (POMC) and decrease the activity of the HPA axis resulting in low glucocorticoid levels (Rittenhouse & Redei, 1997). However, the interaction between stress and sepsis in the neuroendocrine response in animals with hypothyroidism has not yet been described. In the experiments reported here, we investigated the effect of sepsis, through the LPS-induced inflammatory response, on plasma corticosterone and PRL levels during acute immobilization stress in normal and thyroidectomized rats.

METHODS

Animals

Male Wistar rats (200–260 g), obtained from the Oswaldo Cruz Foundation, Salvador, Bahia and maintained in the animal colony of the Health Sciences Center, Federal University of Bahia, were housed under controlled conditions of temperature (23 ± 2 °C) and exposed to a daily 12–12 h light–dark cycle (lights on 06.00–18.00 h). Tap water and standard rat chow were available *ad libitum*, except during the stress session. The experiments were performed in accordance with the ethical principles of animal experimentation adopted by the Brazilian College of Animal Experimentation and with Brazilian legislation.

Induction of hypothyroidism

Bilateral thyroidparathyroidectomy was performed 15 days before the experiments on animals anaesthetized with 2.5 % 2,2,2-tribromoethanol (Aldrich; 1.0 ml (100 g body wt)$^{-1}$, I.P.). Sham-operated animals were submitted to exactly the same procedures as the thyroidectomized animals except that their thyroid glands were not removed. To avoid possible disorders of calcium metabolism induced by parathyroidectomy, thyroidectomized animals were given tap water containing 1 % calcium lactate.

Experimental design

Thyroidectomized (TX) or sham-operated (N, normal or euthyroid) rats were subjected to 120 min of acute immobilization stress. Immobilization was performed using cylindrical, polypropylene rodent-restraining tubes. These tubes provide adequate ventilation for breathing and do not cause pain or discomfort to the animals.

The day before the experiments, animals were anaesthetized as before and a Silastic cannula was implanted into an external jugular vein for blood sample collection. Rats were cannulated according to the method described by Harms & Ojeda (1974). After insertion, the cannula was filled with a 0.9 % NaCl solution containing heparin (500 i.u. ml^{-1}). The indwelling cannula was well tolerated by the rats with no obvious signs of discomfort or infection.

Normal or thyroidectomized rats were treated with an I.P. injection of either LPS (Sigma Chemical Co.; *E. coli* serotype 055:B5, 250 μg (100 g body wt)$^{-1}$), or the same volume of vehicle (saline solution) 90 min before stress induction (Fig. 1).

Blood samples (0.5 ml) for hormone assays were collected into heparinized tubes, before sepsis and stress induction for baseline measurements (−90 min) and at −85, −75, −60, −30, 0, 5, 15, 30, 60 and 120 min of immobilization for measurement of prolactin concentration. Corticosterone concentration was measured at −90 min (baseline) and −60, 0, 30, 60 and 120 min of immobilization (Fig. 1). Plasma was obtained by centrifugation for 15 min at 1870 *g* at 5 °C and immediately frozen and stored at −20 °C until required for assay. To avoid hypovolaemia, the same volume of red blood cells, from a pool of donor rats, was resuspended in sterile physiological saline solution and returned to each rat through the jugular venous cannula.

At the end of the experiments, the rats were killed with an injection of thiopental sodium (50.0 mg kg^{-1}, I.V.; Abbott do Brasil, São Paulo, Brazil), in accordance with the ethical principles of animal experimentation already provided.

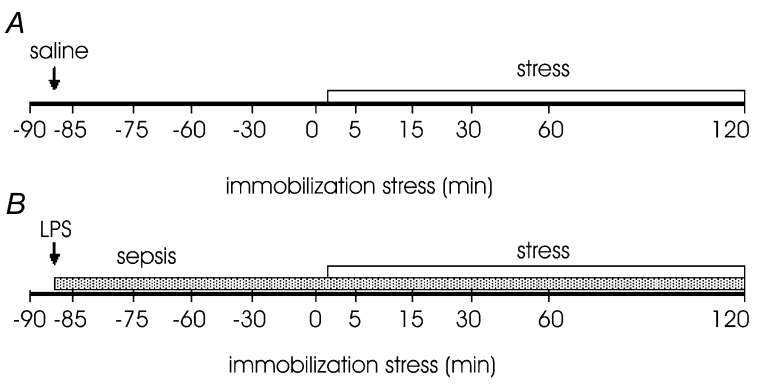

Figure 1

Experimental design for stress (*A*) and sepsis induction together with stress (*B*) in thyroidectomized and normal rats. In both designs lipopolysaccharide (LPS) or saline were injected 90 min before stress induction, immediately after baseline measurements (−90 min). Blood samples were collected during sepsis alone (−85, −75, −60, −30, 0 min) and during 120 min of immobilization stress alone (*A*) and together with sepsis (*B*) (5, 15, 30, 60 and 120 min) for prolactin and corticosterone measurement. Periods of stress and sepsis are shown by horizontal bars.

Hormone assays

Plasma corticosterone levels were measured in duplicate by radioimmunoassay using an antibody supplied by the Laboratory of Neuroendocrinology, Faculty of Medicine, Ribeirão Preto, São Paulo, Brazil and [^{3}H]corticosterone (N.E.N., Dupont Net 182). Plasma prolactin levels were determined using a highly sensitive double-antibody radioimmunoassay kit supplied by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK, USA), containing ratPRL.RP3, ratPRL I_6 and anti-ratPRL S9. Intra- and inter-assay coefficients of variation for the assays varied between 3.0 % and 12.0 %, respectively.

In order to confirm the induction of hypothyroidism, plasma T_4 levels were measured by time-resolved fluoroimmunoassay using commercial kits (EG & G do Brasil, Wallac Division, Brazil) and thyroid-stimulating hormone (TSH) levels were determined using a radioimmunoassay kit (NIDDK, USA) containing ratTSH-RP-3, ratTSH-I-9 and anti-rat TSH-RIA-6.

Statistical analysis

Data are expressed as means ± S.E.M. and were analysed by the non-parametric Mann-Whitney *U* test, to determine the significance of differences between two groups, and by the Wilcoxon test for comparisons of stress-induced changes in each group of animals, with the level of significance set at 5 %. A computer statistics package was used for statistical analyses (SPSS, version 9.0).

RESULTS

Induction of hypothyroidism

Fifteen days after bilateral removal of the thyroid and parathyroid glands, plasma TSH levels in hypothyroid rats exceeded 25.0 ng ml^{-1}, while those of euthyroid animals were less than 1.5 ng ml^{-1}. T_4 levels in hypothyroid rats were below 1.3 μg dl^{-1} while those of euthyroid animals were above 4.5 μg dl^{-1}.

Effect of immobilization stress on the release of corticosterone

There was no significant difference between the baseline values (−90 min) of plasma corticosterone concentration in saline-treated normal (N-SL) and hypothyroid (TX-SL) rats (5.5 ± 1.2 and 4.4 ± 1.0 μg (100 ml)$^{-1}$, respectively; Fig. 2). Levels of plasma corticosterone increased dramatically in both normal and thyroidectomized animals (44.5 ± 1.6 and 21.2 ± 2.6 μg (100 ml)$^{-1}$, respectively) 30 min after the onset of stress and were significantly elevated compared to pre-stress values ($P < 0.01$).

Although plasma corticosterone levels decreased after 120 min of immobilization, they remained significantly higher than baseline levels in both groups ($P < 0.05$). It is worth noting that in hypothyroid animals, stress-induced corticosterone release was partially blocked during immobilization stress; thus, after 30 min of restraint, plasma corticosterone concentrations in TX rats were significantly lower than in sham-operated animals ($P < 0.01$).

Effect of sepsis on release of corticosterone

Figure 3 shows that during 90 min of sepsis before stress induction, plasma corticosterone levels increased continuously in both groups (N-LPS and TX-LPS; $P < 0.05$ compared with baseline). Furthermore, corticosterone secretion during this period was similar for normal and hypothyroid rats.

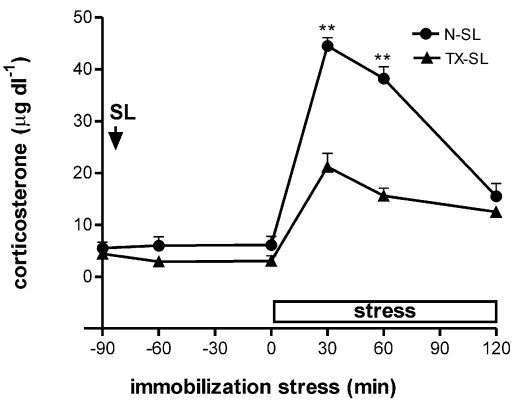

Figure 2

Effects of thyroidectomy on the release of plasma corticosterone in rats subjected to immobilization stress. Blood samples were collected before immobilization: −90 (baseline), −60 and 0 min and during 120 min of stress. The immobilization stress period is represented by the horizontal bar. The arrow represents saline (SL) injection. Results are reported as means ± S.E.M. for 13 animals in each group and were analysed by the non-parametric Mann-Whitney *U* test. ** $P < 0.01$ indicates significance of differences between sham-operated (N-SL) and thyroidectomized (TX-SL) rats.

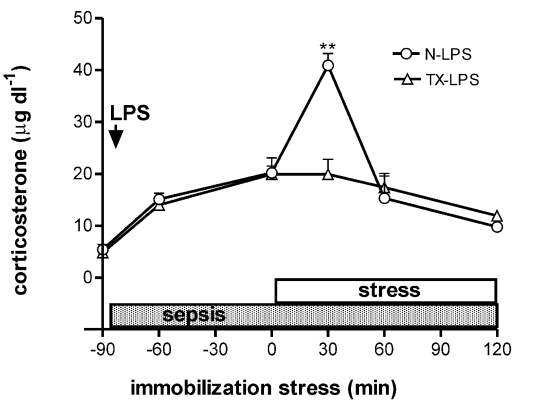

Figure 3

Effects of thyroidectomy on the release of plasma corticosterone in septic rats subjected to immobilization stress. Blood samples were collected before immobilization: −90 (baseline), −60 and 0 min and during 120 min of stress. The shaded bar represents the period of sepsis and the open bar the period of immobilization stress. The arrow represents LPS injection. Results are reported as means ± S.E.M. for 13 animals in each group and were analysed by the non-parametric Mann-Whitney *U* test. ** $P < 0.01$ indicates significance of differences between N-LPS and TX-LPS.

Thirty minutes after the beginning of immobilization stress, plasma corticosterone levels were significantly higher in normal compared to hypothyroid rats (40.9 ± 2.3 and 19.9 ± 2.9 μg (100 ml)$^{-1}$, respectively; $P < 0.01$). Corticosterone release after stress induction in euthyroid septic rats was twice the pre-stress level, while in hypothyroid septic animals it was unchanged from the pre-stress values. Nevertheless, plasma corticosterone levels after 120 min of immobilization stress were higher than baseline levels in both normal and hypothyroid septic rats.

Effect of immobilization stress on the release of prolactin

Thyroidectomy did not alter PRL levels in non-stressed rats. Plasma PRL levels in normal and hypothyroid saline-treated rats were 5.6 ± 0.7 and 3.9 ± 0.3 ng ml^{-1}, respectively. However, as shown in Fig. 4, plasma PRL was dramatically increased after 5 min of immobilization in both groups (N-SL, 22.9 ± 4.8 ng ml^{-1}; TX-SL, 8.8 ± 1.2 ng ml^{-1}); plasma PRL levels in euthyroid rats were 2.5-fold higher than in the hypothyroid group ($P < 0.01$). Despite a slight decrease at 15 min, PRL secretion persisted at high levels throughout the period of immobilization. After 120 min of immobilization, the plasma PRL concentration in both groups was significantly higher than the pre-stress value (N-SL, 17.4 ± 0.8 ng ml^{-1}; TX-SL, 8.0 ± 1.2 ng ml^{-1}; $P < 0.01$). Moreover, the prolactin secretion in saline-injected rats displayed a pattern identical to that of corticosterone secretion; thus, stress-induced prolactin release was partially blocked during immobilization in hypothyroid animals.

Effect of sepsis on the release of prolactin

Figure 5 shows that 90 min of sepsis caused by the injection of LPS stimulated prolactin release in a similar way to stress induction; thus, after 5 min of sepsis, plasma PRL levels increased dramatically in normal (N-LPS) and thyroidectomized (TX-LPS) rats (60.1 ± 17.2 and 16.3 ± 2.8 ng ml^{-1}, respectively; $P < 0.01$). After 15 min of sepsis, PRL secretion declined markedly but still remained significantly above starting values, until the onset of stress.

Treatment with LPS had highly significant effects on plasma levels of PRL throughout the period of stress. LPS enhanced the PRL response to immobilization stress in normal rats. Although the hypothyroid septic rats were not able to achieve the PRL secretion observed in control animals, LPS did enhance the PRL response to stress at 5 min when compared to saline-injected controls. Moreover, hypothyroid rats showed a reduced PRL response to sepsis.

DISCUSSION

In the present study, baseline levels of plasma corticosterone in saline-injected hypothyroid rats were slightly lower than those in normal rats; however, this difference was not significant. Meanwhile, in hypothyroid animals, stress-induced corticosterone release was partially blocked during immobilization. These data support our hypothesis that thyroid hormones are necessary for a proper response of corticosterone release during stress.

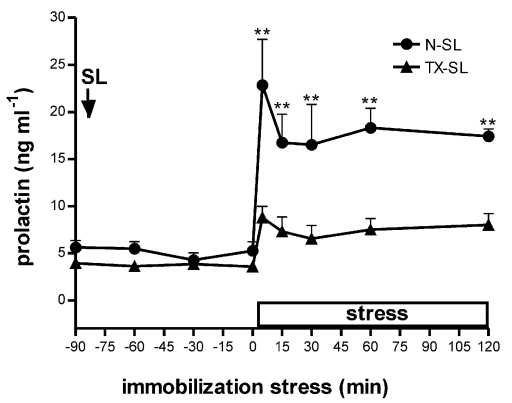

Figure 4

Effects of thyroidectomy on the release of plasma prolactin in stressed rats. Blood samples were collected before immobilization: −90 (baseline), −60, −30 and 0 min and during 120 min of stress. The horizontal bar represents the period of immobilization stress. The arrow represents saline (SL) injection. Results are reported as means ± S.E.M. for 13 animals in each group and were analysed by the non-parametric Mann-Whitney *U* test. ** $P < 0.01$ indicates significance of differences between N-SL and TX-SL.

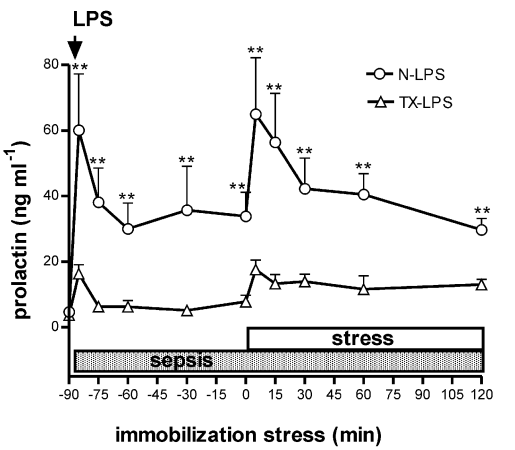

Figure 5

Effects of thyroidectomy on the release of plasma prolactin in septic rats subjected to immobilization stress. Blood samples were collected before immobilization: −90 (baseline), −60, −30 and 0 min and during 120 min of stress. The shaded bar represent s the period of sepsis and the open bar represents the period of immobilization stress. The arrow represents LPS injection. Results are reported as means ± S.E.M. for 13 animals in each group and were analysed by the non-parametric Mann-Whitney *U* test. ** $P < 0.01$ indicates significance of differences between N-LPS and TX-LPS.

Therefore, in order to reveal disturbances caused by hypothyroidism it was necessary to present an acute challenge to homeostasis in the form of immobilization stress.

The mechanisms that account for the interaction between the thyroid and adrenal axes are unknown. It has been proposed by Shi *et al.* (1994) that the effect of hypothyroidism on circulating corticosterone is mediated centrally and not through a direct influence on adrenal corticosterone production. Ascending catecholamine pathways from the brainstem that project to the paraventricular nucleus (PVN) are potential sites for this effect (Shi *et al.* 1994). Another model of the interactions between the HPA and HPT axes proposes that the reduced levels of thyroid hormones result in an increased synthesis of hypothalamic preproTRH 178–199 as a putative corticotropin release-inhibiting factor, which inhibits ACTH synthesis (Redei *et al.* 1998).

However, it is well known that stress is associated with increased corticosterone secretion in normal rats (Gaillard, 1994; Heninger, 1995; Besedovsky & del Rey, 1996). Stimulation of the HPA axis by stress is the result of complex communication between the subcortical network and hypothalamic nuclei, as well as centres in the brainstem and limbic system (Mastotakos *et al.* 1995; Rivest *et al.* 1995; Wilder, 1995; Stratakis & Chrousos, 1995; Besedovsky & del Rey, 1996).

It is interesting that LPS alone, that is, before stress induction, stimulated corticosterone secretion to a similar extent in both groups (N and TX). The HPA axis response after an endotoxin challenge is mainly due to released cytokines (IL-1, IL-6 and TNF-α) from stimulated peripheral immune cells, which in turn stimulate different levels of the HPA axis. Controversy exists regarding the main locus of action of endotoxin on glucocorticoid secretion. Although we cannot dismiss the effects of LPS on the adrenal gland directly, several studies confirm that the PVN plays a pivotal role in LPS-stimulated increases in plasma ACTH levels. Rats in which the PVN has been electrolytically lesioned can mount a detectable plasma ACTH response to a large dose of LPS (2 mg kg^{-1} I.P.), but the magnitude is markedly diminished (Elenkov *et al.* 1992). Further studies showed that peripheral administration of IL−1β exerts no effect on corticosterone secretion in hypophysectomized rats (Gwosdow *et al.* 1990). Therefore, we suggest that activation of the HPA axis by LPS in hypothyroid rats may result in significant protection against potentially lethal effects of pro-inflammatory cytokines.

To our knowledge, the present work is the first experimental evidence to demonstrate that challenge by immobilization stress results in a twofold higher corticosterone release in euthyroid compared to hypothyroid septic rats. Therefore, our study provides even more substantial evidence for the critical role of thyroid hormones in mediating the activation of the HPA axis in response to stress.

In our study we observed that the decrease in circulating T_4 levels in hypothyroid rats did not alter PRL levels in non-stressed rats. It is clear that PRL secretion was dramatically affected by stress in both groups (N and TX), although stress-induced PRL release was partially blocked during immobilization in hypothyroid animals.

From the results of several studies, it has been suggested that the stress-induced release of PRL may have positive consequences for the maintenance of the immune system (Dorshkind & Horseman, 2000; Freeman *et al.* 2000), both in stimulating mitogenesis in T lymphocytes and also in counteracting the negative effects of glucocorticoids. Glucocorticoids are critical for survival during stress, but a negative side-effect is their potentiation of apoptosis in lymphoid cells. Thus, positive regulatory signals that counteract this effect may be critical to lymphocyte survival. Growth and lactogenic hormones such as PRL influence the immune system through their general growth regulatory function (Nagy & Berczi, 1994). We suggest that, in our study, a sharp elevation of PRL secretion in the first minutes of immobilization stress plays a fundamental role in maintaining immunocompetence. In addition, its rapid decline may suggest a way to avoid an exaggerated immune response that could predispose towards antibody and cell-mediated autoimmune disease.

In our study the injection of endotoxin elicited a sharp and brief elevation of plasma PRL concentration in both normal and thyroidectomized groups, although sepsis-induced prolactin release was also partially blocked in hypothyroid animals. Sepsis as a unique stressor was a much more potent stimulus of PRL release than immobilization. Furthermore, PRL secretion during immobilization stress was enhanced by the prior injection of LPS. This suggests that PRL is essential to promote a competence signal which enables the immune system to respond to non-specific stimuli that initiate an inflammatory response. According to Nagy & Berczi (1994), the presence of growth and lactogenic hormones is the first signal necessary for lymphocyte activation besides antigen presentation and cytokine release.

In summary, the results of the present study show that the thyroid hormones are necessary for a proper response of PRL and corticosterone release during immobilization stress. It is recognized that high glucocorticoid levels have immunosuppressive effects and these may be counteracted by PRL. In normal rats both glucocorticoid and PRL levels are elevated during immobilization, leading to an adaptive stress response. However, it is debatable whether hypothyroidism leads to a different level of adaptation, since the release of both hormones was partially blocked during stress, or whether this should be considered a maladaptive response of the immune system.

The induction of sepsis enhanced the secretion of PRL, but not that of corticosterone, in both groups of rats (N and TX). The levels of several cytokines are enhanced during sepsis and the rate of their synthesis is regulated by PRL (Nagy & Berczi, 1994). Low levels of thyroid hormones

partially block the release of PRL, but do not block corticosterone secretion during sepsis. Finally, normal levels of corticosterone secretion induced by sepsis in hypothyroid rats may offer protection against pathological overshoot of inflammatory and immune responses and improve survival in these animals.

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