Short communication

Binding of Dihydropyridine Calcium Antagonists to Membranes from Human Skeletal Muscle

O. KRIŽANOVÁ, J. ORLICKÝ and J. ZACHAR

Centre of Physiological Sciences, Slovak Academy of Sciences, Vlárska 5, 83306 Bratislava, Czechoslovakia

L-type of voltage dependent Ca^{2+} channel was reported to be present in skeletal muscles of rabbits (Borsotto et al. 1984; Curtis and Catterall 1984; Flockerzi et al. 1986; Fosset and Lazdunski 1987; Nakayama et al. 1987) and guinea pigs (Glossmann et al. 1987). It is only this type of Ca^{2+} channel that binds dihydropyridine Ca^{2+} antagonists (Nowycky et al. 1985). The aim of our work was to investigate whether the same type of calcium channel is present also in human skeletal muscle.

Supravital skeletal muscle from patients suffering from a trauma was used for the experiments. Owing to problems concerning the supply of experimental material, experiments with muscle tissue of cadavers were also carried out. B_{max} and K_D were estimated. Membranes enriched in T-tubules were isolated according to Rosemblatt et al. (1981). For the ^{3}H PN 200-110 binding assay (Ruth et al. 1985) and for the evaluation of B_{max} and K_D by Scatchard analysis (Scatchard, 1949) 20 mmol/l Tris −HCl pH 7.4, supplemented with 1 mmol/l $CaCl_2$ and protease inhibitors (PMSF, iodacetamid and benzamidine) were used. Molecular weights of the Ca^{2+} antagonist binding proteins were estimated by the method of Ferry et al. (1985). After covalent binding of ^{3}H azidopine, membrane proteins were separated by SDS electrophoresis on 8% gels according to Laemli (1970) under reducing conditions. For the determination of radioactivity bound the gels were cut into small pieces and solubilized in a scintillation coctail (SLT-41, Spolana Neratovice). Radioactivity of the stripes was measured and compared with bands on silver stained gels.

Results of the binding of ^{3}H PN 200-110 to human muscle membranes are shown in Table 1. B_{max} from supravital crude membranes was 12.3 ± 1.2 pmol/mg protein and K_D was 9.96 ± 5.2 nmo/l. Values of B_{max} and K_D from necrotic crude membranes (28.3 ± 7.4 pmol/mg protein and 14.0 ± 0.6 nmol/l) were comparable to those obtained for supravital crude membranes. Differences in binding properties of ^{3}H PN 200-110 (both B_{max} and K_D) were observed between T-tubules of supravital and necrotic tissues. For the supravital T-tubules B_{max} was 232 pmoles/mg protein and the corresponding value for the necrotic tissue was 70.1 ± 9.2 pmoles/mg protein. On the other hand, K_D for the necrotic muscle was higher than that for the supravital material (56.2 ± 0.8 nmoles/l and

Table 1. K_D and B_{max} values determinated from binding assay with ^{3}H PN 200-110. n — number of patients from whose the tissue was obtained. Each value represents the mean of 3 measurements.

Tissue	K_D nmol/l	B_{max} pmol/mg prot.	n
Human fresh muscle crude membranes	9.96 ± 5.2	12.3 ± 1.2	3
Human fresh muscle T-tubules	16.1	232	1
Human cadaver muscle crude membranes	14.0 ± 0.6	28.3 ± 7.4	3
Human cadaver muscle T-tubules	56.2 ± 0.8	70.1 ± 9.2	3

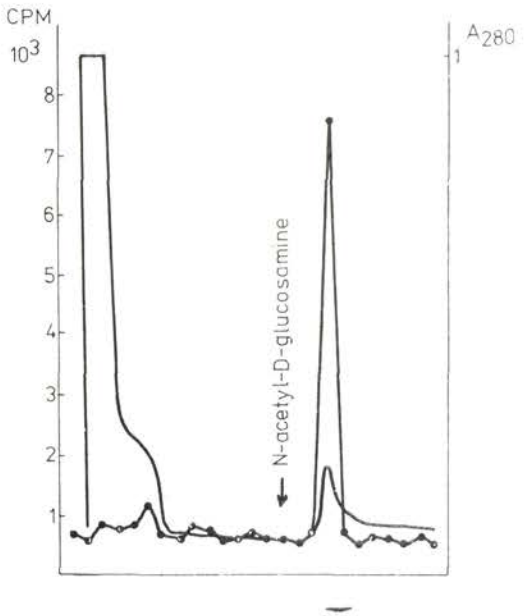

Fig. 1. Protein —— and radioactivity -●-● profiles of WGA-Sepharose column (2 × 1 cm). Glycoproteins were eluted with 250 mmol/l N-acetyl-D-glucosamine. Proteins were measured at 280 nm on continual UV analysator. Radioactivity was measured after addition of Bray's scintillation coctail (Spolana Neratovice) on Beckman counter.

16.1 nmoles/l) respectively. The values of B_{max} and K_D for ^{3}H PN 200-110 binding are very similar to those obtained by Ferry et al. (1985) and Flockerzi et al. (1986). The Ca^{2+} antagonists binding glycoproteins were partially purified by affinity chromatography on WGA-Sepharose. Membrane proteins prelabelled with ^{3}H PN 200-110 and solubilized with 1% digitonin were separated on WGA-Sepharose column (2 × 1 cm). After elution of bound proteins with 250 mmol/l N-acetyl-D-glucosamine the protein elution profile and radioactivity were determined. The protein elution profile and the radioactivity profile are shown in Fig. 1. The radioactivity peak corresponded with the glycoprotein peak. In our experiments molecular weight of this protein was 160—170 kD in purified T-tubules (Fig. 2). This is in good agreement with the values reported for rabbit skeletal muscle by Flockerzi et al. (1986).

Our preliminary studies with polyclonal antibodies support the proposition that human skeletal muscle contains the same binding sites for Ca^{2+} antagonists that appear in rabbit skeletal muscle (unpublished results). Further studies are required to investigate the problem in more detail.

Aknowledgement. We are grateful to Dr. M. Ruščák for valuable advices and to Mrs. D. Chalupková for technical assistance.

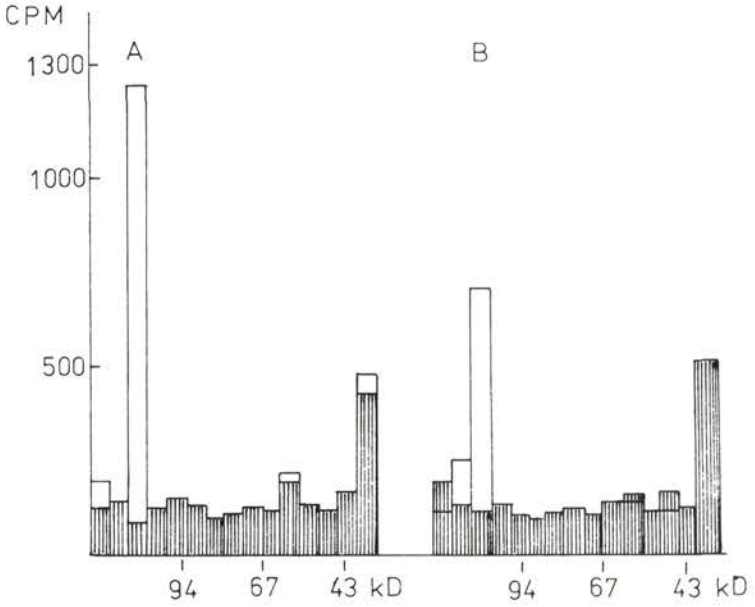

Fig. 2. Determination of molecular weight of protein binding ^{3}H azidopine. Membranes were labelled with ^{3}H azidopine and proteins were separated on 8% gels. After staining the gel was cut into stripes and radioactivity of each stripe was measured. Empty columns — total binding, striped columns — nonspecific binding. *A* — rabbit skeletal muscle T-tubules, *B* — human necrotic skeletal muscle T-tubules. Results have shown that this protein have Mw 160—170 kD.

References

Borsotto M., Barhanin J., Norman R. A., Lazdunski M. (1984): Purification of the dihydropyridine receptor of the voltage-dependent Ca^{2+} channel from skeletal muscle transverse tubules using (+) ^{3}H PN 200-110. Biochem. Biophys. Res. Commun. **122,** 1357—1366

Curtis B. M., Catterall W. A. (1984): Purification of the Calcium Antagonist Receptor of the Voltage-Sensitive Calcium Channel from Skeletal Muscle Transverse Tubules. Biochemistry **23,** 2113—2118

Ferry D. R., Goll A., Rombusch M., Glossmann H. (1985): The molecular pharmacology and structure features of calcium channels. J. Clin. Pharmacol. **20,** 2335—2465

Flockerzi V., Oeken H. J. Hofmann F. (1986a): Purification of a functional receptor for calcium channel blockers from rabbit skeletal muscle microsomes. Eur. J. Biochem. **161,** 217—224

Flockerzi V., Oeken H. J. Hofmann F., Pelzer D., Cavalie A., Trautwein W. (1986): Purified dihydropyridine-binding site from skeletal muscles t-tubules is a functional calcium channel. Nature **323,** 66—68

Fosset M., Lazdunski M. (1987): Biochemical characterization of the skeletal muscle Ca^{2+} channel. In: Structure and Physiology of the Slow Inward Calcium Channel. (Eds. J. C. Venter and D. Triggle) pp. 141—159. Alan R. Liss., Inc.

Glossmann H., Striessnig J., Ferry D. R., Goll A., Moosburger K., Schirmer M. (1987): Interaction between Calcium Channel Ligands and Calcium Channels. Circ. Res. **61,** Suppl. I, I 30—I 36

Laemli U. K. (1970): Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. Nature **227,** 680—685

Nakayama N., Kirley T. L., Vaghy P. L., McKenna E., Schwartz E. (1987): Purification of a Putative Ca^{2+} Channel Protein from Rabbit Skeletal Muscle. Determination of amino-terminal sequence. J. Biol. Chem. **262,** 6572—6576

Nowycky M. L., Fox A. P., Tsien R. W. (1985): Three types of neuronal calcium channel with different calcium agonist sensitivity. Nature **316,** 440—443

Rosemblatt M., Hidalgo C., Vergara C., Ikemoto N. (1981): Immunological and Biochemical Properties of Transverse Tubule Membranes Isolated from Rabbit Skeletal Muscle. J. Biol. Chem. **256,** 8140—8148

Scatchard G. (1949): The attraction of protein for small molecules and ions. Annu. N. Y. Acad. Sci. **51,** 660—672

Final version accepted February 26, 1988