The crooked neck dwarf muscular dysgenesis in fowl
is due to a selective alteration of the somitic myogenic cell line

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Summary. Muscular dysgenesis in trunk and limb regions of the crooked neck dwarf (cn/cn) fowl is characterized by a complete disorganization of the muscles, starting at 7.5 days of incubation and resulting, at the end of the incubation period, in a profound muscular atrophy. It has previously been attributed to progressively extending defects of the myotubes. In this paper, embryonic cn/cn head and neck muscles were subjected to histological and ultrastructural analysis. The mononucleated myoblasts of the skeletal muscles are not diseased. Pathology is only expressed in the multinucleated cells, mainly by impaired sarcomerogenesis and distortion of the sarcoplasmic reticulum. In the non-skeletal (cardiac or smooth) muscles, the connective tissue scaffolding and the ultrastructural features are similar to those of normal muscles at the same age. The present report confirms that the cn defect is confined to the skeletal muscle cells. All of them belong to the same lineage, which is contained in the somitic mesoderm, whether the latter becomes segmented or not during embryogenesis.

Key words: Avian hereditary muscular dysgenesis – Skeletal/non-skeletal musculature – Ubiquitous ultrastructural effects

Introduction

Muscular morphogenesis is the result of cellular movements and of interactive events between the myogenic and non-myogenic partners of the muscle tissue. These events have been documented in birds for the trunk and limb skeletal muscles. It has been demonstrated that these muscles are derived from somitic cells which either differentiate in situ or leave the somite and invade the somatopleure. In the former case, the myogenic cells differentiate in association with the somitic metamerized mesodermal cells (Chevallier 1978, 1979; Kenny-Moobs and Thorogood 1986); in the latter, the myogenic cells differentiate in association with non-somatic connective tissue partners (Chevallier 1979; Chevallier et al. 1976, 1977; Christ et al. 1974, 1977). More recently, similar interactions have been reported in the morphogenesis of the head skeletal muscles. Thus, the myocytes of all the head muscles originate either from the unsegmenting somitomeres or from the occipital somites, but their connective tissue partners are of neural crest and not of mesodermal origin (Noden 1983a, b, 1984; Wachtler and Jacob 1986).

Embryonic hereditary diseases are of great help in analysing the mechanisms of development. During the last decade, the study of a muscular disorder in fowl, the crooked neck dwarf (cn/cn) mutation improved our understanding of normal muscle development. During the second moiety of the incubation period, the cn/cn fowl is characterized by a profound muscular weakness that is conspicuously displayed in appendicular regions (Rosenberg 1947; Wick and Allenspach 1978). The earliest recognition of the mutant phenotype, at 7.5 days of incubation, resides in histological changes in the patterned muscles of the lower leg. These early changes are represented by a partial coalescence of several flexor muscles (Kieny et al. 1983). At that very moment, alterations of the distribution of extracellular matrix components as well as faults in the myotendinous junctions are observed (Mauger et al. 1984); they are accompanied by ultrastructural changes, which are not an all or none phenomenon, but expressed only in a small proportion of myotubes (Kieny et al. 1987). As development proceeds, and fusion involves flexor as well as extensor muscles, these abnormalities become more prominent and more frequent; they gradually affect the whole population of myotubes in the completely disorganized muscle tissue. These abnormalities comprise myofilament and myofibril disarrays, dilated sarcoplasmic vesicles, blebbing of the outer nuclear membrane, nuclear encirclements and inclusions, swollen mitochondria, retraction clots, myofibril conglomeration and finally, at 20 days of incubation, degeneration of all muscle cells. Our recent fine structure study was concerned with several trunk and appendicular skeletal muscles; for all of them the myogenic partners originate from cells that leave the somites. The results showed that the cn/cn pathogenesis is due to concomitantly expressed failures of the contractile system and the sarcotubular system (Kieny et al. 1987). Moreover, studies based on heterogenetic somite-exchange experiments have unequivocally demonstrated that the cn/cn muscular dysgenesis results from a defect of the premyogenic cells originating from the somites (Mauger et al. 1983; Kieny et al. 1986). The question focussed on here is whether the cn/cn myopathy is restricted to the non-cephalic skeletal muscles that are formed by the mobile cells of the segmenting somitic mesoderm, or whether it is an absolute defect that is expressed by any myogenic cell of somitic (unsegmented and metameric) origin. In the latter case, unlike mononucleated

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Materials and methods

Eggs from a cross of parents heterozygous for the *cn* gene were obtained from the mutant fowl stock maintained by P.F. Goetinck at the Department of Animal Genetics, University of Connecticut, Storrs, USA.

Comparative ultrastructural observations were performed on several muscle tissues taken from 13-day embryonic normal (1 embryo) and mutant (3 embryos) siblings, according to the procedure described elsewhere (Kieny et al. 1987). Concerning the head skeletal musculature, the jaw-closing muscles and the tongue muscles were studied. Striated non-skeletal musculature was analysed in the ventricular conotruncal wall. Muscular stomach (gizzard) and brachiocephalic arteries, at the level of the arcus aortae region, were studied for their smooth musculature.

Moreover, the comparative ultrastructural analyses of the cephalic muscles were sustained by a histological study of the head and neck musculature, at incremental stages of development from day 9 through day 15 of incubation. Complete heads with the occipital vertebrae were fixed, paraffin-embedded, sectioned in transverse, sagittal or parasagittal planes and stained with Mallory's triple stain. As the mutant embryo is not externally recognizable before the 12th day of incubation, an *a posteriori* identification was implemented through the histological study of the corresponding lower leg.

Results

Histological analysis of head and neck musculature

Before continuing with the ultrastructural analysis, it was checked whether the neck (Fig. 1a–d) and particularly the head muscles (Fig. 1e–j) were affected in 9- to 15-day *crooked neck dwarf* embryos. Muscular disorganization was discernible from the youngest stages onward. It was characterized by a progressive vanishing of the border of the discrete muscles and by a blurring and subsequent disappearance of the tendons. As muscles were losing their external connective tissue envelope and becoming coalescent owing to a reduction of the intermuscular spaces (Fig. 1h, j), the volume of their bulk shrunk (Fig. 1f) and the remaining scattered and loose myotubes became wavy. Thus the rupture of the muscular architecture occurred according to the same processes as those described in the leg (Kieny et al. 1983) and wing (Mauger et al. 1983) zeugopods.

Concomitantly, skeletal malformations, mainly fusions, were observed from day 12 of incubation onward. They were particularly obvious at day 15. Besides a notable reduction in size (Fig. 1b, d), the skeletal elements of the vertebral column (Fig. 1d) and tongue (Fig. 1f, h) were more or less fused. In the latter, the cartilages of the distal part (entoglossum, basihyal and basibranchial) fused with the branches of the hyoid bone (Fig. 1h).

Ultrastructural analysis

Three types of muscle were investigated at the ultrastructural level: (1) cephalic syncytial striated muscles, where sarcomerogenesis occurs in multinucleated myocytes; (2) striated cardiac muscle, where sarcomerogenesis takes place in nonfusing mononucleated myoblasts; (3) smooth muscles, constituted by mononucleated cells, in which no sarcomerogenesis occurs.

Two kinds of cephalic muscles were investigated, the connective tissue cells of which are, in both cases, of neural crest origin (Noden 1983): the multifasciculated mandibular adductor muscle and the muscles from the mobile part of the tongue. The myogenic cells of the jaw-closing muscles derive from the caudal unmetamerized somites, whereas those of the tongue muscles derive from the rostral somites 2 to 5 (Noden 1984).

Syncytial striated muscles. The same kind of aberrations were observed in *cn/cn* jaw-closing and tongue muscles; therefore the ultrastructural observations of these two muscles were pooled. A striking feature of the dysgenic muscle tissues was the variability of the myotube size (Fig. 2a). As a whole, the ultrastructural abnormalities were the same as those which have been described elsewhere in breast and limb muscles (Kieny et al. 1987). They can be summarized as follows. At 13 days of incubation, the myocyte population was constituted by mononucleated myoblasts and multinucleated myotubes. The normal and mutant myoblasts showed no significant differences, but lesions were present in the mutant myotubes. Myoblasts were numerous and were observed free as well as in apposition with normal-looking or pathological myotubes. In comparison with the well-structured contractile apparatus found in normal myotubes, that of the mutant myotubes was aberrant (Fig. 2). In longitudinal sections of normal muscle cells, myofibrils...