Effects of *Trypanosoma congolense* infection in rams on the pulsatile secretion of LH and testosterone and responses to injection of GnRH

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Changes in pulsatile secretion of LH and testosterone and responses to exogenous GnRH were assessed at different stages of *Trypanosoma congolense* infection in rams. Jugular blood samples were collected every 15 min for 6 h followed by immediate injection of GnRH (20 µg i.v.) and further sample collection after 10, 20, 40, 60, 80, 100 and 120 min. This sampling and injection regimen was performed 5 days before infection (day - 5) and 23 and 52 days after infection. *T. congolense* infection increased ($P < 0.05$) the mean plasma LH concentration over 6 h on day 23 (3.2 ± 0.2 ng ml⁻¹) and decreased ($P < 0.05$) the mean LH concentration on day 52 (1.2 ± 0.2 ng ml⁻¹, $P < 0.05$) compared with day - 5 values (2.0 ± 0.2 ng ml⁻¹). Trypanosome infection induced a rapid decline in plasma testosterone concentration from a mean of 7.5 ± 1.4 nmol l⁻¹ on day - 5 over 6 h to 3.6 ± 0.4 nmol l⁻¹ ($P < 0.05$) on day 23 and 1.7 ± 0.3 nmol l⁻¹ ($P < 0.001$) on day 52. The observed decline in plasma LH concentration in infected rams was not associated with reduced sensitivity of the pituitary to GnRH or its ability to release LH, as the LH response to exogenous GnRH was not impaired throughout the period of infection. However, the testosterone response to GnRH-induced LH stimulation was depressed on both days 23 and 52 after infection. It was concluded that the decline in plasma LH concentration in infected rams was caused by reduced GnRH stimulation of the pituitary, whereas the decline in plasma testosterone was partly caused by reduced sensitivity of the Leydig cells to circulating LH.

Introduction

Trypanosomiasis is a serious, often fatal parasitic disease of animals and humans which occurs throughout the tropical regions of Africa, the Middle East, Asia and Latin America. However, its greatest socio-economical effects are seen in sub-Saharan Africa, an area particularly suited to survival of the tsetse fly which is the vector responsible for cyclical transmission of both animal (i.e. *Trypanosoma congolense*, *T. vivax* and *T. brucei brucei*) and human (*T. b. rhodesiense* and *T. b. gambiense*) trypanosomes (Losos, 1986). *T. congolense* and *T. vivax* are both regarded as haematic parasites, confined to the circulatory system, although *T. congolense* does undergo early development extravascularly at the site of tsetse bite and in associated lymph nodes (Gray and Luckins, 1980). Similarly, *T. vivax* has been found extravascularly in the pituitary gland (Fiennes, 1950), cerebrospinal fluid and aqueous humour (Whitelaw *et al.*,

1988) and in cardiac muscle (Kimeto *et al.*, 1990). Trypanosomes of the *T. brucei* group are primarily considered to be extravascular parasites (Losos and Ikede, 1972; Losos, 1986).

Although a considerable amount of research has been conducted on trypanosomiasis, less attention has been paid to the pathophysiology and pathogenicity of trypanosome-induced reproductive dysfunction, although it is known that reproductive disorders are consistently present among the clinical features of both human and animal trypanosomiasis (Losos, 1986). Some aspects of semen and testicular pathological changes associated with trypanosome infection in ruminants have been reported (reviewed by Ikede *et al.* (1988)). A few studies have described reproductive endocrine changes in male ruminants and a decline in plasma testosterone concentration during *T. congolense* (Waindi *et al.*, 1986) and mixed *T. congolense* and *T. b. brucei* (Adeyemo *et al.*, 1990) infections were observed. However, in these two studies, blood samples were collected infrequently at a rate of one sample (Adeyemo *et al.*, 1990) or three samples (Waindi *et al.*, 1986) per week, hence the accuracy of interpretation of their findings remain questionable in view of the known pulsatile nature of gonadal hormone secretion. The literature on pituitary endocrine changes after *T. brucei* infection is confusing since Emeh and Nduka (1983) showed that the infection in humans induces a

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decline in serum concentration of LH and FSH, but Boersma *et al.* (1989) showed in humans and Hublart *et al.* (1990) in rats, that serum gonadotrophin concentrations remain stable during *T. b. brucei* infections, despite an increase in pituitary LH content and a decline in plasma testosterone concentration. Again single, infrequent samples may be the cause of the discrepancies in the findings.

No comparable studies on gonadotrophic function have been reported in animals infected with haematic trypanosomes (*T. congolense* or *T. vivax*). The objectives of the present investigation were to assess the effects of *T. congolense* infection on the pulsatile secretion of plasma LH and testosterone at different stages of infection in rams and to determine the effect of infection on pituitary responsiveness to exogenous GnRH.

Materials and Methods

Animals

Five twin pairs of Scottish Blackface rams, approximately six months old and of body mass 28–35 kg, were obtained from a local farm. On arrival they were housed in an open pen, vaccinated against pasteurella and clostridial infections using Ovivac-P® (Hoechst Animal Health, Milton Keynes) and treated with anthelmintic using Ivomec® (MSD Agvet, Hertfordshire). They were allowed to acclimatize for 2 months, during which time they were examined regularly and exposed to routine handling such as collection of blood samples twice a week for routine haematological analyses and weighing once a week. Seven days before infection, in November, the rams were transferred to a flyproof isolation unit, where they were maintained under artificial light consisting of cycles of 8 h light:16 h dark (lights on 08:00–16:00 h) to simulate the natural photoperiod. Rams received approximately 500 g concentrate ration day⁻¹ (306 Ewbol Store Lamb Pellets, BOCM Silcock) before and throughout the experiment with hay and water provided *ad libitum*.

Animal allocation and infection

On the day of infection rams were allocated to two groups of five control and five infected animals, one twin ram to each group. These groups were closely matched on the basis of livemass (control 30.8 ± 1.6 kg, infected 30.3 ± 1.3 kg) and haematocrit (control 31.4 ± 0.6%, infected 32.1 ± 0.9%). Rams in the infected group were each injected i.v. with approximately 4 × 10⁵ *T. congolense* organisms raised from stabilate 57/11, originally imported from ILRAD (Kenya) as IL 1180 (Dwinger *et al.*, 1987). The trypanosomes were harvested from irradiated mice (infected 7 days previously) by cardiac puncture under chloroform anaesthesia at the first peak of parasitaemia. The experimental period lasted 58 days from the day of infection (day 0).

Clinical assessment and routine blood examination

Starting from the day of infection, all infected animals were closely observed for changes in demeanour and general body condition. Rectal temperatures (°C) were also recorded three

times a week and animals weighed at intervals of two weeks. Starting one month before infection, blood (5 ml) was obtained by jugular venepuncture in heparinized vacutainers between 09:00 and 10:00 h twice a week, until the day of infection, for estimation of packed cell volume (PCV) using the standard microcapillary method. The plasma was subsequently stored at -20°C to provide baseline hormonal data. After infection, jugular blood samples were taken daily and examined for the presence of trypanosomes using the buffy coat–darkground method described by Murray *et al.* (1977). Once parasitaemia was detected, blood samples were taken three times a week for estimation of PCV and parasitaemia levels. The number of trypanosomes was estimated by a slight modification of the scoring method described by Paris *et al.* (1982) at ×400 magnification. A score of 1+ (1–3 trypanosomes per preparation) represented approximately 10²–10³ trypanosomes ml⁻¹; a score of 3+ (1 trypanosome per field) represented 5 × 10³–5 × 10⁴ trypanosomes ml⁻¹, and a score of 5+ (10–100 trypanosomes field) represented > 5 × 10⁵ trypanosomes ml⁻¹. The blood from control rams was checked once a week as a precautionary measure to monitor accidental infections.

Frequent blood sampling and GnRH injection

Five days before infection and on days 23 and 52 after infection, frequent blood samples were collected from all rams using an indwelling jugular cannula (i.d. 0.86 mm, o.d. 1.27 mm) as described by Jeffcoate (1992). Samples were collected at intervals of 15 min for 6 h (11.00–17.00 h). Synthetic GnRH (L-7134: Sigma, Poole) dissolved in 2 ml sterile saline was then injected from the cannula (20 µg per ram) and further blood samples were collected at 10, 20, 40, 60, 80, 100 and 120 min. The dose of GnRH used was intended to provide an LH response lasting 2–3 h in sheep (Siddall and Crighton, 1977). The blood was promptly centrifuged at 1500 g at room temperature for 10 min and the plasma was decanted and frozen at -20°C until needed for LH and testosterone measurements.

Hormonal measurements

Plasma LH concentration was determined by a double antibody ¹²⁵I-radioimmunoassay (RIA). LH standard was NIH-oLH-S25 (National Hormone and Pituitary Program, NIADDK, Bethesda, MD) and oLH (LER-1056-C2 donated by L. E. Reichert, Albany Medical College, NY) was used for iodination. The characteristics of the antibody raised against ovine LH and the assay procedure were described by Jeffcoate and Lindsay (1989). Displacement of tracer by serial dilutions of plasma from an infected and a control ram paralleled the ovine LH standard curve. The intra-assay coefficient of variation (CV) was 4.9% (*n* = 15) at 3.0 ng ml⁻¹ and 3.9% (*n* = 15) at 10.7 ng ml⁻¹. The interassay CV for 14 assays was 11.6% at 2.7 ng ml⁻¹ and 6.2% at 12.1 ng ml⁻¹. The assay limit of detection, defined as 2 × SD of the zero standard was 0.1 ng ml⁻¹ (*n* = 15).

The plasma testosterone concentration was determined by an ether extraction, second antibody ¹²⁵I-radioimmunoassay

described by Cook and Beastall (1987). The rabbit-anti-testosterone antibody (AB-1030) which was purchased from Bioclinical Services (Cardiff) was raised in rabbits against a testosterone-3-carboxy methyl oxime-BSA conjugate and shows the following crossreactivity: testosterone, 100%; 5 α -dihydrotestosterone, 16%; 5 α -androstane-3 α -17-diol, 5.8%; 5 α -androstane-3 β -17 β -diol, 3.7%; androstenedione, 2.1%; dehydroepiandrosterone, 0.04% and cortisol <0.01%. The standards were purchased from Steraloids (Croydon) and ¹²⁵I-labelled histamine-testosterone was kindly provided by C. E. Gray, Royal Infirmary, Glasgow. There was no effect of plasma from infected or control rams on the displacement of tracer from antibody after extraction. The second antibody reagents, donkey anti-rabbit serum and normal rabbit serum, were obtained from the Scottish Antibody Production Unit (Carlisle). The intra-assay CV was 7.8% at 3.3 nmol l⁻¹ ($n = 16$) and 5.9% at 12.4 nmol l⁻¹ ($n = 16$). The interassay CV for 16 assays was 9.6% at 2.9 nmol l⁻¹ and 3.1% at 25.3 nmol l⁻¹. The assay limit of detection at 2 $\times$ SD of zero standard was 0.1 nmol l⁻¹ ($n = 16$).

Statistical analyses

Group data were averaged and are presented here as means $\pm$ SEM. In samples collected frequently for 6 h, the pulsatile variations in hormone concentration were analysed using the criteria of Canny *et al.* (1990) that a pulse was present when (i) a value exceeded the previous value by at least three SDs of the estimate of the previous value calculated from duplicate sample results and (ii) when the peak value was followed by either a decline or no significant increment. The pulse amplitude was calculated by subtracting the concentration at the onset of the pulse from the peak concentration. Mean hormone concentration was calculated by averaging the concentrations over the 6 h sampling period and the pulse frequency by counting the number of peaks (Naylor *et al.*, 1990). In addition, the area under the curve was calculated using the trapezoid rule (Schreiber *et al.*, 1988). Log-transformed LH and testosterone data were also analysed by calculating the integrated squared second derivative for each 6 h sampling sequence using Minitab and a Microsoft Excel 3.0 computer program as used under similar circumstances by Jeffcoate (1992). This analysis provides a variability index between sequential samples and permits fluctuations in hormone concentrations within a sampling window to be measured and compared. Hormone and clinical data were tested by analysis of variance with repeated measures design (Animal Designs 1, V 1.21 5/6, Data International Service, Glasgow) or by the Newman-Keul multiple range test.

Results

Clinical observations

Trypanosomes were identified in the blood of infected sheep within 5–9 days after infection. The first peak of parasitaemia (mean score 3.1 ± 0.4) occurred, on average, 12 days after infection and infected rams remained parasitaemic

throughout the experiment. Infected rams developed a fluctuating pyrexia (rectal temperature range $39.5 \pm 0.1^\circ\text{C}$ to $40.2 \pm 0.2^\circ\text{C}$) from day 13 onwards. These temperatures were significantly higher ($P < 0.05$ to $P < 0.001$) than those measured in control rams (range $38.9 \pm 0.2^\circ\text{C}$ to $39.4 \pm 0.2^\circ\text{C}$). The PCV values of the infected rams declined rapidly from a preinfection mean value of $32.1 \pm 0.9\%$ to $29.2 \pm 0.7\%$ on day 12, which was significantly lower ($P < 0.05$) than the mean value in control rams ($31.9 \pm 0.5\%$). PCV values continued to decline in infected rams and were significantly lower ($P < 0.001$) than in control rams until day 58 when the study ended. Livemass gain was reduced to 83 g day^{-1} in infected rams compared to 173 g day^{-1} in the control rams over the study period. All infected rams survived up to the end of the study period.

LH concentration

A pulsatile pattern of LH secretion was observed in control rams on each sampling occasion and in infected rams on days –5 and 23, but significantly reduced pulsatile activity was seen in infected rams on day 52 (Fig. 1). LH pulse frequency and amplitude did not change over the experimental period in control rams and were not significantly different in infected rams on days –5 and 23 (Table 1). However the mean plasma LH concentration and area under the LH curve were higher in infected rams 23 days after infection ($P < 0.05$). On day 52 after infection, a lower mean LH concentration was observed in infected rams compared with controls on day 52 ($P < 0.01$) and infected rams on day 23 ($P < 0.05$). LH pulse amplitude was too low at day 52 to permit calculation of amplitude or frequency.

Testosterone concentration

At least one surge in plasma testosterone concentration was observed in each sampling sequence in the control rams and in the infected rams on day –5 (Fig. 1). The mean testosterone concentration over 6 h in the infected rams had decreased on days 23 and 52 ($P < 0.001$) (Table 1). A surge in plasma testosterone was seen in one infected ram on day 52 but concentrations remained at basal values in the other four rams.

LH and testosterone concentration after GnRH injection

GnRH injection evoked significant increases in plasma LH and testosterone in both control and infected rams and at each stage of the experiment (Fig. 2). There was no significant effect of infection or stage of infection on the LH response, as shown by analysis of the area under the GnRH response curves (Table 2). In all cases, plasma LH concentration reached peak values within 1 h of injection. Plasma testosterone concentration was quite variable within each group before GnRH injection but the mean concentration invariably increased 10–20 min afterwards, although this increase was not statistically significant until 20–40 min. The sampling period of 2 h after GnRH injection was not long enough to monitor the entire testosterone response, as an upward trend was still apparent in most cases in

Table 1. Changes in plasma LH and testosterone (group mean $\pm$ SEM) in samples collected at intervals of 15 min for 6 h in uninfected control rams and rams infected with *Trypanosoma congolense* on day 5 before infection (day -5) and days 23 and 52 after infection

Parameter	Day -5		Day 23		Day 52	
	Control (n = 5)	Infected (n = 5)	Control (n = 5)	Infected (n = 5)	Control (n = 5)	Infected (n = 5)
LH for 6 h						
Pulse amplitudes (ng ml ⁻¹)	2.2 $\pm$ 0.3 ^a	2.3 $\pm$ 0.4 ^{ab}	2.5 $\pm$ 0.4 ^{ab}	3.4 $\pm$ 0.3 ^b	2.1 $\pm$ 0.2 ^a	NC
Pulse frequencies	4.8 $\pm$ 0.4 ^a	4.6 $\pm$ 0.2 ^a	5.0 $\pm$ 0.4 ^a	4.2 $\pm$ 0.2 ^a	4.8 $\pm$ 0.2 ^a	NC
Mean (ng ml ⁻¹)	2.1 $\pm$ 0.2 ^a	2.1 $\pm$ 0.2 ^a	2.2 $\pm$ 0.3 ^a	3.2 $\pm$ 0.2 ^b	1.8 $\pm$ 0.2 ^a	1.2 $\pm$ 0.2 ^c
Area under response curve (ng ml ⁻¹ in 6 h)	11.8 $\pm$ 0.8 ^a	11.6 $\pm$ 1.1 ^a	12.6 $\pm$ 1.9 ^a	17.4 $\pm$ 1.0 ^b	10.0 $\pm$ 0.2 ^a	6.6 $\pm$ 0.8 ^c
Variability index	5.1 $\pm$ 0.7 ^a	5.5 $\pm$ 0.7 ^a	5.8 $\pm$ 0.8 ^a	4.6 $\pm$ 1.0 ^a	4.4 $\pm$ 0.1 ^a	2.8 $\pm$ 0.5 ^b
Testosterone for 6 h						
Mean (nmol l ⁻¹)	6.5 $\pm$ 1.1 ^a	7.5 $\pm$ 1.4 ^a	8.2 $\pm$ 1.8 ^a	3.6 $\pm$ 0.4 ^b	6.7 $\pm$ 0.6 ^a	1.7 $\pm$ 0.3 ^c
Area under response curve (nmol l ⁻¹ in 6 h)	37.4 $\pm$ 6.3 ^a	42.9 $\pm$ 7.8 ^a	49.3 $\pm$ 10.6 ^a	20.9 $\pm$ 2.2 ^b	30.8 $\pm$ 3.7 ^a	10.2 $\pm$ 2.2 ^c
Variability index	1.0 $\pm$ 0.2 ^a	1.0 $\pm$ 0.4 ^a	0.9 $\pm$ 0.1 ^{ab}	1.1 $\pm$ 0.2 ^a	1.2 $\pm$ 0.3 ^a	0.7 $\pm$ 0.1 ^b

Significance of difference between group means in the same row are denoted by superscripts ^{ab} $P < 0.05$, ^{bc} $P < 0.01$ and ^{ac} $P < 0.001$.

NC: not calculated owing to absence of pulsatile secretion.

Fig. 1. Pattern of plasma LH (□) and testosterone (●) concentrations in a representative uninfected control ram (a–c) and a *Trypanosoma congolense* infected ram (d–f) in samples collected at 15 min intervals for 6 h, 5 days before infection (a and d) and on days 23 (b and e) and 52 (c and f) after infection.

the last sample (Fig. 2). There was no significant change in the area under the testosterone response curves in the control rams from -5 to 23 and 52 days from infection (Table 2). However, the testosterone response to GnRH injection was affected by trypanosome infection, and mean area under the curve in the infected rams was significantly less in controls on days 23 and 52 ($P < 0.05$, Table 2).

Discussion

The present studies have demonstrated for the first time that alterations in pulsatile secretion of LH and testosterone and in pituitary–testicular responsiveness to exogenous GnRH occur in rams experimentally infected with *T. congolense*. Plasma LH and testosterone concentrations varied in a characteristically

Fig. 2. Mean ($\pm$ SEM) plasma LH and testosterone concentrations for 1 h before and 2 h after injection of GnRH (20 μ g per animal) 5 days before infection (a and d), and 23 (b and e) and 52 days (c and f) after infection in control (■) and *Trypanosoma congolense* infected rams (□).

Table 2. Index of plasma LH and testosterone (mean $\pm$ SEM) secreted during a 2-h period after GnRH injection (20 μ g per animal) to groups of uninfected rams ($n = 5$, control) and rams infected with *Trypanosoma congolense* ($n = 5$, infected) 5 days before infection and 22 and 52 days after infection

Parameter	Day -5		Day 23		Day 52	
	Control	Infected	Control	Infected	Control	Infected
LH: area under curve (ng ml ⁻¹ in 2 h)	44.9 $\pm$ 9.4	41.6 $\pm$ 6.1	39.5 $\pm$ 5.4	43.8 $\pm$ 7.5	41.1 $\pm$ 5.4	31.4 $\pm$ 9.0
Testosterone: area under curve (nmol l ⁻¹ in 2 h)	36.8 $\pm$ 2.5	42.9 $\pm$ 7.4	44.7 $\pm$ 10.3	22.0 $\pm$ 1.5*	37.1 $\pm$ 4.4	22.9 $\pm$ 1.2*

*Significant difference ($P < 0.05$) between control and infected groups.

pulsatile manner in the control rams throughout the experimental period. LH pulse amplitude tended to be higher in infected rams on day 23, resulting in a significantly higher mean LH concentration at this time. In contrast, LH pulses were not detectable by 52 days after infection and, accordingly, mean LH concentration was significantly lower than control values at this time. However, on both occasions, plasma testosterone concentration was lower in infected rams 23 and 52 days after infection as shown by a reduction in pulse amplitudes and, later, an absence of testosterone pulses in some animals. Increased LH secretion 23 days after infection could have been caused by this low plasma testosterone concentration, which would reduce the negative feedback constraints on the pituitary and hypothalamus and increase plasma LH concentrations, as reported previously in intact rams immunized against testosterone (Schanbacher, 1982) or in castrated rams (Lee *et al.*,

1978; Caraty, 1983). However, by 52 days after infection, plasma LH had declined concomitantly with testosterone and this would appear to have been caused by trypanosome infection impairing hypothalamo-pituitary function.

The progressive decline in plasma testosterone after infection indicates that *T. congolense* induced an impairment of testicular steroidogenesis that was rapid in onset and progressive. As testosterone plays an important trophic role in the function of the testis during spermatogenesis, it is likely that the rapid decline in testicular steroidogenesis induced by the infection contributes to the degenerative changes of the spermatozoa which accompany this disease and which were observed in the infected rams (B. H. Mutayoba, unpublished). Primary and secondary spermatocytes and spermatids have been found to degenerate rapidly during trypanosome infection (Anosa and Isoun, 1980; Kaaya and Oduor-Okelo, 1980).

This may be explained by the present study, since spermatogenesis depends on normal testicular androgen secretion (Setchell, 1978; Cameron *et al.*, 1993).

Pituitary responsiveness to GnRH, as measured by the LH response, was not impaired in infected rams at any stage of the experimental period, strongly suggesting that the observed decline in spontaneous LH secretion after infection was not due to impaired pituitary function *per se* but to defects in hypothalamic GnRH secretion or transport. However, testicular responsiveness, as measured by the testosterone response to GnRH-induced LH stimulation, was decreased in infected rams compared with controls. This, and the rapid reduction in plasma testosterone following trypanosome infection, may at least be partly attributable to desensitization of testicular LH receptors as has been observed in rats after *T. b. brucei* infection (Soudan *et al.*, 1992).

Although dynamic changes in GnRH secretion were not investigated in the present study, simultaneous reductions in plasma LH and testosterone concentrations are more likely to occur when there is a reduction in the secretion or release of hypothalamic GnRH (Jeffcoate *et al.*, 1982). The latter is controlled by the activity of neurones present in the hypothalamus and higher brain centres the activity of which, in turn, involves the release of several monoamines, including noradrenergic and dopaminergic agonists and 5-hydroxytryptamine (5HT) (Donoso *et al.*, 1971; Iversen, 1975). Studies with mice chronically infected with *T. b. brucei* (Amole *et al.*, 1989) and rabbits (Stibbs, 1984) voles and mice (Stibbs and Curtis, 1987) chronically infected with *T. b. gambiense* have shown marked disturbances in several monoamine neurotransmitters including dopamine, 5HT and noradrenaline in the midbrain, thalamus and hypothalamus. Such neurochemical changes may alter the hypothalamic GnRH secretory pattern and may also be involved in the behavioural symptoms that frequently accompany trypanosomiasis in humans and animals (Stibbs, 1984). Brain neurotransmitter studies have not been reported in *T. congolense*-infected animals, but changes in brain neurotransmitter concentrations could occur during chronic trypanosome infections as a result of localization of large numbers of the organism in the brain microvasculature causing vasodilatation of brain capillaries and development of pressure on the surrounding brain parenchyma (Mwambu and Losos, 1978). The findings obtained in the present study along with those summarized above (Stibbs, 1984; Stibbs and Curtis, 1987; Amole *et al.*, 1989) indicate that the central control mechanisms regulating GnRH secretion were affected by 52 days after infection.

Other mechanisms that might have been involved in the pituitary and testicular endocrine dysfunctions observed in the present study include trypanosome-induced fever and cytokine activation (Mitchell *et al.*, 1986; Sileghem *et al.*, 1993; Winstanley *et al.*, 1993). Trypanosome-induced pyrexia has been associated with impairment of Leydig cell steroidogenesis (B. M. Mutayoba, unpublished), and several cytokines which are stimulated during trypanosome infection are also known to impair the function of the hypothalamic-pituitary axis (reviewed by Kennedy and Jones, 1991). In addition, proteases originating from trypanosomes have been shown to reduce serum concentrations of testosterone and increase pituitary LH content (Hublart *et al.*, 1990; Huet *et al.*, 1992). One

unresearched effect of such proteases may be the degradation of GnRH in the hypophyseal vasculature, especially since GnRH is known to be particularly susceptible to degradation in plasma (Swift and Crighton, 1979). Furthermore, the hypothalamo-pituitary-adrenal axis is stimulated by *T. congolense* infection, resulting in increased plasma cortisol concentration (Mutayoba and Gombe, 1989; Soudan *et al.*, 1992; Abebe *et al.*, 1993). This may exacerbate hypothalamo-pituitary-testicular dysfunction since increased plasma cortisol concentration has been shown to decrease androgen secretion and Leydig cell sensitivity to gonadotrophins (Charpenet *et al.*, 1981).

In conclusion, the present study has demonstrated two major defects in the hypothalamo-pituitary-gonadal axis as a result of *T. congolense* infection in rams. First, testosterone secretion was reduced within about two weeks of infection, despite adequate plasma LH concentrations, possibly because of reduced Leydig cell sensitivity to LH. Second, there was a longer-term reduction in plasma LH concentration, perhaps associated with impairment of some aspect of endogenous GnRH activity, since exogenous GnRH evoked normal LH secretion. It is postulated that these defects in hypothalamo-pituitary function might have been induced either directly, by trypanosome-borne factors or indirectly, after activation of the host defence system.

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