SHORT PAPER
The inheritance of non-response to noradrenaline in newborn Scottish Blackface lambs

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Summary

The normal response to injection of noradrenaline (NA) in newborn lambs is an increase in metabolic rate and rectal temperature, due to the stimulation of non-shivering thermogenesis. In a previous study 6 out of 7 lambs born to a sire previously selected for low resistance to cold failed to show this characteristic response and were termed non-responders. The sire, 2 of his male offspring and 6 sires selected randomly from the flock were mated to several ewes and the response to NA stimulation recorded in 116 newborn lambs. Control sires produced only normal responder lambs whereas the remaining sires all produced both responder and non-responder lambs. The proportion of non-responders was not significantly different from 0·50, which is consistent with a dominant major gene. Analysis of the quantitative traits, peak metabolic rate and peak rectal temperature following NA injection confirms that a major gene is segregating in the study population, but is unable to distinguish between dominant and recessive modes of inheritance. Absence of non-responders in other studies suggests that dominance is more likely. This new genetic resource could aid our understanding of brown adipose tissue metabolism and the effect of catecholamines on metabolic pathways.

1. Introduction

Lamb mortality in Britain is a major economic problem. Pre-weaning mortality averages 12–15% nationally and can be as high as 40% on individual farms in some circumstances (Slee, 1981). A high proportion of perinatal deaths is attributable to hypothermia, or starvation, which can be caused by cold exposure inhibiting the suckling drive (Alexander & Williams, 1968). Any genetically determined differences in cold resistance which could be selected upon may reduce lamb mortality. Slee, Griffiths & Samson (1980) developed a technique for measuring the cold resistance of newborn lambs in a water bath. Divergent selection lines were formed using neonatal cold resistance as the selection criterion. After 3 years, significant differences in cold resistance were found between the lines, showing that cold resistance was moderately heritable, with a heritability of around 0·3 (Slee & Stott, 1986). While skin thickness, fleece type and heat production from shivering all contribute towards cold resistance, another major factor in lambs under 2 days old is non-shivering thermogenesis (NST). NST, which comprises about half of the total cold-induced heat production in Scottish Blackface lambs (Slee, Simpson & Wilson, 1987*a*), is normally activated by cold exposure which stimulates the production of endogenous noradrenaline (NA). This in turn stimulates brown adipose tissue (BAT) to produce heat from the oxidation *in situ* of free fatty acids derived from triglyceride (e.g. Alexander, 1979). In a subsequent trial (Slee, Simpson & Woolliams, 1987*b*) exogenous NA was administered, by subcutaneous injection, to newborn lambs from the high and low selection lines. The response to NA was typified by large increases in metabolic rate and rectal temperature, which reached a peak on average 30 min after injection, followed by a decline. Offspring from 11 of the 12 sires in the study (6 from the high line and 5 from the low line) all showed this characteristic pattern, which has been invariably observed in many studies involving hundreds of lambs, at this laboratory and elsewhere. However, 6 of the 7 tested offspring sired by one of the low-line sires, 83S314, showed no response to NA. Metabolic rates and rectal temperature all remained at or very near base levels. This led to speculation that non-response to NA may be governed by a major gene.

The aim of this study was to examine whether non-response to NA was controlled by a major gene and to

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determine the mode of inheritance of the trait more precisely. Such a gene, if naturally present in sheep populations, could have a detrimental effect on viability, as an important component of cold resistance, NST, would presumably be absent in the affected lambs.

2. Materials and Methods

(i) *Animals*

Three Scottish Blackface sires were mated to Scottish Blackface ewes during November 1984. Sire 83S314 had previously been selected for low cold resistance and had produced offspring which did not respond to NA (non-responders). He was mated to 16 ewes from the high-cold-resistance selection line, 16 ewes from the low-cold-resistance selection line, 11 control ewes and the 10 ewes he had been mated to in the previous year. The remaining two sires (84S347 and 84T248) were the only male offspring of sire 83S314 mature enough for mating. One of these (84S347) had been classed as a non-responder at birth and the other (84T248) had not been included in the previous study and had not been tested. Each sire was mated to high-line and low-line ewes. Also included in the study were 6 control sires selected at random from the flock and mated to control ewes and ewes from the high and low selection lines. Lambing took place during April and May 1985.

(ii) *Experimental procedure*

After suckling, newborn lambs were weighed and clipped on a small mid-dorsal patch. They were then placed in a water bath maintained at 38 °C, the approximate thermoneutral temperature for newborn lambs (Slee *et al.* 1980). Lambs were gently restrained in a standing position with the water at neck level. A face mask was used to collect expired air, which was then dried. Metabolic rate (in W/kg) was calculated from the oxygen consumption, estimated as the difference in oxygen concentration in expired and fresh inhaled air (Slee *et al.* 1987*a*). The airflow rate through the system was maintained at 15 l/min. Rectal temperature was recorded using a copper–constantan thermocouple inserted 6 cm and the water-bath temperature was monitored using two additional thermocouples. Data from the thermocouples, oxygen analyser and flowmeter were recorded at 1 min intervals on a Solartron data logger.

Base-line rectal temperature and oxygen consumption (resting metabolic rate) were measured during an initial 15 min equilibration period, after which noradrenaline bitartrate (*Levophed*) was administered subcutaneously in the mid-dorsal region at a dose equivalent to 150 μg/kg, diluted in sterile saline to a solution of 1 mg:1 ml vol. Following injection, changes in oxygen consumption and rectal temperature were recorded. If a metabolic-rate response was elicited the lamb was removed from the water bath when the metabolic rate, having first increased, fell 10% below the peak value. If no response resulted, the lamb was removed from the water bath after 45 min. Time of onset of response was variable but, in a previous study (Slee *et al.* 1987*a*), onset averaged about 10 min after injection and was always less than 30 min using the same dose and route of administration.

Response curves for metabolic rate and rectal temperature either showed a definite steady increase or fluctuated around the base level. Those that fluctuated around the base level never achieved metabolic rates above 10 W or rectal temperatures over 41 °C.

(iii) *Genetic analyses*

The genetic analyses are based on three traits: non-response to NA challenge, which is qualitative, and the two quantitative traits, peak metabolic rate and peak rectal temperature. Because animals were classified as responders and non-responders using arbitrary criteria based on the shapes of the response curves it was decided that the associated quantitative traits should also be analysed. Animals not tested were regarded as having unknown phenotype in the analyses.

Maximum likelihood was used to test several genetic models taking all the family relationships of individuals in the study into account using the package PAP (Hasstedt, 1981). Models fitted include polygenic inheritance, a major gene (dominant, recessive and intermediate with complete or partial penetrance) and a non-genetic model in which non-response is random. A detailed account of the models and model fitting can be found in Cannings, Thompson & Skolnick (1978).

For quantitative traits governed by a major gene, the observations are assumed to be normally distributed within each genotype. The parameters to be estimated are the gene frequency of the allele for non-response (p), the means within each of the three genotypes (μ_1, μ_2, μ_3) and the within-genotype standard deviation (σ). For the qualitative trait of non-response a major gene is modelled using the following parameters: the allele frequency (p) and the penetrance of each genotype (f_1, f_2, f_3), i.e. the probability of expression of the trait given the genotype. Likelihood ratios are used to test the goodness of fit of each model, noting that $-2 \times \log_e$ likelihood difference is asymptotically χ^2 with degrees of freedom equal to the difference in the number of parameters estimated.

3. Results

A total of 116 lambs was NA tested in 1985. Sire 84S347 was already known to be a non-responder

whilst sires 83S314 and 84T347 were of unknown phenotype. Table 1 gives the numbers of responders born in 1985. All the lambs born to control sires gave a clear response to NA. Sires 83S314, 84S347 and 84T248 produced both non-responder and responder offspring, with 0·50, 0·50 and 0·38 of their offspring failing to respond, respectively. These segregation ratios are not significantly different from 0·5, which is consistent with each sire being heterozygous for a dominant gene for non-response and all the ewes being normal homozygotes. The segregation ratio was independent of whether the ewes were controls or from the high- or low-cold-resistance selection lines.

Inspection of the segregation ratios seems to indicate that a major gene is involved but does not provide a sufficiently powerful test to differentiate between different genetic models. Using a maximum-likelihood approach we are able to take the family structure into account. Table 2 gives the maximum-likelihood estimates for the model parameters for the qualitative trait, NR. A dominant model ($f_1 = f_2 < f_3$) and a recessive model ($f_1 < f_2 = f_3$) both fit significantly better than a random model ($f_1 = f_2 = f_3$) ($P < 0·05$) in which there is no genetic transmission of the trait. The dominant model fits the data marginally better than the recessive model. A fully intermediate model ($f_1 \neq f_2 \neq f_3$) does not fit the data significantly better than the dominant and recessive models. No improvement in fit is found by allowing the penetrances to be sex-dependent or depend on the selection line of the ewe. The results from the analysis of the qualitative trait strongly suggest the presence of a major gene segregating in the population but are inconclusive in distinguishing between dominant and recessive models.

Non-responders had a mean rectal temperature of 39·83 °C (S.D. = 0·37) and a mean peak metabolic rate of 6·27 W (S.D. = 1·56), whereas responders had a mean rectal temperature of 41·47 °C (S.D. = 1·56) and a mean peak metabolic rate of 14·49 W (S.D. = 3·75). The means are significantly different ($P < 0·001$) and the traits are positively correlated ($r = 0·44$, $P < 0·001$) after accounting for response and non-response. Maximum-likelihood estimates of the model parameters for the two quantitative traits, peak metabolic rate and peak rectal temperature, are given in Table 3. Although a polygenic model was fitted to the data for both traits, the results are not presented in the table because the estimated heritability was zero for both traits and the resulting likelihoods the same as for the random model. If a major gene is segregating in this population these results are as would be expected since there would be greater variation within segregating sibships than between sibships. Dominant ($\mu_1 = \mu_2$) and recessive ($\mu_2 = \mu_3$) models for peak metabolic rate and peak rectal temperature fit much better than the random model ($\mu_1 = \mu_2 = \mu_3$). The intermediate model ($\mu_1 \neq \mu_2 \neq \mu_3$) does not fit significantly better than the dominant or recessive models. Allowing the standard deviations within genotypes to differ

Table 1. *Numbers of responder and non-responder lambs following challenge by exogenous noradrenaline*

	Sire							
	84S314		84S347		84T248		Controls	
Ewe type	NR	R	NR	R	NR	R	NR	R
HR	4	7	5	9	6	7	0	5
LR	13	8[a]	10	6	4	11	0	2
CTL	5	7	—	—	—	—	0	7
	22	22 (0·50)	15	15 (0·50)	10	18 (0·38)	0	14 (0·00)

NR = non-responder, R = responder, HR = high cold resistance, LR = low cold resistance, CTL = control
Values in parentheses denote proportion not responding.
[a] Includes repeat matings of ewes previously mated to 84S314.

Table 2. *Maximum-likelihood estimates of genetic parameters for the trait of non-response to noradrenaline*

Model	P	f_1	f_2	f_3	$-2 \log_e L + const$
Random	—	0·41	0·41	0·41	58·41
Recessive	0·53	0·87	0·00	0·00	51·72
Dominant	0·05	0·88	0·88	0·00	50·47
Intermediate	0·05	1·00	0·88	0·00	50·45

Table 3. *Maximum-likelihood estimates of the genetic parameters of the traits peak metabolic rate and peak rectal temperature*

Model	P	μ_1	μ_2	μ_3	σ	$-2 \log_e L + \mathrm{const}$
Peak metabolic-rate						
Random	—	10·95	10·95	10·95	4·56	53·70
Dominant	0·06	6·50	6·50	14·58	2·19	22·35
Recessive	0·51	6·54	14·61	14·61	2·19	22·47
Intermediate	0·06	6·38	6·51	14·58	2·19	22·35
Peak rectal temperature						
Random	—	40·86	40·86	40·86	1·00	77·24
Dominant	0·06	39·94	39·94	41·68	0·50	46·49
Recessive	0·53	39·97	41·71	41·71	0·50	46·76
Intermediate	0·06	39·83	39·94	41·68	0·50	46·47

significantly improves the fit of the model, but it does not help in discriminating between hypotheses.

The results give an overall estimate of gene frequency of about 0·05 for the dominant hypothesis and about 0·50 for the recessive hypothesis. Using the penetrances in Table 2 this gives expected incidences of non-response in the flock of 0·09 and 0·22 respectively, for the two hypotheses under random mating. Previous studies have used NA as a challenge for non-shivering thermogenesis by infusion and subcutaneous injection in over 100 lambs (e.g. Slee *et al.* 1987*a*) yet the only non-responder lambs found have descended from sire 83S314, indicating a very low incidence and on the basis of this we find the dominant hypothesis more tenable.

4. Discussion

A major gene has been found which prevents the normal thermogenic response to NA challenge in newborn lambs. From current data we are unable to distinguish conclusively between dominant and recessive modes of inheritance but a dominant mode of inheritance is considered more likely due to the lack of observed non-responders outside this study. The genetic defect which causes non-response to NA is unknown but currently under study. Dissections have shown that BAT is present in non-responder lambs but the error could be in BAT metabolism, the NA receptors or elsewhere in the BAT stimulatory pathway.

The widespread incidence of a recessive gene preventing the normal BAT-mediated heat production in response to cold exposure would have a major effect on lamb survival. The non-shivering component of heat production comprises between 30 % and 60 % of the total heat production in newborn lambs (Alexander & Williams, 1968; Slee *et al*, 1987*a*). During the first few hours after birth, while the coat is still drying, the lamb is vulnerable to cold and NST is often necessary to ensure survival. On the other hand the defect may be the consequence of a single, recent

mutant gene that has been artificially maintained in this population as a consequence of selection for low cold resistance (Slee *et al* 1987*b*). The implications for the farming industry then become less important. However, a new genetic resource has been found which could enable us to improve our understanding of BAT metabolism and the action of catecholamines in sheep. This is important, for example, in the light of growing interest in catecholamines as carcass-repartitioning agents and because possible genetic relationships between BAT and subsequent white-fat metabolism in older lambs could have important implications for carcass quality. Furthermore, the discovery of the gene is of particular importance as very few single genes have been found in livestock and until now the only major genes identified in sheep have been genes controlling coat colour, presence of horns, dwarfism and prolificacy (e.g. the Booroola gene).

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