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A light microscopic study of denervation atrophy in serial sections[1]

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Summary. Type I and type II muscle fibers from rat extensor digitorum longus muscle were sequentially followed in serial sections after various time intervals following denervation. The type II fibers exhibited the greatest degree of atrophy as well as showing a systematic diameter variability along its length.

In studies of experimental denervation and in various neuromuscular disorders, such as the spinal muscular atrophies, many authors have reported regional differences in fiber sizes, both in the same cross-section as well as in the longitudinal axis of the muscle[2,3]. Such regional differences may lead to problems in sampling error. A basic premise underlying these studies has been that of assuming both diameter and structural homogeneity throughout the length of a given muscle fiber. It has been recently shown that systematic diameter and morphological variability may occur along the length of certain fiber populations in normal and dystrophic extraocular muscle[4,5] as well as in dystrophic peripheral muscle[6]. In these studies, fibers were followed and their identities were retained in serial sections. In the present investigation, a serial sectioning method[4] was used to analyze by light microscopy rat extensor digitorum longus (EDL) muscle which had been denervated for various time intervals in order to sequentially follow the course of atrophy in type I and type II muscle fibers.

Materials and methods. 20 male Wistar rats with an average b.wt of 200 g were used in this study. The right hind limb of 15 rats were surgically denervated under ether anesthesia by excision of a 0.5 cm segment of the sciatic nerve just proximal to the bifurcation of the tibial and peroneal nerves. The cut end of the proximal portion of the nerve was capped with Silastic tubing to prevent subsequent reinnervation. At 14, 21, and 28 days following surgery, a group of 5 rats was sacrificed and the EDL muscles were fixed in situ by dripping 4% glutaraldehyde onto the muscles for 10–15 min. The muscles were then removed whole, fixed overnight in 4% glutaraldehyde and processed by standard electron microscope methods and embedded whole in Epon 812. 5 nonoperated animals served as controls and were sacrificed at the beginning of the experiment. The experimental and control muscles were transversely sectioned at 15 µm by steel knife on a sliding microtome. Such sections were viewed by phase contrast microscopy, the osmium postfixation providing adequate stain to differentiate type I and type II muscle fibers[7]. By phase contrast, the type I fibers stain more intense than the type II fibers. The type I fibers also exhibit larger and more abundant mitochondria, evidenced as granulation, than the type II muscle fibers. From each muscle, several fascicles containing at least 100 muscle fibers were followed throughout their length by the taking of frequent photomicrographs at closely spaced intervals. The diameters of the muscle fibers (both type I and type II) were measured in the muscle's proximal, middle, and distal thirds, according to Brooke and Engel[8]. The fiber diameters were expressed as means ± SD. The significance of the differences between the various regions within each group and time interval was determined by Student's t-test. All p-values greater than 0.01 were considered to signal nonsignificance.

Results. The results of this study are summarized in the table. It is apparent from the table that there is no significant difference for the control muscles in the mean fiber diameters of type I and type II fibers in the various regions examined. 14 days following denervation, both type I and type II fibers exhibited diameter decreases of approximately 25% ($p < 0.001$) and 42% ($p < 0.001$), respectively, as compared to the control. The mean diameters of both fiber populations were not significantly different in the 3 regions examined. After 21 and 28 days post-denervation, the type II muscle fibers exhibited extensive atrophy (approximately 70%) and significant diameter variability (table). These fibers were largest in their middle third and highly atrophic both proximally ($p < 0.001$) and distally

Means and SD (in µm) of diameters of type I and type II muscle fibers from control, 14, 21, and 28 days post-denervated rat extensor digitorum longus muscles. Measurements were made in cross-sections from the proximal, middle, and distal thirds of the muscle's length

	No. of rats	Proximal	Middle	Distal
Control	5			
Type I		27.6 ± 1.5	28.4 ± 2.7	27.8 ± 2.4
Type II		37.2 ± 5.4	36.2 ± 5.2	36.4 ± 6.1
14 days post-denervation	5			
Type I		21.0 ± 2.5	21.1 ± 3.0	19.9 ± 2.0
Type II		20.1 ± 3.3	20.4 ± 2.8	20.2 ± 3.2
21 days post-denervation	5			
Type I		21.8 ± 2.6	21.5 ± 2.3	22.9 ± 3.9
Type II		10.3 ± 3.5	13.1 ± 2.6	9.9 ± 2.9
28 days post-denervation	5			
Type I		25.6 ± 3.1	22.4 ± 4.1	22.8 ± 3.5
Type II		9.6 ± 3.6	16.0 ± 3.0	9.8 ± 3.1

($p < 0.001$) in the muscle. We have termed this diameter variability exhibited by the type II fibers to be a 'centripetal neurogenic atrophy'. The type I fibers did not show any further atrophy, but did exhibit an increase in diameter distally after 28 days post-denervation. Fiber splitting was not observed in any of the muscle fiber populations sampled and therefore could not account for the atrophy observed.

Discussion. Both human and animal muscle can be broadly subdivided into 2 fiber types, type I fibers (red muscle fibers) and type II fibers (white muscle fibers). Morphologically, the type I fibers have more mitochondria and lipids than the type II fibers which are rich in glycogen and have more sarcoplasmic reticulum than the type I muscle fibers[9]. Physiologically, the type I muscle fibers have slow-twitch properties, whereas the type II muscle fibers have fast-twitch properties. There is a growing debate in the literature regarding which muscle fiber population atrophies following denervation. The present study is in agreement with those investigators which have described a preferential type II atrophy[3,10]. This implies that both fiber populations atrophy, however, the type II cells atrophy to a much greater extent than the type I fibers. The apparent susceptibility of the type II fibers to denervation atrophy suggest that they are in greater need of neural control than the type I fibers. The observed centripetal neurogenic atrophy of the type II fibers was completely unexpected and might possibly be due to several causes. It has been reported that 3-4 days following denervation, there is an increased synthesis of acetylcholine (ACh) receptors along the entire sarcolemma of the muscle fiber; this results in a concurrent sensitivity to ACh[11]. It has been shown that this chemosensitivity to ACh spreads from the endplate region towards the tendon ends[12]. This sensitivity appears to peak in about 2 weeks and after approximately 3 weeks declines in a centripetal direction, resulting in only the endplate region exhibiting any ACh sensitivity[13]. It is of interest to note in this regard, that it is at 3 weeks following denervation that we observed a centripetal neurogenic atrophy of the type II fibers. Another possible explanation for the observed systematic diameter variability of the type II fibers is that these cells might exhibit a differential stretch effect, whereby their proximal and distal thirds are under greater stretch than their middle third. It would appear that fiber size varies according to the area selected for biopsy and measurement. This variability of fiber size along the longitudinal axis of the muscle can cause considerable problems in interpretation of clinical severity and prognosis.

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Neuronal cell deficits following maternal exposure to methadone in rats[1]

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Summary. Rat offspring were maternally subjected to methadone hydrochloride during gestation or lactation. At 21 days of age, the area of the pyramis (cerebellar lobule VIII) in prenatally and postnatally exposed groups was reduced 45% and 36%, respectively, from control levels, and the total number of internal granule neurons/section was reduced 49% and 46%, respectively; the number of granule neurons/mm^2 in both groups was 75% of control values. Based on histological evaluation the timetable of cerebellar morphogenesis was unaltered in rats prenatally exposed to methadone, suggesting a permanent neuronal deficit, but cerebellar development was markedly delayed in animals subjected postnatally.

Methadone, a narcotic analgesic, is widely used in detoxification programs for narcotic-addicted pregnant women[2]. This synthetic opioid crosses the placenta and enters the fetal circulation of humans[3] and laboratory animals[4], and has been detected in the milk of lactating humans on methadone maintenance[3]. Clinical reports suggest that infants maternally subjected to narcotics are at risk for neurodevelopmental dysfunction[5,6], but the mechanisms underlying these problems have not been defined. The developing nervous system of laboratory animals also appears particularly sensitive to opiates, and a variety of physiological, biochemical, and behavioral abnormalities have been reported in drug-exposed offspring[7-12]. In an earlier study[7], conducted in rats at postnatal day 21 when cerebellar neurogenesis is normally completed, animals maternally subjected to methadone during either gestation or lactation had cerebella that weighed 25% less than that of controls and contained 11-18% fewer cells. In a morphological investigation, we now demonstrate a loss of cerebellar neurons in 21-day-old rats perinatally exposed to methadone. Moreover, we have found that the sequence of neuro-ontogenic events responsible for these neuronal deficits is dependent on the timing of drug exposure; the temporal course of cerebellar morphogenesis proceeds unaltered in rats prenatally subjected to methadone, but is markedly delayed in animals postnatally-treated with this synthetic opiate.

Female Sprague-Dawley rats (180-200 g) were housed