Effects of fasting and an opioid antagonist on food intake in lambs infected with intestinal parasites

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ABSTRACT

This study was carried out to investigate central nervous system pathways which may be involved in the depression of food intake exhibited by parasitised lambs.

Ten lambs with intake depression (c.20%; $p<0.05$); as a result of chronic infection with 4000 *T.colubriformis* larvae/day together with 10 non-infected animals were used in a factorially designed experiment. The factors were a 24 h fast or no fasting followed by; saline solution (control), or 0.6 mg/kg brotizolam (blocks satiety signals at the ventromedial hypothalamus), or 0.125 mg/kg of naloxone (an opioid antagonist), all in a 2 ml volume given i.v. immediately prior to feeding.

Trickle infection depressed mean daily intake by 20% ($p<0.05$), likewise short term intake was depressed by infection ($p<0.05$). Fasting stimulated both short term and daily intake by approximately 100% ($p<0.001$) and 12% ($p<0.05$) respectively. Naloxone depressed cumulative intake for up to 2 h ($p<0.01$- $p<0.001$) while brotizolam stimulated intake at both 60 and 120 minutes ($p<0.001$).

These results show that opioid signals stimulate food intake in lambs and that fasting will overcome the effects of parasitism on appetite in the short term. A lack of opioid signals may be a factor in parasite induced food intake depression.

Keywords Food intake, naloxone, brotizolam, fasting, *T.colubriformis*, parasite, lambs.

INTRODUCTION

The most significant effect of gastro-intestinal parasitism on the host is a depression in voluntary food intake, the extent of which is dependent on the number of larvae ingested and the helminth species (Steel *et al.*, 1980; Sykes *et al.*, 1988).

The mechanism of this depression remains poorly understood although some authors have implicated elevated blood levels of compounds such as cholecystokinin (Symons, 1985), gastrin and pepsinogen (Fox *et al.*, 1989) as associated factors.

Previously we have shown that lambs chronically infected with the gastro-intestinal parasite *T.colubriformis* had depressed food intake but that this reduced rate of food consumption was temporary/ reversible (Dynes *et al.*, 1990). In that study a centrally acting satiety blocker, brotizolam, temporarily increased food consumption and treatment with an anthelmintic resulted in a permanent recovery in the rate of consumption and in total daily food intake.

These findings indicate a lack of hunger drive in parasitised sheep. Some recent studies (Della-Fera and Baile, 1984) have implicated endogenous opiates as factors which stimulate hunger in sheep. In the present study brotizolam and naloxone, an opioid antagonist, were used to investigate the role of central appetite control and of opiates in particular.

MATERIALS AND METHODS

Twenty female sheep (Coopworth x Dorset Down) which had been weaned at 7 weeks of age (liveweight 19 ± 2kg) were drenched with ivermectin (200ug/kg Ivomec®) and maintained indoors on a pelleted diet. At approximately 12 weeks of age (25 kg liveweight), animals were randomly allocated to parasite infected and to non-infected groups (n=10).

The infected group were dosed orally with 4000 infective *Trichostrongylus colubriformis* larvae per day, administered 3 times per week (9333 larvae per dose). For the first 4 weeks of the trial non-infected animals were pair-fed with infected animals to minimise liveweight difference between groups. In the first 2

weeks a pattern of short term intake was established for each animal by recording intake following a 2 h food deprivation period at 10,20,30,40,50,60,75,90,105,120 min post feeding, then hourly for 8 h and finally 22 h after feeding.

Treatments were applied factorially ($2x2^3$) so that within infected and non-infected groups, each lamb experienced fasting and no fasting in combination with saline (vehicle), brotizolam and naloxone. Fasting consisted of a 24 h period without food, water was available *ad libitum*.

Brotizolam (Boehringer Ingleheim, New Zealand) was administered by slow i.v. injection (2 mg in 2 ml solution) immediately prior to feeding. Naloxone (Sigma) was administered i.v. at a rate of 0.125 mg/kg in a 2 ml 0.9% saline solution 15 min prior to feeding. Treatments were applied on alternate days to minimise any carry-over effects. Short term intake was recorded as outlined earlier.

Statistical analysis was carried out using SAS statistical package to compute ANOVA on mean cumulative intake at 30, 60 and 120 minutes.

RESULTS

Trickle infection of lambs with 4000 *T.colubriformis* larvae/day depressed mean daily food intake by 20% ($P<0.05$) compared with the non-infected group, i.e. a 10% ($P<0.05$) depression in terms of intake per kg metabolic ($W^{0.75}$) liveweight. Likewise short term intake was depressed by infection ($P<0.05$)(Fig. 1).

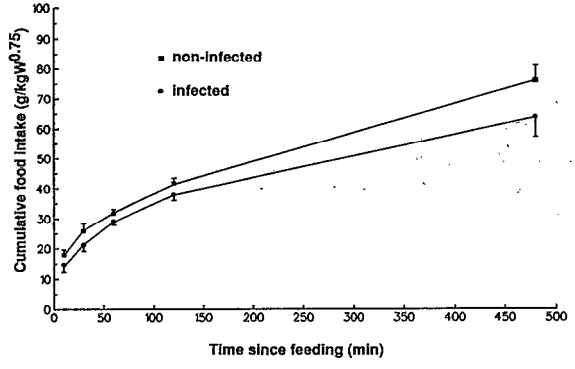

FIG 1 Cumulative food intake ($g/kgW^{0.75}$) for infected and non-infected lambs.

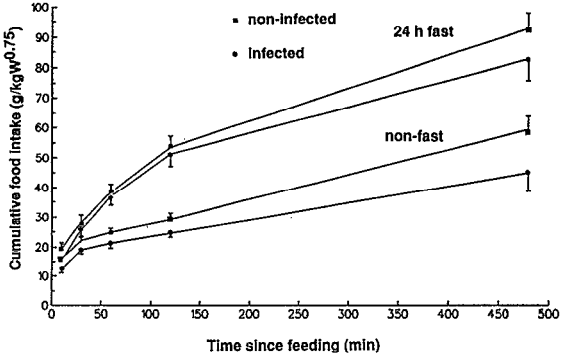

FIG 2 Cumulative food intake ($g/kgW^{0.75}$) for infected and non-infected lambs not fasted or fasted for 24 h.

Fasting increased ($P<0.05$) food intake (Fig. 2) in both infected and non-infected lambs. The effect of fasting on cumulative food intake generally lasted between 30 and 120 minutes (Table 1).

Naloxone treatment depressed short-term food intake for up to 2 h ($P<0.01$-$P<0.001$) compared with saline treatment (Fig. 3) but there was no effect after this initial period. Brotizolam treatment initially stimulated food intake compared with saline treatment (Fig. 3), the increase being significant at 60 and 120 minutes ($P<0.001$), but the effect did not continue beyond 2 h.

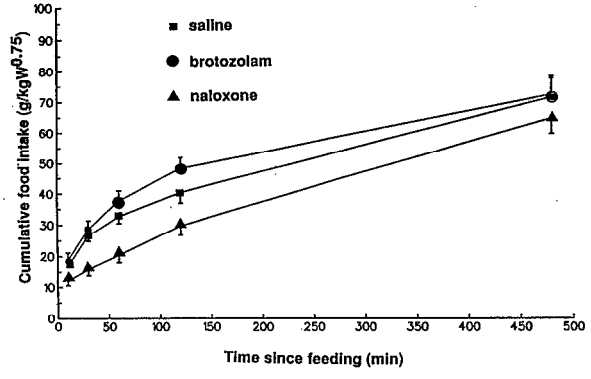

FIG 3 Cumulative food intake ($g/kgW^{0.75}$) for lambs treated with 2 ml saline 2 mg brotizolam or 0.125 mg/kg naloxone.

TABLE 1 Mean (± s.e.m) cumulative food intake (g/kgW$^{0.75}$) of parasite infected and non-infected lambs not fasted or fasted for 24 h and treated with 2 ml saline solution 2 mg brotizolam or 0.125 mg/kg naloxone. Data are pooled for parasite infected and non-infected lambs.

		Time since feeding (min)		
		30	60	120
saline	24 h	32±1.4	42±1.7	52±2.2
	no fast	21±1.5	23±1.7	28±2.3
brotizolam	24 h	31±1.5	46±1.7	65±2.2
	no fast	26±1.4	29±1.7	31±2.3
naloxone	24 h	16±1.6	24±1.7	38±2.2
	no fast	15±1.5	16±1.7	21±2.3

DISCUSSION

These findings demonstrate that fasting will stimulate food intake in lambs even when they have been suffering inappetance due to parasite infection. Fasting was a powerful stimulant to both short term (100% increase in intake by 120 minutes) and daily intake (12 % increase).

Suppression by naloxone of short term food intake of all lambs in the present study supports a role for the involvement of opioids in the regulation of intake which has been suggested by the work of Scallett *et al.* (1985), Baile *et al.* (1981) and Morley *et al.* (1983). Although Baile *et al.* (1981) showed that naloxone depressed food intake of sheep in the 2 h period of their study, the effect lasted only 30 minutes in the present case. This short-duration action may result from naloxone treatment having a short biological half-life or from an overriding effect of the 24 h fast on food intake.

Brotizolam was used principally to demonstrate the short-term responsiveness of both infected and non-infected animals to an established intake stimulus. This showed that the intake depression observed in naloxone-treated lambs was achieved in animals with competent food intake regulatory pathways, which increases the validity of the observation. Brotizolam stimulated intake more in infected than in non-infected animals in our earlier study (Dynes *et al.*, 1990) and in cattle suffering from gastro-intestinal disorders (Fanneau de la Horie and Vaugon, 1986), however in this study the trend was not significant. The lack of effect of brotizolam on 24 h intake was similar to the findings of Fanneau de la Horie and Vaugon (1986) who concluded this was due to the short biological half-life (0.3 h) of the compound. Nevertheless increases in daily feed intake following brotizolam have been reported previously (Baile and McLaughlin 1979; Breier, 1985) but in both of these instances treatment was administered when animals were already satiated whereas in the present case animals had been deprived of food for 2 h at the time of treatment. A reduction of 20% in voluntary food intake in the infected group of lambs is similar to the 16% depression reported by Sykes and Coop (1976) and Steel *et al.* (1980) but quantitatively less than that recorded in our previous study (Dynes *et al.*, 1990). Also the pattern of eating (initiation and cessation of meals) was not markedly altered by parasite infection although the rate of intake expressed as cumulative food intake was significantly depressed. This suggests that the signals for initiating and stopping a meal were similar in both groups but that the drive to eat or hunger was suppressed in the infected lambs. This indicates the necessity to record cumulative food intake for at least 2 h to reveal the level of food intake depression caused by gastro-intestinal parasitism.

In conclusion this study has established that parasite-induced depression of feed intake in lambs can be overcome in the short term by fasting or by a centrally acting stimulant. In addition the results show that fasting may produce its effects on intake by an opioid pathway. It is possible that this pathway is impaired in parasite-infected sheep.

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