A comparative pharmacologic-histologic study of slow and twitch fibers in the superior rectus muscle of the rabbit

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The superior rectus muscle of the rabbit can be separated into two plates: One is comprised solely of Felderstruktur ("slow") fibers and the other almost exclusively of Fibrillenstruktur ("twitch") fibers. Responses of isolated portions of the two muscle types to acetylcholine were compared under identical experimental conditions. The results indicate that the Felderstruktur fibers are responsible for tonic contractions of the extraocular muscles of the rabbit. It is reasonable to assume that they serve the same function in the extraocular muscles of other mammals. Felderstruktur fibers, although striated, demonstrate certain histologic and pharmacologic characteristics, which are intermediate between those of striated and smooth muscle.

Two distinct varieties of striated muscle fibers have been demonstrated morphologically and pharmacologically in amphibians and birds, such as frog and chick.1-7 The Felderstruktur or "slow" fibers are distinguished by large, irregular, and poorly defined fibrils and "en grappe" nerve endings. These fibers respond by a tonic contraction to either repetitive electric nerve stimulation or exposure to acetylcholine; they do not contract to a single neural stimulation. The Fibrillenstruktur or "twitch" fibers, on the other hand, demonstrate histologically a regular and distinct pattern of fibrils and only one nerve ending of the "en plaque" type.8 They react by a twitch to a single electric nerve stimulation, but do not maintain tension to acetylcholine; a repetitive neural stimulation induces a tetanic contraction.

Both types of fibers have also been found in various muscles of several mammalian species.5,10 Recently, they have been demonstrated morphologically in the extraocular muscles of the guinea pig, rat, cat, dog, monkey, and man;11-16 and both morphologically and pharmacologically in the superior oblique muscle of the cat.16 Dietert17 identified the presence of the two fiber types in the human extraocular muscles by electron microscopy. In accompanying light microscopic studies he suggested that the terminals of the "en plaque" type usually occur singly; however, two "en plaque" nerve endings per fiber could occasionally be seen.

The purpose of the present study was to ascertain the role of the tonic function of the "slow" fibers in mammalian extraocular muscles. A systematic examination of their morphology revealed that the superior rectus muscle of the rabbit is uniquely suited for a comparison of the pharmacologic responses of both fiber types: In this

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muscle the "slow" and "twitch" fibers are separated from each other into two layers within the muscle plate. It is planned to extend the pharmacologic studies to electrophysiologic examinations.

Material and methods

Albino rabbits (1.2 to 1.6 kilograms) were used in all of the experiments. For in vitro pharmacologic studies the superior rectus muscle was carefully prepared in situ, under the dissecting microscope, to obtain strips of muscle fibers exclusively of the Felderstruktur and predominantly of the Fibrillenstruktur type. With the animal under general anesthesia (intravenous pentobarbital sodium), the muscle was freed from the surrounding tissue. The exposure was facilitated by the extracapsular extraction of the lens, which moderately reduced the volume of the globe. Loss of vitreous had to be avoided, as the resulting infolding of the sclera made the preparation of the two muscle layers more difficult. Narrow marginal strips were cut away with a razor blade from both sides along the entire length of the muscle. This procedure was necessary because of the topographic relation of the two plates of histologically different fibers. Brief additional ether inhalation was found useful to produce a transient hyperemia in the vascularized connective tissue which separates the upper from the deeper muscle leaf. About 10 mm. proximal to the insertions had to be discarded. The spatula, carefully maintained in the same plane, was then moved toward the apex of the orbit. The separated muscle leaves were secured by No. 6-0 silk sutures at their distal and proximal ends at identical distances from the point of insertion. The strips were then cut free and transferred to separate muscle baths for simultaneous recording in the pharmacologic assay. The animals were killed by an overdose of sodium pentobarbital.

The muscle baths were filled with 10 ml. of a modified Krebs-Ringer solution, continuously bubbled with a mixture of 95 per cent O₂ and 5 per cent CO₂ and maintained at a constant temperature of 36.0° C. ± .5° C. To sensitize the muscles 1 mg per milliliter eserine was added to the baths. A Sanborn force displacement transducer, Model FTA-1-1, was used to measure the quasi-isometric contractions induced by the added drugs. The transducer was connected to a Sanborn carrier preamplifier, Model 150-1100 AS, and the responses recorded on an Esterline-Angus milliammeter. The sensitivity of the recording system allowed the measurements to a minimum of ±3 mg. As the reactions of the two muscle preparations had to be compared, subsequent muscle preparations were placed alternately in one or the other muscle bath to minimize the interference of mechanical differences of the selected system. The dissected muscle strips were placed initially under a tension of 100 mg. They were stimulated with acetylcholine, the concentration of which was varied from 0.1 to 0.5 mg per milliliter bath solution. At the completion of the experiment the muscles were blotted in a standardized fashion and weighed on a Roller-Smith balance.

The muscle preparations to be used for histologic studies were fixed in situ without stretching by dropping Susa solution on the tissue before dissection and transfer into the fixative. After dehydration the material was embedded in paraffin, and serial cross sections were subjected to various staining procedures (hematoxylin and eosin, iron and hematoxylin, van Gieson, and Bodian silver technique). Divided muscle strips were also fixed in Susa solution and processed similarly to the intact preparations.

Results

Histology. A cross section through the belly of the superior rectus muscle of the rabbit (Fig. 1) shows a superficial thin layer of small muscle fibers (diameter 7 to 26 μ, average 18 μ). They appear to be distributed in a loose connective tissue which is rich in capillaries and reticular fibers. The muscle fibers are of the Felderstruktur type with irregularly clumped fibrils which are not sharply delineated even under high light-microscope magnification (Fig. 2). Midway along the length of the muscle a great number (15 to 20 per cent) of the fibers have centrally or slightly eccentrically placed nuclei, in addition to peripheral nuclei (Fig. 3).

A thin layer of richly vascularized connective tissue (arrow in Fig. 1) separates the upper sheet of Felderstruktur fibers from a deeper, much thicker leaf, comprised of bundles of large muscle fibers (diameter: 27 to 53 μ, average 40 μ) of the

*Modified Krebs-Ringer solution (all values are in moles per liter) NaCl, .11612, KC1, .00465, CaCl₂, .00248, KH₂PO₄, .00152, MgSO₄, .00239, NaHCO₃, .00245, Glucose, .01088.
Fibrillenstruktur type, with regularly separated fibrils (Fig. 4). As a rule their nuclei are located peripherally and only rarely are found in the axial part of the fiber (Fig. 5). Fig. 6 demonstrates the clear distinction between the two fiber types. In the marginal portion of the muscle, however, the superficial layer increases in thickness and partially enwraps the deeper muscle plate.

Therefore, these marginal portions of the muscle were always cut off before the two layers were separated.

Fig. 7 shows the two plates of the muscle after careful separation with the uniform Felderstruktur fibers above and a predominant Fibrillenstruktur pattern below.
Fig. 3. Felderstruktur muscle fibers with a central or slightly eccentric nucleus in addition to peripheral nuclei. (Hematoxylin and eosin. A, ×300, B, ×1,030, C, ×2,400.)
The deeper layer usually also includes a few Felderstruktur fibers. The average area of cross sections of the upper layer was 0.248 sq. mm. and of the lower portion 1.318 sq. mm. (Fig. 8); the average wet weight of 100 dissected muscle pieces amounted to 1.3 mg. for the upper (Felderstruktur) and to 5.5 mg. for the lower (Fibrillenstruktur) layer (Fig. 9). These measurements were made on histologic preparations of muscles which had been suspended in the bath for four hours and repeatedly exposed to acetylcholine; they were not significantly different from similar measurements of cross sections of unexposed muscles. The exposed tissue, how-

Fig. 4. Fibrillenstruktur muscle fiber from lower leaf. Within the fiber bundles the muscle fibers are close to one another. (Hematoxylin and eosin. ×2,400.)

Fig. 5. Fibrillenotyp muscle fiber with slightly eccentric nucleus, a rare finding. (Hematoxylin and eosin. ×1,650.)
Fig. 6. Intermediate layer with adjoining muscle leaves demonstrating the sharp separation of Felder- from Fibrillenstruktur muscle fibers, which do not differ in diameter in the zone of the intermediate layer. (Hematoxylin and eosin. x1,000.)

Fig. 7. Dissected muscle, showing the separation between Felderstruktur fibers in the upper part and Fibrillenstruktur fibers in the lower part. The venules on the inner surface of the Fibrillenstruktur portion are the marks for the advancement of the spatula. A few Felderstruktur fibers remain attached to the border of the Fibrillenstruktur portion of the muscle. (Hematoxylin and eosin. x100.)
ever, histologically showed swelling of the fibers with beginning vacuolization and pyknosis of the nuclei. These degenerative changes, however, did not impair the pharmacologic responses of the muscle strips, even after a bath exposure of 36 hours.

In the other extraocular muscles a topographic separation of Felder- and Fibrillenstruktur fibers was not detected although the same techniques for fixation and staining were used.

**Pharmacology.** Spontaneous "contractions" were observed in 8 of 50 Felderstruktur muscle strips at intervals from one to three per minute. The amplitudes varied between 5 and 30 mg. (Fig. 10). Twitch type contractions were never observed.

When the muscle strips were exposed to a low dose of acetylcholine (0.05 µg per milliliter), those of the Felderstruktur type responded by a nearly immediate "contraction" of a magnitude of approximately 80 mg., and with a duration of more than 6 minutes. In contrast, the majority of the Fibrillenstruktur preparations (38 of 50)

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**Fig. 8A.** Felderstruktur piece of dissected muscle after a bath exposure for approximately four hours. (Hematoxylin and eosin. x50.)

**Fig. 8B.** Fibrillenstruktur piece of dissected muscle after a bath exposure for approximately four hours. (Hematoxylin and eosin. x50.)
Fig. 9. Wet weight of 50 Felder and Fibrillen type preparations, respectively, after exposure to acetylcholine.

Fig. 10. Spontaneous "contractures," found in Felder type preparations. Calibration: 20 mg., 10 seconds. (Recorded on the Sanborn.)

demonstrated only a small rise in tension (amplitude ~ 20 mg.), which developed slowly and was followed by a similar fall (Fig. 11). Approximately one fifth of the Fibrillenstruktur strips (12 of 50) showed no response at all to acetylcholine at this dose level. Increased concentrations of acetylcholine (.1 to 1.0 µg per milliliter) induced faster and higher rises of tension in both Felder- and Fibrillenstruktur preparations. However, the responses of the Felderstruktur strips remained proportionately greater at all drug levels. The tension of the Fibrillenstruktur strips again returned rapidly to the base-line level, whereas that of the Felderstruktur preparations remained elevated for longer than 10 minutes. After the removal of the added drug by washing out the baths, the tensions always returned to the initial levels.

Discussion

The pharmacologic examination of "slow" and "twitch" fiber preparations from the superior rectus muscle of the rabbit was greatly helped by their topographic interrelationship. Fibrillenstruktur fibers comprising the deeper leaf of the muscle have the clear characteristics of striate muscle with well-delineated separated fibrils, a
small amount of endomysium, and, as a rule, peripherally located nuclei. It is further known that these fibers have "en plaque" type of nerve endings. The Felderstruktur fibers of the superficial muscle plate also exhibit cross striation, but in some respects these fibers appear to be intermediate between striated and smooth muscle. Their nuclei are usually located both peripherally and axially, and a continuous network of reticular fibers surrounds each muscle cell. Multiple end plates of "en grappe" form are characteristic for these fibers.

The Felderstruktur preparations responded to acetylcholine with a "contracture" and sustained maintenance of the induced tension. This effect was elicited by a dose as low as .05 μg per milliliter. The Fibrillenstruktur fibers, which comprise approximately four fifths of the bulk of the muscle, were either refractory to this dose of acetylcholine or reacted only transiently and slightly. It is plausible to explain this minimal reactivity by the admixture of "slow" fibers in the Fibrillenstruktur muscle plate. Thus, an acetylcholine-induced "contracture" has to be ascribed entirely to the responses of the "slow" or Felderstruktur fibers.

Hess and Pilar have reported that Felderstruktur fibers of the superior oblique muscle of the cat are physiologically of the "slow" fiber type. By selective block of the "twitch" fibers they were able to demonstrate indirectly that the "slow" fibers are responsible for the acetylcholine-induced contracture of the muscle. In the present study, a direct relation could be made between the morphology and phar-
macologic responses of the two fiber types because of their segregation within the superior rectus muscle of the rabbit.

The concentration of acetylcholine required to elicit a response from the Felderstruktur preparations of the superior rectus muscle is similar to the threshold dose required to elicit a response from the Felderstruktur fibers of the frog, J. Physiol. 121: 318-340, 1953.

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The Felderstruktur fibers in the extraocular muscles of the rabbit and the cat might be considered as responsible for the continuance of the tone in these muscles. It is possible that the tonic function of the "slow" fibers in the cat's and rabbit's extraocular muscles is not limited to these two species, since Felderstruktur fibers have been demonstrated in the extraocular muscles of all mammals so far examined.

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