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Short Contribution

Species- and muscle type-dependence of perinatal isomyosin transitions

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ABSTRACT The progressive transition from developmental to adult myosin isoforms during perinatal development was quantified in four muscles (diaphragm, gastrocnemius medialis, masseter and tongue) of four mammals (guinea-pig, hamster, rabbit and rat). It was observed that the timing of transition varied for each muscle, and differed according to the mammal as well. This suggests that the synthesis of adult myosin isoforms may be partly related to the specialized contractile function of a given muscle in a given species.

KEY WORDS: *myosin isoform, perinatal development, mammal muscles*

It is now well established that myosin is developmentally regulated. As shown in the study by Whalen *et al.* (1981), embryonic, neonatal and adult-type myosin isoforms are synthesized sequentially during the perinatal development of rat hind leg muscles. We recently observed this sequential myosin isoform synthesis in a dozen well-defined rat muscles, but showed in addition that each muscle displayed a specific time-dependence; thus only adult-type myosin isoforms were present in the diaphragm at a time when such isoforms were not yet present in the masseter (d' Albis *et al.*, 1989). The factors regulating the synthesis of the diverse myosin isoforms appear to be multiple, having both genetic and epigenetic origins. Our recent results might suggest that in addition to the widely-studied neuronal and hormonal controls, muscle contractile activity could also be involved in regulating the type of myosin isoforms synthesized, since the synthesis of adult-type myosins in the rat diaphragm starts at the time of birth, when the diaphragm, prior to the other muscles, becomes immediately active (d' Albis *et al.*, 1989).

To check this hypothesis, we studied the disappearance with time of embryonic- and neonatal-type myosin isoforms in the diaphragm of three other mammals, the hamster, rabbit, and guinea-pig. In these animals, we also compared the myosin isoform sequence observed in the diaphragm to those in the gastrocnemius medialis, masseter and tongue muscles. Considering in effect the large differences between the myosin transition timings of these muscles in the rat (d' Albis *et al.*, 1989), it was clearly of interest to determine whether they extended to other mammals.

The contents in different myosin isoforms of the four selected skeletal muscles were determined in hamsters, rabbits, and guinea-pigs of several ages. One example of these measurements is given in Fig. 1A, where the myosin isoforms present in the muscles of a 12-day-old rabbit are displayed. The diaphragm and gastrocnemius medialis were found to contain slow-, adult-, and neonatal-type myosin isoforms, while the masseter contained a mixture of adult- and neonatal-type myosin isoforms and the tongue muscle at this stage only adult-type myosins. A second example is given in Fig. 1B, where the myosin isoforms present in the muscles of a 10-day-old hamster are displayed. In addition to the slow-type myosin isoform, which was present in the diaphragm and to a small extent in the gastrocnemius medialis, the four muscles contained a mixture of adult and neonatal myosin isoforms in close relative proportions.

The disappearance with age of embryonic and neonatal myosin isoforms in the four muscles of hamster, rabbit, and guinea-pig was quantified and is shown in Fig. 2; some of the results previously obtained in the rat (d'Albis *et al.*, 1989) are also displayed for comparison.

A certain number of conclusions can be drawn:

- Like rat muscles, the muscles of the three mammals studied in this work displayed developmental myosin isoforms which were distinguishable from the adult myosin isoforms by their electrophoretic properties under their native forms. This had already been observed for the rabbit (Hoh and Yeoh, 1979) and the guinea-pig (Lyons *et al.*, 1986), but had not yet been observed, as far as we know, for the hamster. This result was less expected than might be supposed, since no electrophoretic separation between the neonatal and adult-type myosin isoforms was found in the case of the cat (Hoh *et al.*, 1988), ferret or meryon (unpublished results).
- The myosin isoform transitions in the four muscles did not follow the same pattern of time-dependence in the four mammals (Fig

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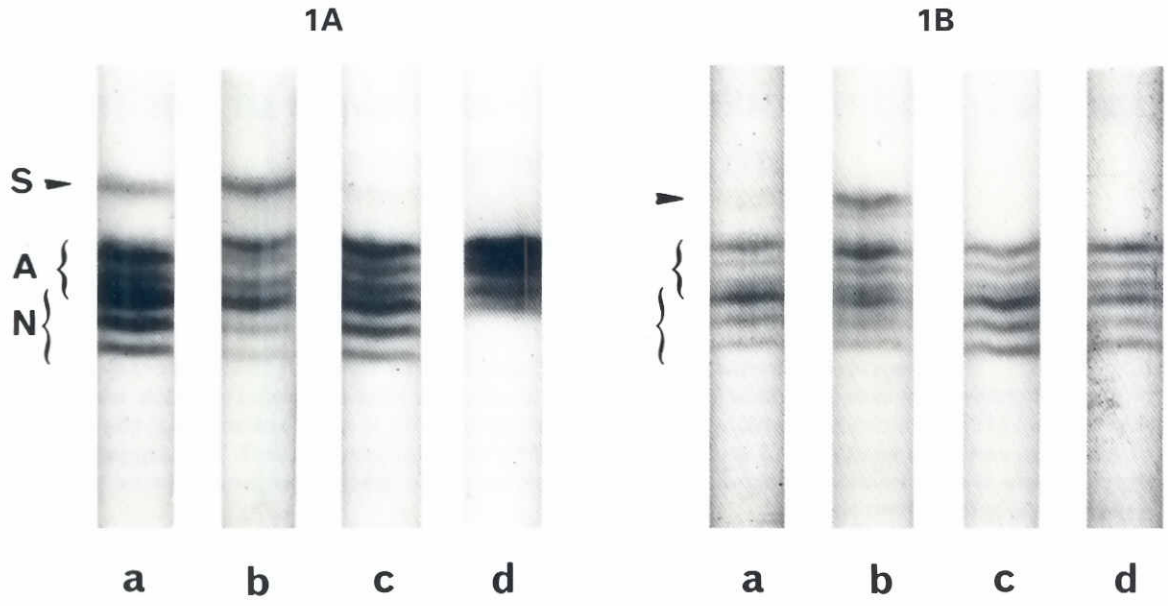


Fig.1. Separation of myosin isoforms in four muscles by electrophoresis under nondissociating conditions. *Muscles were: (a) gastrocnemius medialis, (b) diaphragm, (c) masseter, (d) tongue muscle. S, slow-type myosin, A, adult-type myosins, N, neonatal-type myosins.* **(A)** *12-day-old rabbit.* **(B)** *10-day-old hamster.*

2). While the half-times of the transitions varied from seven to 23 days postnatal for the rat muscles (d' Albis *et al.*, 1989), they occurred in the hamster at about the same time, 12±2 days postnatal, for the four muscles studied. An intermediate situation was observed in the rabbit and guinea-pig. In these animals, three of the muscles displayed about the same time-dependence, while the fourth one, the tongue in the rabbit and the masseter in the guinea-pig, displayed either a more precocious or a later transition.

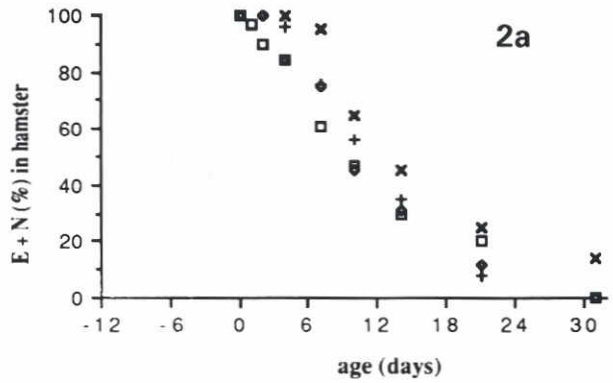
- The comparison of the myosin isoform transitions in the four mammals for a given muscle gave various results (Fig. 3). For the diaphragm, the synthesis of the adult-type myosin isoforms appeared in the hamster and rabbit to be linked to birth as it is in the rat; however, this transition occurred much earlier in the guinea-pig, whose gestation period is the longest. For the gastrocnemius, similar curves were also obtained in the hamster, rabbit, and rat, but again the curve for the guinea-pig indicated a more precocious transition. On the other hand, for both the tongue muscle and the masseter, the transition curves displayed very different patterns of time-dependence for the four mammals. In addition, the persistence of neonatal myosin isoforms in the masseter of the adult animal, which had been previously observed in the rat (d' Albis *et al.*, 1986) mouse (d' Albis *et al.*, 1986) and human (Butler-Browne *et al.*, 1988), was found, though to a smaller degree, in the hamster, but not in the guinea-pig (d' Albis *et al.*, 1986) or the rabbit.

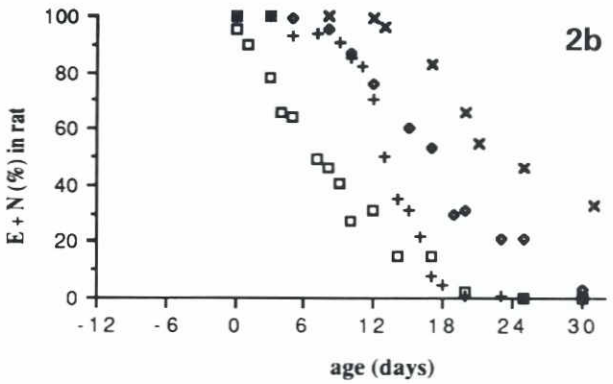
Myosin polymorphism allows muscle to adapt to changing physiological conditions. In particular, the synthesis of adult-type myosin isoforms during development is linked to a higher contraction velocity of adult animal muscles and to an increase in the myosin ATPase activity (Houadjeto *et al.*, 1990). As early as 1966, Trayer and Perry established that the pattern of ATPase increase varied with the species studied, adult values being reached at birth in the guinea pig, soon after birth in the rabbit, and two weeks after birth in the rat.

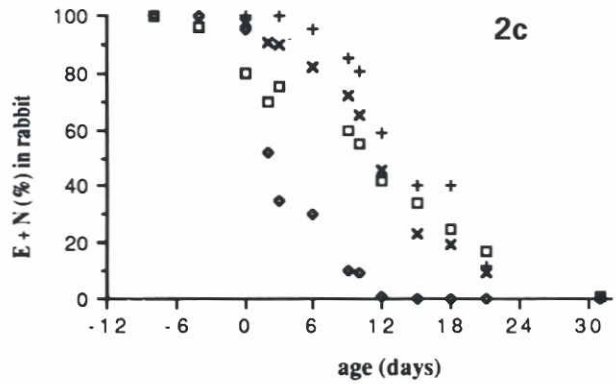
We have shown here that the time at which the adult myosin pattern was reached strongly depended on the particular muscle examined and that it differed for the four mammal species studied. With the exception of the guinea-pig, which displayed for all muscles the most precocious transitions to the adult myosin isoforms, no direct correlation was found between the duration of gestation and the timing of the transitions. The most precocious muscles were the

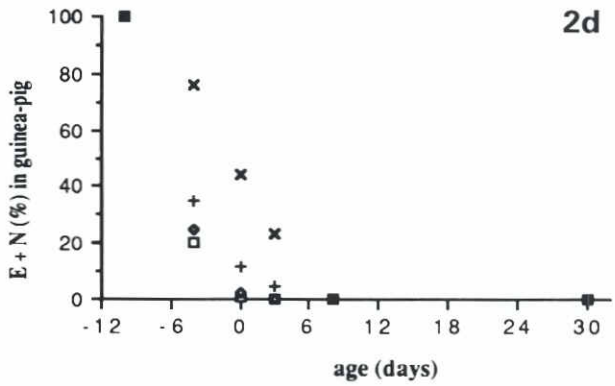
Fig.2. Proportions of embryonic (E) and neonatal (N) myosin isoforms combined in relation to total myosin isoforms versus the age of the hamster (2a), rat (2b), rabbit (2c), and guinea-pig (2d). *Negative numbers correspond to embryos whose ages relative to birth were calculated for average gestation durations of 30 and 68 days for the rabbit and guinea-pig, respectively. Muscles were: (+) gastrocnemius medialis, (❑) diaphragm, (x) masseter, (◊) tongue muscle.*

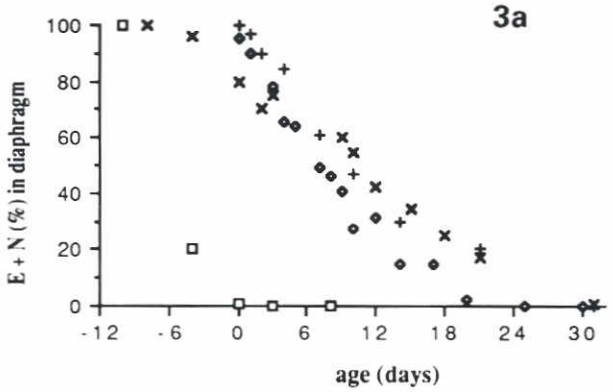
Fig.3. Proportions of embryonic (E) and neonatal (N) myosin isoforms *combined in relation to total myosin isoforms in the diaphragm* **(3a)**, *tongue muscle* **(3b)**, *gastrocnemius medialis* **(3c)**, *and masseter* **(3d)** *versus the age of the hamster (+), rat (◊), rabbit (x), and guinea-pig(❑). Negative numbers correspond to embryos whose ages relative to birth were calculated for average gestation durations of 30 and 68 days for the rabbit and guinea-pig, respectively.*

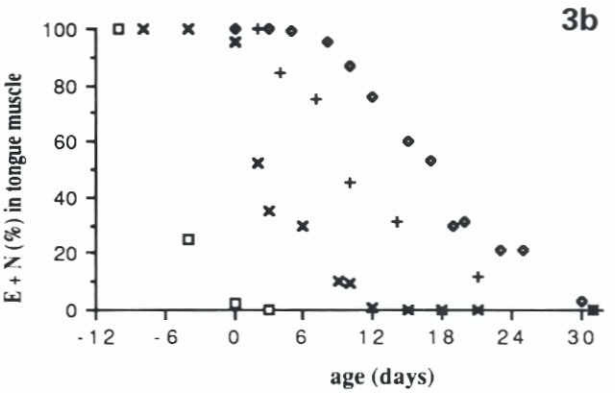

2a
E + N (%) in hamster
100
80
60
40
20
0
-12
-6
0
6
12
18
24
30
age (days)

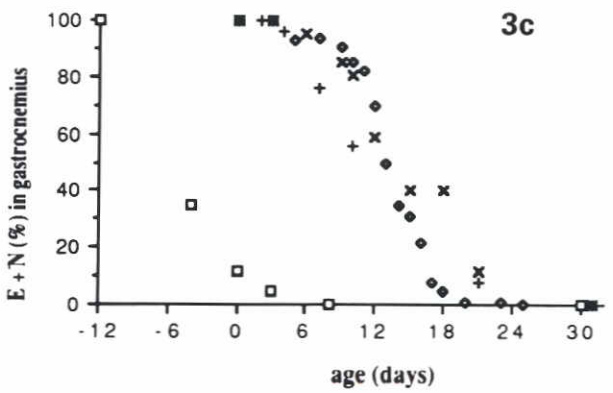

2b
E + N (%) in rat
100
80
60
40
20
0
-12
-6
0
6
12
18
24
30
age (days)

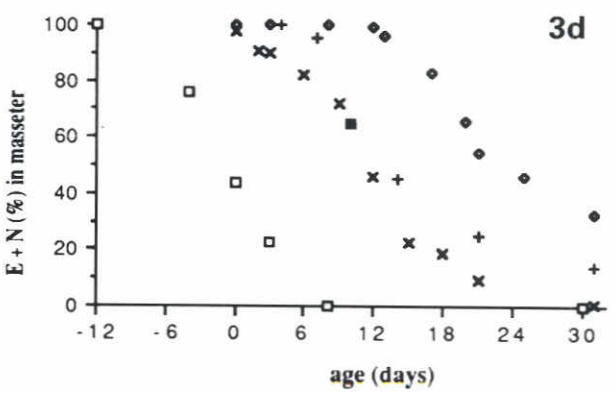

2c
E + N (%) in rabbit
100
80
60
40
20
0
-12
-6
0
6
12
18
24
30
age (days)


2d
E + N (%) in guinea-pig
100
80
60
40
20
0
-12
-6
0
6
12
18
24
30
age (days)


3a
E + N (%) in diaphragm
100
80
60
40
20
0
-12
-6
0
6
12
18
24
30
age (days)


3b
E + N (%) in tongue muscle
100
80
60
40
20
0
-12
-6
0
6
12
18
24
30
age (days)


3c
E + N (%) in gastrocnemius
100
80
60
40
20
0
-12
-6
0
6
12
18
24
30
age (days)


3d
E + N (%) in masseter
100
80
60
40
20
0
-12
-6
0
6
12
18
24
30
age (days)

diaphragm in the rat, but the tongue muscle in the rabbit.

In addition to possibly different genetic programs, epigenetic regulating factors might intervene differently depending on the muscle and the species. Innervation has been shown not to influence significantly the synthesis of adult myosin isoforms (Butler-Browne *et al.*, 1982; Gambke *et al.*, 1983). On the other hand, the increase in the thyroid hormone concentration which occurs during development plays a decisive role (Gambke *et al.*, 1983). This hormone concentration reaches a peak at about 1 week postnatal in the rabbit (Devaskar *et al.*, 1986) and 2 weeks postnatal in the rat (Dubois and Dussault, 1977), which might explain, in part, why the tongue muscle and masseter were more precocious in the rabbit than in the rat. However, all muscles do not respond in the same way to thyroid hormone (Izumo *et al.*, 1986; d' Albis *et al.*, 1990), which might explain why they do not display the same transition curves in a given animal.

The contractile function could also be a determinant of adult myosin isoform synthesis, as has been shown for muscle growth (Zak, 1981). This was our hypothesis in the case of the rat diaphragm, in which, indeed, the synthesis of adult myosin isoforms started at birth time, when the muscle has to function immediately and repetitively. This turned out to be true as well for the hamster and rabbit. On the other hand, the synthesis of adult myosin isoforms started during fetal life in the guinea-pig, which is already highly developed at the time of birth. One may, in addition, wonder why the tongue displayed such an early synthesis of adult myosin isoforms in the rabbit. It might possibly be related to a particularly precocious function of this muscle in this mammal. More work is in progress to determine the regulatory factors, which confer to a muscle its specific development pattern.

Experimental Procedures

Pregnant hamster *(Golden Hamster)*, rabbit *(New Zealand)*, and guinea-pig *(Tricolor)* females were provided by Lessieux, and pregnant rat females *(Wistar)* by Janvier. The duration of gestation was on the average 16, 30, 68, and 22 days, respectively. The muscles were dissected from embryos and from young animals up to the age of 1 month and myosin was extracted as previously described (d' Albis *et al.*, 1989). Myosin isoforms were separated by gel electrophoresis under nondissociating conditions and quantified by densitometry (d' Albis *et al.*, 1989).

Acknowledgements

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References

BUTLER-BROWNE, G.S., BUGAISKY, L.B., CUÉNOUD, S., SCHWARTZ, K. and WHALEN, R.G. (1982) Denervation of newborn rat muscles does not block the appearance of adult fast myosin heavy chain. *Nature 299:* 830-833.

BUTLER-BROWNE, G.S., ERIKSSON, P.O., LAURENT, C. and THORNELL, L.E. (1988). Adult human masseter muscle fibers express myosin isozymes characteristic of development. *Muscle Nerve 11:* 610-620.

D'ALBIS, A., CHANOINE, C., JANMOT, C., MIRA, J.C. and COUTEAUX, R. (1990). Muscle-specific response to thyroid hormone of isomyosin transitions during rat postnatal development. *Eur. J. Biochem. 193:* 155-161.

D'ALBIS, A., COUTEAUX, R., JANMOT, C. and ROULET, A. (1989). Specific programs of myosin expression in the postnatal development of rat muscles. *Eur. J. Biochem. 183:* 583-590.

D'ALBIS, A., JANMOT, C. and BÉCHET, J.J. (1986). Comparison of myosins from the masseter muscle of adult rat, mouse and guinea-pig. Persistence of neonatal-type isoforms in the murine muscle. *Eur. J. Biochem.* 156: 291-296.

DEVASKAR, U.P., DEVASKAR, S.U., SADIQ, F. and CHECHANI, V. (1986). Ontogeny of plasma-free thyroxine and triiodothyronine concentrations during the perinatal period and maternofoetal transfer of thyroid hormones in the rabbit. *Dev. Pharmacol. Ther. 9:* 115-123.

DUBOIS, J.D. and DUSSAULT, J.H. (1977). Ontogenesis of thyroid function in the neonatal rat. Thyroxine (T4) and triiodothyronine (T3) production rates. *Endocrinol. 101:* 435 - 441.

GAMBKE, B., LYONS, G.E., HASELGROVE, J., KELLY, A.M. and RUBINSTEIN, N.A. (1983). Thyroidal and neural control of myosin transitions during development of rat fast and slow muscles. *FEBS Lett. 156:* 335-339.

HOH, J.F.Y., HUGHES, S., HALE, P.T. and FITZSIMONS, R.B. (1988). Immunocytochemical and electrophoretic analyses of changes in myosin gene expression in cat limb fast and slow muscles during postnatal development. *J. Muscle Res. Cell Motil. 9:* 30-47.

HOH, J.F.Y. and YEOH, G.P.S. (1979). Rabbit skeletal myosin isoenzymes from fetal, fast-twitch and slow-twitch muscles. *Nature 280:* 321-322.

HOUADJETO, M., BÉCHET, J.J. and D' ALBIS, A. (1990). Comparative structural and enzymatic properties of skeletal muscle myosin from neonatal and adult rabbits. *Eur. J. Biochem. 191:* 695-700.

IZUMO, S., NADAL-GINARD, B. and MAHDAVI, V. (1986). Thyroid hormone receptor alpha isoforms generated by alternative splicing differentially activate myosin HC gene transcription. *Science 231:* 597-600.

LYONS, G.E., KELLY, A.M. and RUBINSTEIN, N.A. (1986). Testosterone-induced changes in contractile protein isoforms in the sexually dimorphic temporalis muscle of the guinea pig. *J. Biol. Chem. 261:* 13278-13284.

TRAYER, I.P. and PERRY, S.V. (1966). The myosin of developing skeletal muscle. *Biochem. Zeitsch. 345:* 87 - 100.

WHALEN, R.G., SELL, S.M., BUTLER-BROWNE, G.S., SCHWARTZ, K., BOUVERET, P. and PINSET-HÄRSTRÖM, I. (1981). Three myosin heavy chain isozymes appear sequentially in rat muscle development. *Nature 292:* 805-809.

ZAK, R. (1981). Contractile function as a determinant of muscle growth. In *Cell and Muscle Motility*, Vol. 1 (Eds. R.M. Dowbett and J.W. Shay). Plenum Publish. Corp., pp. 1-33.

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