Histol Histopath (1990) 5: 213-217

Histology and Histopathology

Type IIC fibres in certain muscles of the adult rat (sedentary and exercised)

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Summary. Samples were taken, at fixed levels, of the vastus lateralis, the caput lateralis of the gastrocnemius muscle and the longissimus lumbaris of 72 Wistar rats which were either sedentary or subjected to various exercise schedules. The samples were analyzed using the histochemical technique of myosin ATPase (m-ATPase) after preincubation at pH 4.2, and the fibre-types I, II (IIA and IIB) and IIC were identified, calculating the percentage of type IIC fibres as well as their minimum diameter.

The percentages of these IIC fibres found in the red and mixed parts of the gastrocnemius (caput lateralis) and the longissimus lumbaris were between 0.7% and 2.6%. However, their presence was not detected in the vastus lateralis or in the white part of the gastrocnemius (caput lateralis). The lack of differences in this fibre type between the males and females of the population was shown statistically. Likewise, no significant modification of the IIC fibres between sedentary and exercised animals was seen. With regard to fibrillar size, females showed a smaller minimum diameter than males, the results showing a small increase in the size of these fibres in both sexes after exercise, although in most cases this was not statistically significant.

Key words: Muscle fibre types, Histochemistry, Exercise

Introduction

Brooke and Kaiser (1970), using preincubation in an acid medium with the myosin ATPase technique, classified human and rat muscle cells as fibre types I, IIA, IIB and IIC. Staron and Pette (1986) considered that, with this technique, type IIC fibres, on the basis of the different proportions they have of heavy chains of slow or type I myosin and fast or type II myosin, show a variable intensity of staining, and that this fibrillar population could be further divided into subtypes IC and IIC. Jansson (1975), Jansson and Kaiser (1977), Hintz et al. (1982, 1984), McMiken (1986) and Diz (1987) consider type IIC fibres to have an intermediate capacity for oxidation, falling between type I and type II, in addition to a density of mitochondrial volume slightly greater than that of type I fibres (Hoppeler et al., 1983; Howald et al., 1985).

IIC fibres are proportionately low in adult mammals but common in very young animals, referring here to undifferentiatd cells in the process of development (Essén et al., 1980). As these fibres have intermediate characteristics, in both their contractile proteins and their metabolic profile, lying between fibre types I and II, they may be considered as precursor or transitory fibres which appear or increase proportionately in order to adapt the muscle to new demands (Sréter et al., 1974; Jansson and Kaiser, 1977; Jansson et al., 1978; Müntener, 1982; Engel and Barker, 1986). However, the presence of these fibres also appears to be greater in regenerative processes, so it is also probable that their appearance conforms to a transitory state in the development of new fibres from satellite cells (Hikida et al., 1983; Salminen, 1985). Another tendency in which the presence of these fibres is involved is related to the fibrillar transformations brought about by exercise. With respect to this, it is worth studying the work of Jansson et al. (1978). Here, human volunteers were subjected to relatively prolonged aerobic and anaerobic exercise, and studies showed that after anaerobic exercise there was a low percentage of type I fibres and an increased percentage of type IIC fibres, whereas after aerobic exercise a greater percentage of type I fibres was found, as well as an increase in type IIC fibres. This led them to consider IIC fibres as transitory fibres which become types I or IIA. Studies done by Howald (1982) and Pierobon-

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Bormioli et al. (1981), amongst others, lead to similar conclusions.

Anyway, it continues to be a subject for debate (Howald, 1982; Engel and Barker, 1986; McMiken, 1986) whether type IIC fibres are at a stage of general development between satellite and mature cells, in which case they would be undifferentiated fibres, or in a state of direct transition between two fibrillar types due to external influence.

The following results for these muscular fibres come from an experiment which was not principally concerned with this topic (Morales-López, 1989), but it seems worthwile to make this information available in the hope of helping to clarify what is known about this particular fibre. Thus, the purpose of the present experiments was to study the size and number of type IIC fibres of some muscles in the adult rat both in normal conditions and after training exercises.

Materials and methods

The experiment involved 72 adult Wistar rats, with an average weight of 230 g (females) and 350 g (males), which were killed at 20 weeks. Their diet consisted of standard homogenized feed and water, ad libitum. The animals were divided into seven groups, each comprising six males and six females, except for groups VI and VII of six rats each.

Group I (control) was sedentary, while groups II to VII were subjected to different programmes of exercise running on a treadmill (Letica). The exercise procedures for each group are shown in Table 1.

Once each group has finished its training programme, the rats were killed and samples were taken of the longissimus lumbaris, vastus lateralis of the femoral quadriceps, and the caput lateralis of the gastrocnemius.

The samples were quick-frozen lollowing the technique of Dubowitz and Brooke (1973), and then a series of 10 μm thick sections was made in a cryostat at -20 °C. These sections were stained with myofibrillar adenosinetriphosphatase (m-ATPase) after acid incubation (pH 4.2) (Brooke and Kaiser. 1970), and fibre types I (light), II (IIA and IIB) (clear) and IIC (intermediate) wee identified (Fig. 1). The quantitative study was based on the mixed part of the vastus lateralis and the red and the mixed parts of the gastrocnemius (caput lateralis) (Armstrong and Phelps, 1984), in addition to the entire longissimus lumbaris. Percentages of each fibre type were calculated by counting a minimum of 500 fibres.

The size (minimum diameter) of the type IIC fibres was calculated using a computerized image analyzer, counting all the IIC fibres present in each sample.

The quantitative results are expressed as mean (±SD). Differences among groups were evaluated using the Snedecor F test and Tukey's studentized range test.

Results

Table 2 shows the statistical values for the percentage of IIC fibres present in the control group for each muscle or part of muscle analyzed. The mixed gastrocnemius muscle showed the greatest percentage of this type of fibres, while the vastus lateralis muscle showed no type IIC fibres at all.

Table 3 shows the corresponding values ($\bar{x}$ ± SD) obtained for each experimental group, as well as the results of the variance analysis. As can be seen in this table, no differences in the percentage of type IIC fibres were found among the different groups in any of the muscles or muscle portions studied.

Table 4 shows the mean minimum diameter found for these fibres - for each sex, and muscle or muscle part in the control group, and Table 5 shows the results obtained for the experimental groups. Both of these tables include the results of the variance analysis performed for each sex, muscle or muscle portion. The minimum diameter of type IIC fibres was always higher in male than in female rats. The red gastrocnemius muscle showed the greatest diameter of these fibres. A slight increase in the size of IIC fibres was noted after exercise training.

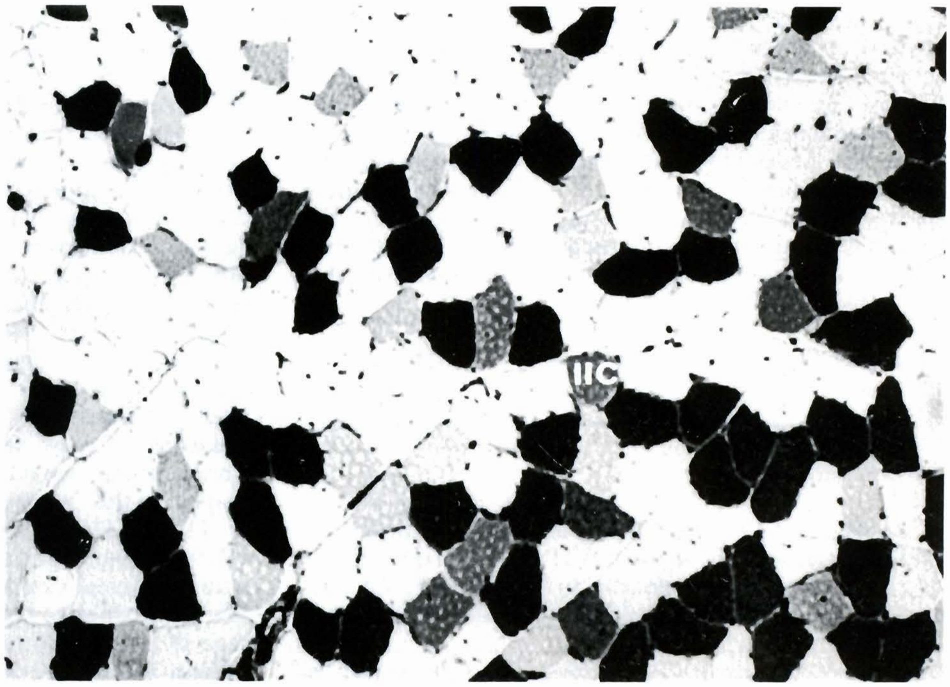

Fig. 1. Micrograph showing fibre type identification in the areas of transition between the red and white portions of the gastrocnemius muscle (caput lateralis). m-ATPase after acid preincubation -pH 4.2. × 100

Table 1. Diagram showing the exercise schedules to which the different groups were submitted.

GROUPS	EXERCISE	SCHEDULE
Group I	CONTROL	
Group II	SPEED	gradual (35-55 m/min), 5 runs/day, 4 weeks
Group III		initial (55m/min), 5 runs/day, 4 weeks
Group IV	ENDURANCE (30 m/min)	2 weeks, 10 min/day
Group V		group IV + 2 weeks, 20 min/day
Group VI		group V + 2 weeks, 30 min/day
Group VII	MIXED	group V + group III

Table 2. Statistics for the percentage of type IIC fibres in each muscle of the control group (group I).

VARIABLES / STATISTICS	TYPE IIC FIBRES			
	GASTROC-RED	GASTROC-MIX.	VASTUS L.	LONGISSIMUS
N	12	12	12	12
MEAN	1.19	2.59	0.00	0.65
STANDARD ERROR	0.24	0.36	-	0.16
STANDARD DEVIATION	0.84	1.25	-	0.54
MINIMUN VALUE	0.39	1.10	-	0.08
MAXIMUN VALUE	3.30	5.85	-	1.73
C.V. 100	70.96	48.10	-	82.82

Table 3. Results ($\overline{x} \pm$ SD) for the percentage of type IIC fibres in each muscle.

VARIABLES / GROUPS	TYPE IIC FIBRES			
	GASTROC-RED	GASTROC-MIX.	VASTUS L.	LONGISSIMUS
Group I	1.2±0.8	2.6±1.3	0.0±0.0	0.7±0.5
Group II	2.0±1.0	1.2±0.9	0.0±0.0	1.1±0.6
Group III	1.4±0.9	1.9±1.1	0.0±0.0	1.0±0.7
Group IV	1.9±1.1	2.4±2.0	0.0±0.0	0.7±0.9
Group V	2.3±1.1	1.6±1.5	0.0±0.0	1.3±0.5
Group VI	1.3±0.4	1.2±0.8	0.0±0.0	0.4±0.3
Group VII	1.4±0.7	1.9±0.9	0.0±0.0	0.2±0.2

Table 4. Results of the variance analysis done to study the differences between muscles, with respect to the minimum diameter of type IIC fibres in males and females of Group I ($\overline{x} \pm$ SD).

VARIABLE / MUSCLES	MINIMUM DIAMETER	
	FEMALES	MALES
Gastrocnemius (red)	39.6 ± 0.8	45.6 ± 2.8
Gastrocnemius (mixed)	33.4 ± 1.4 ^{A}	39.7 ± 3.7 ^{A}
Vastus Lat. (mixed)	–	–
Longissimus Lumbaris	29.0 ± 1.7 ^{AB}	40.7 ± 3.4

The indices A and B show a significant difference ($P < 0.05$) with respect to the red portion of the gastrocnemius and the mixed portion of the same muscle, respectively.

Table 5. Results of the variance analysis done to study the differences between muscles, with respect to the minimum diameter of type IIC fibres in males and females ($\overline{x} \pm$ SD).

VARIABLE / GROUPS	MINIMUN DIAMETER					
	GASTROCNEMIUS (red)		GASTROCNEMIUS (mixed)		LONGISSIMUS LUMBARIS	
	FEMALES	MALES	FEMALES	MALES	FEMALES	MALES
Group I	39.6 ± 0.8	45.6 ± 2.8	33.4 ± 1.4	39.7 ± 3.7	29.0 ± 1.7	40.7 ± 3.4
Group II	47.4 ± 0.9 ^{A}	48.2 ± 4.1	39.2 ± 3.9 ^{A}	46.5 ± 2.4 ^{A}	34.2 ± 1.6 ^{A}	38.3 ± 3.2
Group III	43.2 ± 4.9	50.4 ± 1.4	39.5 ± 3.3 ^{A}	43.9 ± 2.1	33.1 ± 1.3	42.9 ± 1.9
Group IV	42.9 ± 0.9	51.6 ± 3.5	31.2 ± 1.1 ^{A}	46.0 ± 3.1 ^{A}	37.5 ± 3.3 ^{A}	37.7 ± 2.9
Group V	48.2 ± 3.8 ^{AD}	43.4 ± 5.9 ^{D}	40.9 ± 3.3 ^{A}	40.7 ± 1.4 ^{B}	34.5 ± 5.0 ^{A}	37.6 ± 2.7
Group VI	43.9 ± 2.5	48.5 ± 7.4	39.7 ± 0.7 ^{A}	44.2 ± 6.8	35.6 ± 2.8 ^{A}	38.5 ± 1.9
Group VI	44.8 ± 1.2	46.8 ± 1.9	39.2 ± 1.5	41.8 ± 1.7	32.1 ± 1.7	38.5 ± 4.0

The indices A, B and D show significant differences ($P < 0.05$) with respect to groups, I, II and IV, respectively.

Discussion

As can be seen from what was said in the introduction, the basic facts concerning the nature, presence and functional or generic role of type IIC fibres (Broke and Kaiser, 1970) have still not been established. In this way, it is worth pointing out that this type of fibre can be clearly identified in rats by using the technique of m-ATPase after preincubation at pH 4.2, whereupon they show staining which ranges from dark to light. This fact —in accordance with the research of Staron and Pette (1986)— could be related to the different proportions of slow and fast heavy chains shown by this type of fibre. Their size did not turn out to be entirely uniform either, although it is true that, in every case, the mean values are similar to or less than those of type I fibre from the corresponding muscle (Morales-López, 1989). The technique of m-ATPase after preincubation at pH 4.2

also permits identification of the latter fibre type, although its colouring is rather close to that shown by type IIB fibres; in any case, this preincubation does not seem advisable for their differentiation.

It is also interesting to note the presence of these type IIC fibres in the muscles studied, although it should first be made known that in every case the samples were taken from adults, some of which were inactive (control group) and others submitted to progressive exercise. Its presence, therefore, cannot be considered as either the widespread result of myogenesis (Brooke et al., 1971; Essén et al., 1980; Engel and Barker, 1986; Gil, 1986) or the consequence of neuromuscular alterations (Dubowitz and Brooke, 1973; Engel and Barker, 1986; Noneus and Essén-Gustavsson, 1986). All of this lead to the supposition that, in the control group at least, there would be a low incidence of this type of fibre (Dubowitz and Brooke, 1973; Curless and Nelson, 1976; Andersen and Henriksson, 1977). Despite this, in the longissimus lumbaris and in the red and mixed parts of the caput lateralis of the gastrocnemius, between 1.2% and 2.6% was measured, both in the control group and in the experimental groups. However, it should be pointed out that in not one of the seven groups were any fibres of this type identified in the white portions of the gastrocnemius or the vastus lateralis, and very exceptionally in the deeper zones of the vastus lateralis.

In this way, it could be noted that -at least in the muscles and muscle portions analyzed- no appreciable numerical difference was observed among the groups in the percentage of type IIC fibres. Despite this, there are authors (Sréter et al., 1974; Jansson et al., 1978; Müntener, 1982; Schantz et al., 1982) who assert that the presence of IIC fibres increases with exercise. Besides, these authors consider this fibrillar type to be undifferentiated fibres, destined to evolve into whatever kind of fibre may be required by the muscles, in a kind of adaptive phenomenon.

Bearing this in mind, it is worth pointing out another observation, although it was not measured. In the caput lateralis of the gastrocnemius, in the areas of transition between the red and white portions, an abundant presence of type IIC fibres was observed (Fig. 1). It is as if the gain or loss of type I fibres between the two portions at the area of transition were implicitly linked to the type IIC fibres. These observations have also been reported as appearing between the red and the mixed portions of the carporadial extensor muscle of the dog.

In conclusion, the results of this study indicate that the proportion of type IIC fibres in some skeletal muscles of the adult rat is very low and highly variable. Besides, this proportion of fibres does not change after training exercise of different intensity and duration. Nonetheless, this training produces a slight hypertrophy of these miofibres.

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Accepted November 25, 1989