Neuromuscular spindles in bovine foetuses with hereditary muscle fiber hyperplasia

H.J. Swatland

Department of Animal and Poultry Science, University of Guelph, Guelph (Ontario, Canada N1G 2W1), 20 October 1981

Summary. In cattle with genetic hyperplasia of skeletal muscle fibers, foetuses have an increased number of intrafusal muscle fibers in their neuromuscular spindles.

Neuromuscular spindles are sensory organs which are involved in the regulation of muscle length and movement[1]. The miniature muscle fibers inside neuromuscular spindles are called intrafusal fibers, and normal skeletal muscle fibers are then called extrafusal fibers. Hereditary hyperplasia of extrafusal muscle fibers in cattle is due to a single pair of genes with incomplete dominance[2], and the numbers of extrafusal muscle fibers are approximately doubled in the postcranial skeletal muscles[3]. In adult cattle with hyperplasia of their extrafusal muscle fibers, the functional terminal innervation ratio is increased[4,5]. Prenatally, intrafusal muscle fibers can develop as far as the myotube stage without innervation, but the completion of differentiation is dependent on both the sensory and motor innervation[6]. The preliminary study reported here was undertaken to determine whether the gene for extrafusal fiber hyperplasia also causes an increase in the number of intrafusal muscle fibers.

Methods and materials. A bull which was known from breeding records to be homozygous for the gene causing fiber hyperplasia was bred to 4 homozygous cows so as to produce 4 homozygous foetuses with muscle fiber hyperplasia. One cow was killed, and the other 3 foetuses were removed by Caesarian section. The 4 foetuses were matched by crown-rump length (CR) with 4 normal foetuses obtained from an abattoir. The CR lengths reported here correspond approximately to 115, 125, 135 and 155 days gestation. Foetuses were fixed in 10% formalin by arterial perfusion. A sartorius muscle was removed from each foetus and cut into blocks so that, after paraffin embedding and sectioning (10 µm), transverse sections of the whole muscle could be obtained at positions of ¼, ½ and ¾ the way along the length of the muscle. Sections were stained with Harris's haematoxylin and eosin. Each section was methodically searched so as to identify and measure all its neuromuscular spindles.

Results. In both normal and hyperplastic muscles, the numbers of spindles and the numbers of intrafusal fibers per spindle increased with age. The numbers reported here are not absolute because each muscle was searched in only 3 planes of sectioning. It is possible that some spindles were missed, and it is also possible that some spindles were seen twice, if they extended through 2 planes of sectioning. The minimum diameter of inner spindle capsules was similar in both normal and hyperplastic muscles and it showed no consistent growth during the foetal period examined (mean 17.9 ± 3.9 µm). The same was found for the minimum internal diameter of external spindle capsules (mean 35.9 ± 11.7 µm).

Foetuses with hyperplastic muscles had more intrafusal fibers than normal foetuses (table 1). Both the numbers of spindles and the numbers of intrafusal fibers per spindle were increased, but in a rather irregular manner. Without serially sectioning through the complete length of a spindle it is difficult to distinguish between nuclear bag and nuclear chain fibers in foetal muscle. However, many of the supernumerary intrafusal fibers in hyperplastic muscles were without axial nuclei and had a solid core of myofibrils, as indicated by the ratio of fibers with axial nuclei to fibers without axial nuclei (table 2). Fibers with axial nuclei were larger in diameter than fibers without axial nuclei. Intrafusal fibers of both types in hyperplastic muscles were smaller than those in normal muscles.

Discussion. This preliminary study showed that the bovine gene for hyperplasia of extrafusal fibers does affect the neuromuscular spindles, and that further investigation is justified. The data presented in tables 1 and 2 indicate that the spindles in hyperplastic muscles may differ in their number or composition. The 3 main differences were, 1. variable increases in numbers of spindles, 2. variable increases in numbers of intrafusal fibers per spindle, and 3. a slight decrease in intrafusal fiber diameters. In this preliminary study, statistical analysis of these data was not attempted because the variable responses to the gene were superimposed on already complex curvilinear patterns of growth. However, the very unusual muscle spindles of hypeŕplastic bovine muscles, with up to 12 intrafusal fibers in a spindle, could provide a unique oportunity to study developmental neuromuscular interactions.

Table 1. Numbers of intrafusal fibers and neuromuscular spindles in normal bovine foetuses and in foetuses with hyperplastic muscles

Normal				Hyperplastic			
CR	Fiber number	Spindle number	Fibers per spindle	CR	Fiber number	Spindle number	Fibers per spindle
26	6	2	3.0 ± 0.0	26	7	2	3.5 ± 0.7
29	21	5	4.2 ± 1.3	29.5	61	7	8.6 ± 2.4
33	29	6	4.8 ± 1.8	33	37	7	5.3 ± 3.0
43	36	7	5.1 ± 2.3	42	69	13	5.2 ± 2.3

Foetal age is given by CR length, and means are followed by SD.

Table 2. Minimum diameters (µm) of intrafusal fibers with axial nuclei (AN) and intrafusal fibers without axial nuclei, and the ratio of intrafusal fibers with axial nuclei to those without axial nuclei

Normal				Hyperplastic			
CR	Diameter		Ratio	CR	Diameter		Ratio
	With AN	Without			With AN	Without	
26	5.3 ± 0.6	3.0 ± 1.2	1:1.3	26	4.5 ± 0.6	3.0 ± 1.0	1:0.8
29	7.4 ± 3.5	4.5 ± 1.1	1:3.2	29.5	4.9 ± 1.1	3.9 ± 1.8	1:2.8
33	5.3 ± 1.8	4.7 ± 1.5	1:0.9	33	5.1 ± 1.1	4.4 ± 1.2	1:3.6
43	6.1 ± 1.7	5.9 ± 1.7	1:0.6	42	5.8 ± 1.6	4.3 ± 1.6	1:2.5

Foetal age is given by CR length, and means are followed by SD.

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