Aetiology of thyroidal dysfunction in murine toxoplasmosis

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(*Received 2 February 1998; revised 24 March 1998; accepted 25 March 1998*)

SUMMARY

Mice infected with *Toxoplasma gondii* manifest a rapid decline in serum thyroxine (T_4) levels. To locate the locus of the hypothyroxinaemia, the integrity of the pituitary–thyroid axis of infected mice was assessed by a thyrotropin-releasing hormone (TRH) assay. A rise in serum T_4 after inoculation of TRH implies the release of thyrotropin (thyroid-stimulating hormone) from a functionally intact pituitary. Administration of a single, large-dose (1 μg) bolus of TRH to infected mice induced a positive, although subnormal, T_4 response. In contrast, when infected mice were pre-treated with a series of low-dose (5 ng) pulses of TRH prior to the bolus challenge, the T_4 response was markedly enhanced. We suggest that the multiple inoculations of low-dose pulses of TRH 'primed' the pituitary (and secondarily the thyroid) and led to replenishment of their readily available hormone reserves and the heightened response to stimulation. These observations indicate that the locus of thyroid dysfunction is in the hypothalamus, not the pituitary or thyroid, and apparently involves impairment of the pulsatile release of TRH.

Key words: *Toxoplasma gondii*, thyroxine, thyrotropin-releasing hormone (TRH), pulsatile stimulation, pituitary, hypothalamus.

INTRODUCTION

Female Nya:NYLAR mice, infected with *Toxoplasma gondii*, exhibit a rapid decline in serum thyroxine (T_4) levels within the first week post-infection (p.i.) (Stahl & Turek, 1988). The absence of discrete pathological changes in the thyroid suggested that a physiological impediment likely had developed within the hypothalamus–pituitary–thyroid (HPT) axis and was interfering with normal thyroidal function. In an attempt to locate the site of the presumptive defect, components of the HPT axis were tested for responsiveness to hormonal stimulation. Although thyroidal responsiveness was demonstrated, the magnitude of the induced responses was noticeably diminished, inferring lack of thyroidal reserves rather than primary thyroidal malfunction (Stahl & Kaneda, 1998).

In the experiments reported herein, pituitary responsiveness to TRH stimulation was evaluated indirectly by monitoring T_4 release from the thyroid, rather than by directly monitoring TSH release from the pituitary. We show that the infected-mouse pituitary clearly was responsive to stimulation, especially so when first primed by multiple low doses of TRH administered in repetitive, pulsatile fashion, thereby confirming pituitary integrity and implicating the hypothalamus as the likely site of the putative HPT impediment.

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MATERIALS AND METHODS

Mice

Details concerning the source and maintenance of the Nya:NYLAR strain of albino mice have been reported previously (Stahl & Turek, 1988). Only female mice, approximately 3 months of age and weighing 20–22 g, were used.

Toxoplasma

The Cornell (CS) strain of *T. gondii* was maintained, quantitated, and administered as previously described. Mice were infected with 8 cysts, inoculated i.p. in 0·5 ml aliquots of brain emulsion. The procedure for obtaining cysts and for preparing both infective and control inocula were exactly as described in the preceding paper (Stahl & Kaneda, 1998).

Reagents

Thyrotropin-releasing hormone (TRH) was purchased from Sigma Chemical Co., (St Louis, MO, USA). The TRH was dissolved in sterile physiological saline and inoculated in 0·2 ml aliquots. Control mice received 0·2 ml of saline. When multiple injections of TRH were given over a 2-day period, a fresh solution was prepared each morning, divided into aliquots, frozen, then thawed and appropriately diluted prior to use. All inoculations were given i.p.

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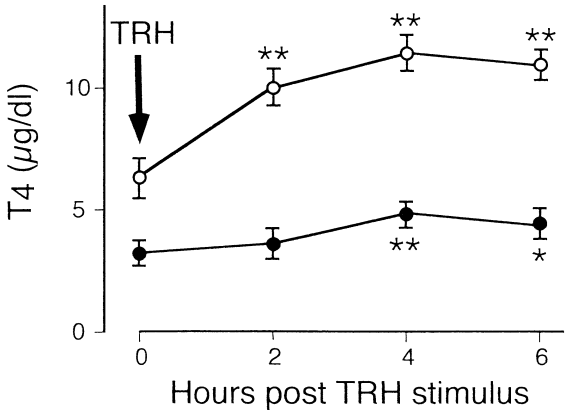

Fig. 1. Subnormal T_4 response to a 1 μg bolus of TRH in Nya:NYLAR female mice infected with *Toxoplasma gondii* for 3 weeks. Uninfected controls (○, $n = 8$); Infected (●, $n = 8$). Data expressed as mean ± S.D. Single and double asterisks identify statistically significant differences ($P < 0{\cdot}05$ and $P < 0{\cdot}01$, respectively) between the hourly changes in T_4 levels and the 0 h baseline value.

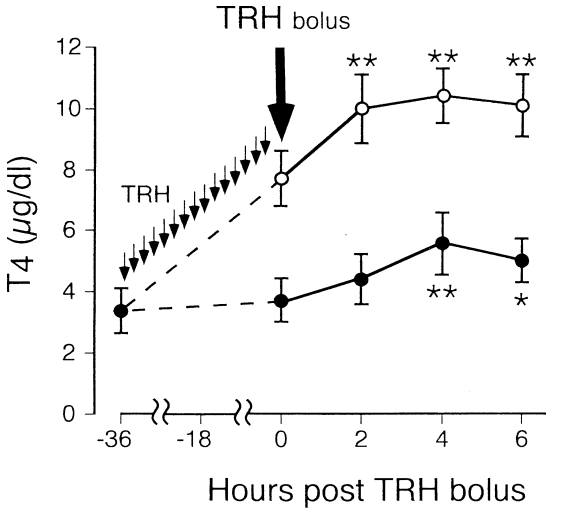

Fig. 2. Effect of 'priming' on the subnormal T_4 response of *Toxoplasma gondii*-infected Nya:NYLAR female mice to TRH. 'Priming' was by the administration of multiple, repetitive inoculations of low-dose (5 ng) aliquots of TRH, at 2 h intervals, prior to challenge with a 1 μg bolus. Infected control mice received multiple inoculations of saline. All mice infected for 3 weeks; data expressed as mean ± S.D. Unprimed (●, $n = 10$); Primed (○, $n = 10$). Single and double asterisks identify statistically significant differences ($P < 0{\cdot}05$ and $P < 0{\cdot}01$, respectively) between the hourly changes in T_4 levels and the 0 h baseline value.

T_4 response to a bolus of TRH

The effect of a single, large-dose bolus of TRH (1 μg) was determined in mice infected with *T. gondii* for 3 weeks. Uninfected littermates served as controls. Prior to treatment, several drops of blood were withdrawn from the periorbital sinus of the mice and spotted on filter paper and processed as described in the previous paper (Stahl & Kaneda, 1998). The purpose of this bleeding was to establish a T_4 baseline. Both groups of mice were then challenged with the 1 μg (50 μg/kg) bolus of TRH, and bled again after 2, 4, and 6 h.

T_4 response to a bolus of TRH following multiple, repetitive inoculations of low-dose aliquots of TRH

Prior to initiating the series of repetitive inoculations, 2 groups of animals, consisting of 8 normal and 8 *T. gondii*-infected mice, were bled to provide pre-treatment (control) T_4 values. After the single bleeding, these mice were not used again in the following experiment.

For 'priming' purposes, a group of 10 mice, infected for 3 weeks, received a total of 15 inoculations of 5 ng (0·25 μg/kg) aliquots of TRH. The first day, the mice received 9 injections at 2 h intervals, beginning at 07.00 and continuing until 23.00. The inoculations were resumed at 05.00 the following morning. A second group of 10 infected mice, serving as unprimed controls, received 15 injections of saline only. After an initial bleeding to establish the pre-bolus challenge T_4 baseline, both groups of infected mice received the 1 μg bolus of TRH and were serially bled after 2, 4, and 6 h.

Assay for T_4

Small, uniformly-sized circles in the dried blood spots were punched out, the blood eluted, and assessed for T_4 by a commercial radioimmunoassay (Neometrics, Inc., Syosset, NY, USA).

RESULTS

T_4 response of infected mice to a bolus of TRH

The results of the first experiment are graphically depicted in Fig. 1. The T_4 baseline of the uninfected normal controls was approximately twice the level of the *T. gondii*-infected mice. Two h after challenge with the 1 μg bolus of TRH, the normal mice showed a sharply elevated T_4, which then plateaued over the next 4 h. The infected mice also demonstrated a T_4 response 2 h after the challenge, but it was markedly reduced in magnitude. After 4 h, the T_4 had belatedly risen to its highest level and thereafter began to decline. These results demonstrated that a 1 μg bolus of TRH, given by the intraperitoneal route, was capable of eliciting a distinct T_4 response, and confirmed the validity of the 'TRH response test' for use in monitoring the HPT axis of infected mice.

T_4 response to the bolus of TRH in infected mice 'primed' with multiple, repetitive inoculations of low-dose aliquots of TRH

The results are presented in Fig. 2. It is readily apparent that the administration of multiple low-

https://doi.org/10.1017/S0031182098003035 Published online by Cambridge University Press

dose inoculations of TRH succeeded in raising the baseline T_4 of infected mice to the normal range (as shown in Fig. 1). This increase in baseline T_4 did not occur in the infected mice that received only the injections of saline. Following the TRH bolus challenge, the primed infected mice responded quickly and vigorously, comparable to the response shown by normal, uninfected mice (see Fig. 1). In contrast, the saline-treated infected mice showed the same delayed and noticeably diminished T_4 response previously exhibited by infected mice (compare with Fig. 1). The markedly improved T_4 responsiveness of the primed mice likely reflects an increase in the readily available reserves of pituitary TSH and thyroidal T_4, resulting from the series of low-dose inocula of TRH administered in pulsatile fashion.

DISCUSSION

The goals of the present study were to determine if the pituitary of the *T. gondii*-infected mouse is responsive to stimulation by TRH, and to seek validation of our working hypothesis that the hypothyroxinaemic state of the infected mice is not due to pituitary failure, but to a disturbance in the pulsatile release of TRH from the hypothalamus. Our experimental results confirm both goals.

The assessment of pituitary function was by the 'TRH response test' (Hall *et al*. 1972; Mitsuma *et al*. 1973; Lothrop, Tamas & Fadok, 1984; Lothrop & Nolan, 1986), in which serum T_4 levels are monitored before and after the administration of TRH. The rationale is that an increase in T_4 after administration of TRH is dependent on the induced release of endogenous TSH from the pituitary and subsequent stimulation of the thyroid. Challenging infected mice with just a single inoculation of TRH, even with pharmacological amounts (the 1 μg bolus), consistently engendered a subnormal T_4 response compared to the response by normal mice. We attribute the weak T_4 response to depletion of the readily available pools of pituitary and thyroid hormone reserves. In contrast, the repetitive administration of multiple low-dose (5 ng) increments of TRH prior to inoculation of the challenge bolus significantly increased the magnitude of the subsequent T_4 response. Apparently, the pulses of repetitive TRH injections effectively 'primed' the pituitary (and secondarily the thyroid), and led to replenishment of the pools of TSH and T_4. Although TSH levels were not monitored *per se*, the enhanced T_4 response of infected mice treated with pulses of TRH was a clear indication of the release of endogenous TSH from the pituitary, thus confirming the integrity and responsiveness of the pituitary thyrotropes to TRH. It is well documented that disruption of TRH pulsatility results in curtailment of *de novo* synthesis of TSH by the pituitary thyrotrope, depletion of the pool of readily available TSH, and disruption of TSH pulsatility (Brabant *et al*. 1991). In turn, the reduction in pituitary reserve and perturbation of the pulsatile release of TSH would adversely affect thyroid function. Each pulse of TSH not only elicits the release of T_4 from the thyroid, but induces the synthesis and storage of new T_4 (Brabant *et al*. 1986; Greenspan *et al*. 1986). We suggest, therefore, that the inadequately primed and depleted pituitaries (and thyroids) of the infected mice were unable to respond to single injections of TRH either as vigorously or as rapidly as the PT axis of normal mice. When the pituitary was first primed with a series of TRH injections administered in pulsatile fashion, the synthesis and replenishment of both pituitary and thyroid reserves ensued, as manifested by the heightened T_4 responsiveness to the bolus of exogenous TRH.

The proposed sequence of events outlined above closely parallels findings in previous studies on reproductive failure in female mice chronically infected with *T. gondii*, a syndrome characterized by cessation of cycling and ovarian atrophy (Stahl, Kaneda & Noguchi, 1994). The integrity of the pituitary–ovarian axis of infected mice was evaluated by administering hypothalamic gonadotropin-releasing hormone (GnRH) to stimulate the release of gonadotropins from the pituitary, then monitoring the secondary effects on the ovary. Although the atrophied ovaries of the infected mice were responsive to single injections of GnRH, thereby confirming the release of endogenous gonadotropins from the pituitary, the ovarian responses were weak and transient, ovarian insufficiency was not ameliorated, and normal cycling did not resume. These observations indicate that inadequate levels of the readily releasable pool of pituitary gonadotropins, believed to result from a central (hypothalamic) inhibition of the pulsatile release of GnRH, are responsible for the weak ovarian responses. Accordingly, when the pituitary of the infected mice was first primed with periodic pulses of GnRH every 4 h for 3 days, a challenge bolus of GnRH then induced a vigorous ovarian response, exemplified by the resumption of cycling, ovulation, and increased secretion of estrogen (Stahl *et al*. 1995). Clearly, priming of the pituitary by the pulsatile administration of GnRH had replenished gonadotropin reserves and effectively reversed the pituitary insufficiency.

We therefore envision a comparable central disruption of the pulsatile release of hypothalamic TRH in the infected mice, leading to inadequate pituitary priming, depletion of TSH reserves, and perturbation of the pulsatile release of TSH from the pituitary. This was followed by thyroid insufficiency and the decline in serum T_4 levels, and by the subnormal T_4 responses to exogenous TRH. Although the events leading to depressed thyroid

https://doi.org/10.1017/S0031182098003035 Published online by Cambridge University Press

function in *T. gondii*-infected mice appear traceable to a putative flaw in hypothalamic function, the reason(s) for the development of the flaw remain to be elucidated. For the interim, however, the following speculations are offered in explanation. Soon after infection, the ensuing tissue invasion and destruction of cells attracted large numbers of macrophages and lymphocytes which, upon exposure to antigen, released a cascade of cytokines that have been shown to play a pivotal role in the pathophysiology of infectious and inflammatory diseases and in modulating host-defence reactions (Beaman, Wong & Remington, 1992; Dinarello, 1992). The cytokines can affect distant organ systems, including the central nervous system. Access to the brain can occur at circumventricular sites, areas where the blood–brain barrier is fenestrated (Dinarello, 1992; Johnson & Gross, 1993). Within the circumventricular sites, innervation of the terminal endings of hypothalamic neurons occurs, resulting in the release of corticotropin-releasing factor (CRF) and activation of the pituitary–adrenal (PA) axis (Sapolsky *et al*. 1987; Tsagarakis *et al*. 1989; Katsuura *et al*. 1990; Shibata, 1990; Shibata & Blatteis, 1991). The release of glucocorticoid hormones from the stimulated adrenal leads to adaptive changes in metabolic activity and in regulation of the immune response. In addition, in reports relevant to this study, it was shown that the release of CRF within the hypothalamus interrupts the pulse generator governing the pulsatile release of TRH (Brabant *et al*. 1991; Adriaanse *et al*. 1993), the effects of which would result in the depressed thyroid function, as outlined above. Furthermore, it has been demonstrated that CRF is also disruptive to the pulsatile release of GnRH (Petraglia *et al*. 1987; Rivier & Vale, 1984, 1990; Rivier & Rivest, 1991), which explains the pituitary and ovarian insufficiency, anovulation, and reproductive failure. It has been suggested that these alterations in hormone levels are part of the process of physiological adaptation to 'stressful' situations, encompassing such diverse conditions as infection, inflammation, trauma, surgery, fever, even vigorous exercise, and may involve the temporary inhibition of 'luxury' functions such as reproduction in order to maintain more vital metabolic processes and overall homeostasis (Rivier, Rivier & Vale, 1986; Chrousos & Gold, 1992; Reichlin, 1993).

There is another aspect to the present study which may have important clinical implications. This concerns the possible involvement of thyroid hormone insufficiency in the central nervous system (CNS) sequelae of congenital murine toxoplasmosis and perhaps in congenital human toxoplasmosis. For example, it is known that normal development of the murine brain is dependent on maternal and fetal T_4 before birth, and on thyroid hormone produced by the pup after birth (Morreale de Escobar *et al*. 1987; Porterfield & Hendrich, 1993). Since adult mice quickly develop hypothyroxinaemia after infection with *T. gondii*, the availability of maternal T_4 during intrauterine development of the fetus would be curtailed. In addition, fetuses infected *in utero* may also exhibit HPT impairment, resulting in fetal thyroid hormone insufficiency before birth and continuing into postnatal life. In view of the well-characterized pathological effects of T_4 deficiency during critical periods of murine brain development, the consequences of both pre- and post-natal hypothyroxinaemia could very likely manifest in developmental anomalies of the CNS ranging in severity from retarded development to gross malformations. Verification of these conjectures will require further elucidation of the complex cascade of neuroimmunoendocrinological reactions of the murine host, both adult and fetus, to the parasitic insult.

We are indebted to Drs R. Bellisario and S. Koerner-Brown, Hypothryoid Screening Laboratory, New York State Department of Health, Albany, New York, for T_4 radioimmunoassay of blood samples. We also thank our colleague, Dr T. Tanaka, Department of Infectious Diseases, Tokai University School of Medicine, for expert computer assistance in the preparation of the graphs.

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