The specificity of seven monoclonal antibodies specific to myosin heavy chain isoforms in rat, dog and human skeletal muscles

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We present our experience with seven monoclonal antibodies specific to myosin heavy chain (MyHC) isoforms. Six antibodies were applied in rat, human and canine skeletal muscle sections, but one of them, 6H1 [1] in human muscle sections only. Since the orthologue MyHC isoforms exhibit higher similarity in their amino acid sequence than paralogues, quite similar staining pattern could be expected among different species. In human and canine muscle samples fibre types were determined according to the reaction for myofibrillar ATPase, in human samples MyHC transcript expression was revealed using in situ hybridization technique as well [2]. The set of four antibodies specific to rat MyHC isoforms, BA-D5 (MyHC-1), SC-71 (MyHC-2a), BF-35, (MyHC-1, -2a, -2b), BF-F3 (MyHC-2b) reacted as declared [3]. One of the commercially available antibodies (Alexis Biochemicals), A4.74, according to the manufacturer specific to MyHC-2a isoform of various species, reliably marked type 2a fibres of rat. But F113.15F4, declared to be specific to MyHC-2a and -2b of various species, actually stained type 2a and 2x fibres (Fig.1a-c). Therefore, using this antibody the presence of rat MyHC-2x can be additionally confirmed, which can be otherwise demonstrated only on the principle of exclusion with BF-35. Using the same set of antibodies the canine and human slow MyHC isoforms can be clearly revealed, but not the fast ones. Namely, SC-71 and A4.74 antibodies intensively stained canine (Fig. 1d-f) and human (Fig.2) type 2a fibres, which expressed 2a MyHC transcripts in humans and moderately type 2x, in humans expressing mostly 2x MyHC transcripts [4]. Such double-intensity labeling of 2a and 2x fibres by SC-71 antibody was found in those mammalian species with only these two fast fibre types present except of horse in which only type 2a fibres were labeled [5]. The 6H1antibody was the only one that selectively labeled human type 2x fibres or fibres expressing 2x MyHC transcripts. F113.15F4 stained both human and canine fast fibre types. The negative results obtained with BF-F3 in human and canine muscle samples are in agreement with the absence of MyHC-2b in the skeletal muscles of these two species. As fast MyHC isoforms (paralogues) have a high degree of homogeneity, we assume that they share the epitopes of the used anti-fast MyHC antibodies. In conclusion, our results imply that the reactivity of antibodies specific to distinct MyHC isoforms, especially the fast ones should be carefully evaluated when used in various species.

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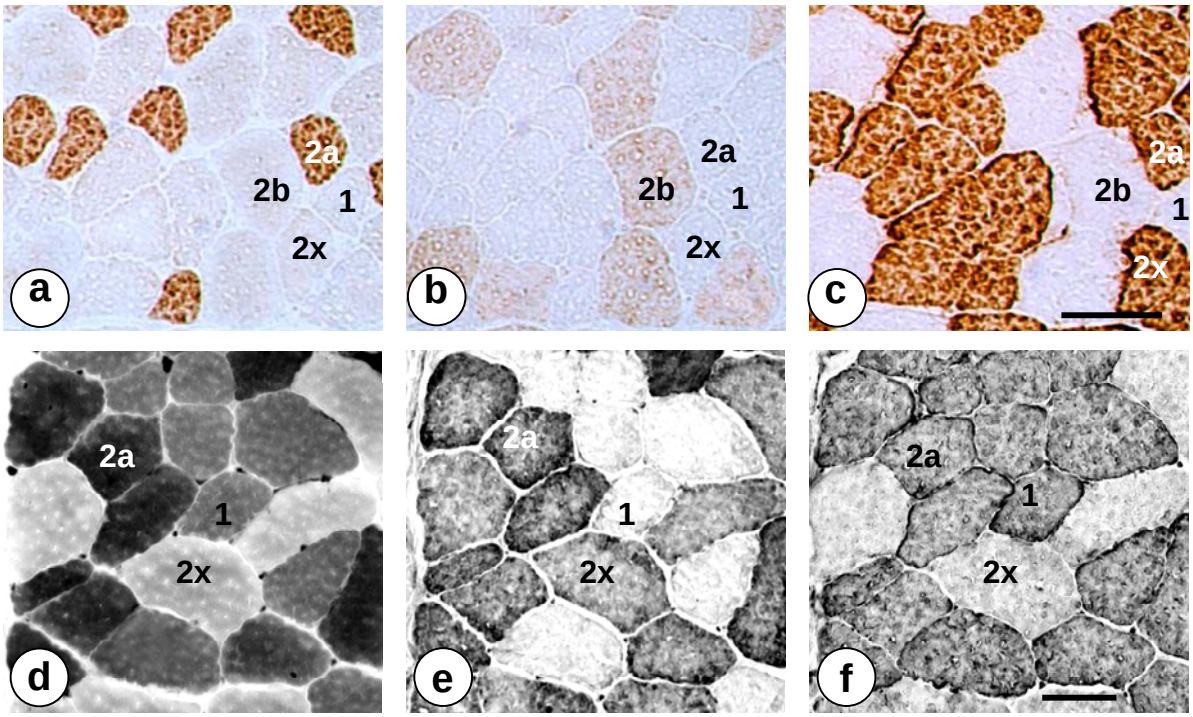

Figure 1. Demonstration of fast MyHC isoforms expression in rat extensor digitorum longus (a-c) and canine triceps brachii (d-f) muscles by antibodies against MyHCs: A4.74 (a), BF-F3 (b), F113.15F4 (c), myofibrillar ATPase reaction at pH 4.6 (d), SC-71 (e), BF-35 (f). Scale bar is 50μm.

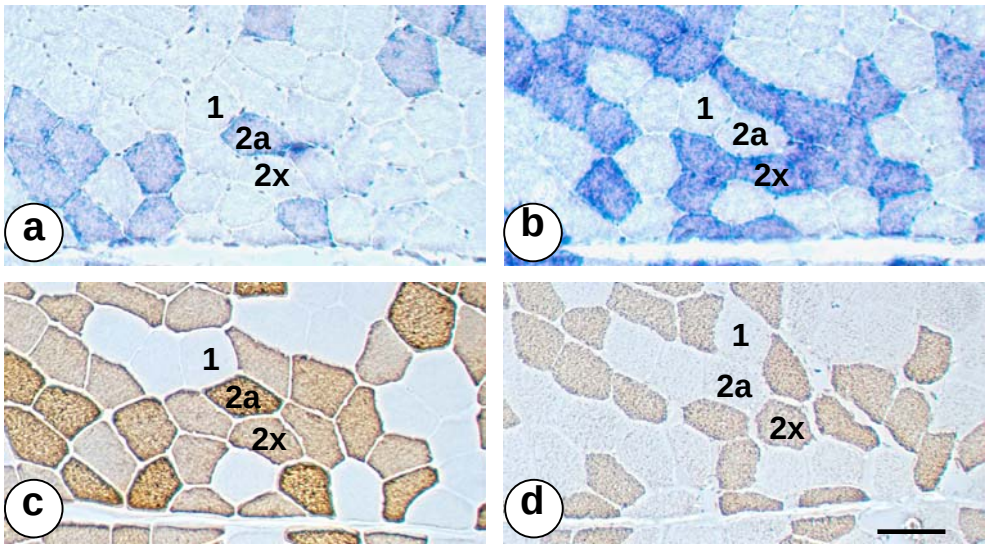

Figure 2. Expression of 2a (a) and 2x (b) MyHC transcripts (mRNA) in human biceps femoris muscle revealed by digoxigenin-labelled RNA probes using in situ hybridisation technique and immunohistochemical differentiation of fast fibre types (2a and 2x) with antibody SC-71 (c) and 6H1 (d). Scale bar is 50 μm.

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