Short communication

Denervation Induced Changes in Level of Ca-Antagonists Binding Cytosolic Protein from White Skeletal Muscle

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Denervation of skeletal muscle is generally associated with an increase of the concentrations of proteins of some outer membrane transport systems, such as acetylcholine and DHP receptors. After denervation, the acetylcholine receptor expression rate was reported to increase about 150-fold (Shieh et al. 1988). The concentration of DHP receptors from T-tubules increased approximately two times (Schmidt et al. 1984; Lehotský et al. 1988). It is not clear, whether the higher levels are due to enhanced expression or to decreased turnover rates.

The cytosolic protein which was shown to bind Ca^{2+} antagonists, was able to transport calcium after its reconstitution into phospholipids, and reacted with polyclonal antibodies against the DHP-sensitive calcium channel of rabbit skeletal muscle (Križanová et al. 1989; 1990). The physiological role of this channel remains unclear. It was suggested (Križanová et al. 1990) that the cytosolic protein is a metabolic product of the DHP-receptor alpha subunit. Some clues concerning the relationship between the membrane bound and the cytosolic DHP-binding proteins could be obtained if the same increases of DHP-binding sites are obtained after denervation.

The fast twitch muscles of the rabbit hind leg were denervated as described earlier (Lehotský et al. 1990). Crude membranes and cytosolic proteins were obtained as described by Križanová et al. (1989), and binding assays were performed with (methyl)-^{3}HPN 200-110. SDS-PAGE analysis was carried out according to Laemmli (1970), and proteins were estimated according to Lowry et al. (1951).

Although the kinetic properties of the DHP receptor have been studied in denervated muscle membranes (Schmidt et al. 1984; Lehotský et al. 1988), the question concerning the cytosolic DHP-binding protein has not been discussed.

Table 1. Specific binding of H-PN 200-110 to crude microsomal membranes and to the cytosolic fraction from denervated and control white rabbit skeletal muscle. Results are means + double SD from 5 independent experiments analysed in triplicate.

Fraction	Control	Denervated
	fmol H-PN 200-110/mg prot. (fmol H-PN 200-110/g wet weight)	
Crude microsomal membranes	57.9 + 12.7 (64.3 + 11.7)	124.0 + 25.5 (151.3 + 29.1)
Cytosolic fraction	2.25 + 0.15 (6.34 + 0.21)	4.72 + 0.73 (15.10 + 0.96)

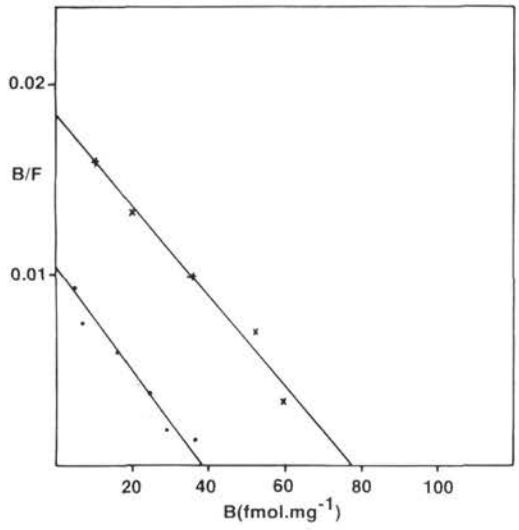

Fig. 1. Scatchard analysis of specific dihydropyridine H-PN 200-110 binding to the soluble fraction from control (—.—) and denervated muscles (— × — × —). Results are means from 4 independent experiments analysed in triplicate by linear regression.

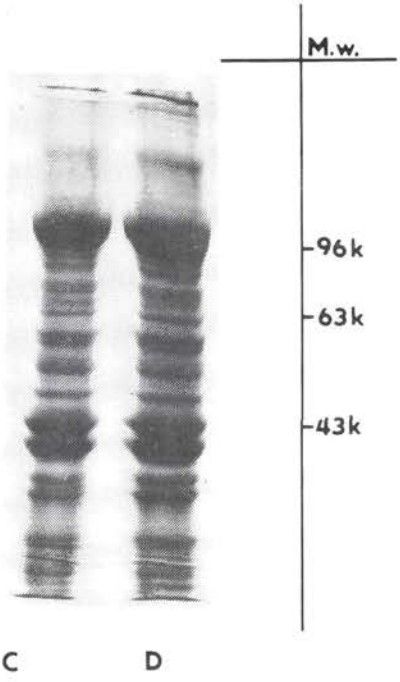

Fig. 2. SDS-PAGE of cytosolic muscle proteins from control (C) and denervated (D) muscles according to Laemmli (1970), using 8 per cent gel. Standards used: phosphorylase b (94 kD), bovine serum albumine (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), soybean trypsin inhibitor (20.1 kD), lactalbumin (14.4 kD) (all from Pharmacia Fine chemicals, Sweden).

The results summarized in Table 1 show that ^{3}H PN 200-110 binds specifically to both the membrane and the cytosolic fractions. Specific binding to microsomes from denervated muscle was approximately twofold that to the control microsomal vesicles (124.0 + 25.5 fmol/mg protein and 57.9 + 12.7 fmol/mg protein). The same ratio of binding ^{3}H-PN 200—110 was observed to cytosolic proteins from denervated and control tissue (4.72 + 0.73 to 2.25 + 0.15 fmol/mg protein), although specific binding to the cytosolic proteins was 25 times lower than to membrane fractions; this proportion was maintained also after when relating the binding to wet weight. Saturation binding experiments showed a single family of ^{3}H-PN 200—110 proteins with maximal binding capacity B_{max} = 77.3 fmol/mg protein for denervated and 38.5 fmol/mg protein for control cytosolic fraction (Fig. 1.), without any differences in the dissociation constant.

One-dimensional SDS-PAGE analysis of cytosolic proteins from denervated and control fractions shows no significant quantitative or qualitative differences (Fig. 2), either in the whole protein profile or in the DHP binding protein with Mr = 90 kD; nevertheless, some distinctions cannot be excluded at higher resolution.

It may be concluded that DHP specifically binds to the cytosolic protein in a manner very similar to that to microsomal membranes. A more detailed study is required to confirm the clear relationship between the cytosolic and the membrane-bound DHP-receptor.

References

Križanová O., Hudecová S., Orlický J., Zachar J. (1989): Is Ca antagonists binding protein from cytosolic fraction the precursor of alpha 1 subunit of Ca channel? Gen. Physiol. Biophys. **8,** 99—111

Križanová O., Hurňák O., Hudecová S., Zachar J. (1990): The Ca-antagonists binding cytosolic protein has properties of the Ca-channel. Gen. Physiol. Biophys. **9,** 343—352

Laemmli U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227,** 680—685

Lehotský J., Drgová A., Dobrota D., Kameništiaková L., Mézešová V. (1988): Content of cholesterol and enzyme activities in ST system from white denervated and normal muscle. 14th International Congress of Biochemistry, Prague, Abstract No. MO—307

Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J. (1951): Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193,** 265—270

Schmid A., Kazazoglou T., Renaud J. F., Lazdunski M. (1984): Comparative changes of levels of nitrendipine Ca-channels, of tetrodotoxin-sensitive Na-channels and of ouabain-sensitive Na,K-ATPase following denervation of rat and chick skeletal muscle. FEBS Lett. **17,** 114—118

Shieh B. H., Ballivat M., Schmidt J. (1988): Acetylcholine receptor synthesis rate and levels of receptor subunit messenger RNAs in chick muscle. Neuroscience **24,** 175—187

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