Volume 328, number 3, 253–258 FEBS 12833 August 1993

Expression of β subunit isoforms of the Na^+,K^+-ATPase is muscle type-specific

Harinder S. Hundal[a,b], André Marette[a,*], Toolsie Ramlal[a], Zhi Liu[a] and Amira Klip[a]

[a]*Division of Cell Biology, The Hospital For Sick Children, 555 University Avenue, Toronto, Ont. Canada* and [b]*Department of Anatomy and Physiology, The University of Dundee, Dundee, DD1 4HN, Scotland, UK*

Received 24 June 1993

Hindlimb skeletal muscles of the rat express two isoforms of the α (α1 and α2) and two isoforms of the β (β1 and β2) subunits of the Na^+,K^+-ATPase. Because several muscles constitute the hindlimb, we investigated if specific isoforms are expressed in particular muscles Northern blot analysis using isoform-specific cDNA probes demonstrated that soleus muscle expressed only the β1 transcript, whereas EDL or white gastrocnemius muscles expressed only the β2 transcript, and red gastrocnemius muscle expressed both mRNAs. All muscles tested expressed both α1 and α2 transcripts, albeit to various degrees: α1 transcripts were present to about the same extent in all muscles but α2 mRNA was 4-fold more abundant in soleus than in EDL for the same amount of total RNA. β subunit protein levels were investigated in purified plasma membrane fractions of pooled red (soleus + red gastrocnemius + red quadriceps) or white (white gastrocnemius + white quadriceps) muscles using isoform-specific antibodies. Red muscles expressed mostly the β1 protein while white muscles expressed mostly the β2 subunit. Both muscle groups had similar levels of α1 or α2 subunits, and crude membranes isolated from red muscles had 30% higher Na^+,K^+-ATPase activity than white muscle membranes. We conclude that oxidative muscles (slow and fast twitch) express β1 subunits, whereas glycolytic, fast twitch muscles express β2 subunits, and that both β isoforms support the Na^+,K^+-ATPase activity of the α subunits.

Sodium pump; Muscle membrane

1. INTRODUCTION

The Na^+,K^+-ATPase (EC 3.6.1.37) is an integral membrane protein complex that catalyses the ATP-dependent transport of Na^+ and K^+ across the plasma membrane of most mammalian cells [1,2]. The Na^+ and K^+ gradients maintained by the Na^+,K^+-pump play a central role in the functioning of secondary active transport processes such as Na^+-coupled amino acid transport, regulation of cell volume and intracellular pH [3–5]. In nerve and skeletal muscle the activity of the Na^+,K^+-ATPase is especially important for maintaining the resting membrane potential and contributes towards its restoration following an action potential [6]. The minimum Na^+,K^+-ATPase unit capable of hydrolysing ATP and undergoing conformational changes in the E_1-E_2 transitional cycle is an αβ heterodimer. Three isoforms of the α subunit (about 110 kDa) have been identified to date (α1, α2 and α3), expressed in a tissue- and developmental stage-specific manner [7]. The α subunit is responsible for the catalytic activity of the enzyme and contains the binding sites for all its substrates (Na^+, K^+, ATP and Mg^{2+}) as well as for the cardiac glycoside ouabain. For a fully functional enzyme unit the α subunit has to associate with a glycosylated β subunit (35 kDa polypeptide) of which three isoforms (β1, β2 and β3) have also been identified [8]. In vitro expression studies have suggested that the β subunits are important in preserving the stability of the α subunit as well as the normal processing and transport of mature enzyme complexes to the plasma membrane [8–10]. Since dissociation of β subunits from α subunits results in a loss of enzyme activity it is conceivable that the β subunit may serve a regulatory function. However, exactly how the β subunit allows the α subunit to become catalytically active and whether specific α and β isoform pairs result in the formation of Na^+,K^+-ATPase units of distinct functional activity remains unknown.

We recently reported the presence of α1, α2, β1 and β2 subunits of the Na^+,K^+-ATPase in mixed skeletal muscles and analyzed their subcellular distribution [11]. Although tissue-specific expression of α-subunits has been well documented (reviewed in [7]) little is known concerning the expression and distribution of specific isoforms within a single tissue, such as skeletal muscle, whose cellular (fibre-type) composition is highly heterogeneous [12]. Histological and physiological techniques provide a useful classification of muscle fibre types which take into account their rate of contractile twitch and their ability to perform aerobic oxidative metabolism, as follows: slow-twitch oxidative (type I); fast-

Correspondence address: A. Klip, Division of Cell Biology, The Hospital For Sick Children, 555 University Avenue, Toronto, Ontario, Canada, M5G 1X8. Fax: (1) (416) 813-5028.

**Present address:* Dept. of Physiology and Endocrinology, CRML, Laval University Hospital Research Centre, 2705 Boul. Laurier, St-Foy, Qc, Canada G1V 4G2.

twitch oxidative-glycolytic (type IIa) and fast-twitch glycolytic (type IIb).

In the present study we investigated the expression at the mRNA and protein levels of the two α and two β isoforms of the pump present in specific muscles composed primarily of type I fibres (soleus), type I and type IIa fibres (red gastrocnemius), type IIa and type IIb fibres (extensor digitorum longus or EDL) or type IIb fibres (white gastrocnemius). We present novel biochemical evidence for a muscle fibre type-specific expression of β subunit isoforms. The β1 mRNA and protein levels were found to correlate with the oxidative phenotype, whereas the glycolytic, fast twitch phenotype correlated selectively with the β2 isoform. The possible implications that such a specific pattern of expression has for Na^+,K^+-ATPase function in skeletal muscle are discussed.

2. EXPERIMENTAL

2.1. *Muscles*

Soleus, EDL, red or white parts of the gastrocnemius and quadriceps were rapidly excised from anaesthetized, decapitated male Sprague–Dawley rats, frozen in liquid N_2 and stored at −80°C until used for subcellular fractionation or RNA extraction. Upon thawing the muscles were cleaned of all visible fat, connective or nervous tissue.

2.2. *RNA extraction and Northern blotting*

Total RNA was extracted from four separate muscles: soleus, red gastrocnemius, EDL and white gastrocnemius, using the single-step RNA isolation with acid guanidinium thiocyanate-phenol/chloroform extraction [13] RNA was quantitated by its 260/280 UV absorbance using a Hitachi U-200 double-beam spectrophotometer. For Northern blots, 15 μg of total RNA was electrophoresed under denaturing conditions in 1.2% (w/v) agarose gels containing 8% (v/v) formaldehyde. The integrity and relative amounts of RNA were confirmed by visualization of ethidium bromide stained ribosomal RNA under ultraviolet light The RNA was then transferred onto nytron membranes (Schleicher & Schuell, CT) as described [14], which were then baked for 90 min at 80°C in a vacuum oven. Blots were pre-hybridized overnight at 42°C in 6 × SSPE, 10 × Denhardt's, 0.5% SDS, 200 μg/ml salmon sperm DNA. Hybridization was carried out in 50% formamide 6 × SSPE, 0.5% SDS, 100 μg/ml salmon sperm DNA, 10% dextran sulphate for 16 h by adding [α-^{32}P]dCTP (ICN, Ont.) labelled isoform-specific cDNA probes. The α1 cDNA probe was generated after restriction of the full-length α1 cDNA with *Nar*I (Pharmacia, Ont.) and *Stu*I (Promega, WI) (to give a probe comprising nucleotides 89–421). The α2 cDNA probe was prepared by incubating the full-length α2 cDNA with *Sca*I and *Nhe*I (Promega, WI) (to give a probe comprising nucleotides 121–502). cDNA probes for the β1 (nucleotides 1–1600) and β2 (nucleotides 1–1331) isoforms of the Na^+,K^+-ATPase were prepared from their respective full-length cDNA's. These selected sequences do not cross-react with the alternative isoform transcript. All full-length cDNAs were kindly provided by Dr. J. Lingrell, University of Cincinnati. Radioactive labelling of cDNA probes was performed by the random primer method [15]. Following hybridization nytron membranes were subjected to three successive 5 min washes with 1 × SSC, 0.1% SDS at 65°C prior to exposure to Kodak X-Omat film at −80°C.

2.3. *Subcellular fractionation of rat skeletal muscle*

Crude membranes (CM), plasma membranes (PM) and intracellular membranes (IM) were isolated from two sources, soleus, red gastrocnemius and red quadriceps, or white gastrocnemius and white quadriceps, following a procedure used for hindlimb muscles [16] essentially as previously described [17]. We have previously characterized the membranes isolated by both enzymatic and immunological approaches [18,19]. Fractionation of CM on discontinuous sucrose gradients yields the PM fraction which contains plasma membrane markers but not transverse tubules, and the IM fraction which is depleted of cell surface markers and of sarcoplasmic reticulum but contains the intracellular pool of insulin-regulatable GLUT4 glucose transporters [16–20].

2.4. *Western blotting*

Muscle membrane fractions (10 μg) were subjected to SDS-PAGE [21] on 10% precast polyacrylamide gels (Novex, CA). Separated proteins were electrophoretically transferred onto 0.2 μm pore size polyvinylidene difluoride sheets (PVDF, Biorad, CA) and then blocked with 3% BSA/Tris–saline–Tween 20 for 1 h at room temperature prior to incubating overnight at 4°C with either a monoclonal anti-α1 antibody (Mck-1; kind gift from Dr. Kathleen Sweadner, Harvard University, [22]) diluted 1:100, or with polyclonal antibodies to the α2, β1 and β2 subunits (Upstate Biotechnology, NY) of pre-established specificity [11,23], diluted 1:500 PVDF sheets were washed 3 × in Tris–saline–Tween 20 and incubated for 1 h at room temperature with 1 μCi/ml of either [^{125}I]protein A (for anti-α2, -β1 and -β2) or ^{125}I-labelled sheep anti-mouse (for anti-α1), then washed 3 × in Tris–saline–Tween 20 for 15 min, air-dried and exposed to XAR-5 Kodak film at −80°C. Films were quantitated by a PDI DNA scanner with one-dimensional gel analysis software (1.3 discovery)

2.5. *Na^+,K^+-ATPase activity*

Potassium stimulated *p*-nitrophenyl phosphatase (KpNPPase) is an accepted measure of Na^+,K^+-ATPase activity [24] more compatible with skeletal muscle than is ATP hydrolysis. Leaky CM fractions were generated by a freeze/thaw cycle and 20 μg protein were pre-incubated in 50 mM Tris-HCl, pH 7.6, 5 mM $MgCl_2$, 1 mM EDTA in the absence or presence of 20 mM KCl for 5 min on ice The reaction was started by addition of *p*-nitrophenyl phosphate (final concentration 5 mM) and allowed to proceed at for 30 min 37°C, stopped with ice-cold 1 N NaOH and *p*-nitrophenol content quantitated from the sample absorbance at 405 nm.

3. RESULTS AND DISCUSSION

3.1. *Expression of Na^+,K^+-ATPase subunit mRNAs in red and white muscles*

The presence of transcripts of the β subunits of the Na^+,K^+-ATPase was investigated in the soleus (84% type I and 16% type IIa fibres) and EDL (60% type IIa and 37% type IIb) muscles (see [25] for fibre composition). A representative Northern blot is shown in Fig. 1A. The β1 cDNA reacted with a major transcript of 2.7 kb and two very minor ones of 2.3 and 1.9 kb (as seen in kidney and brain, likely arising from differential polyadenylation). These transcripts were detected predominantly in the soleus, whereas β2 transcripts (2.8 kb) were detected mainly in the EDL. In four separate RNA preparations β1 mRNA was found to be 3.1 ± 0.7-fold higher in the soleus than in the EDL muscle, whereas β2 mRNA was at least 8-fold higher in the EDL, being practically not measurable in the soleus. This suggests that the more oxidative and slow-twitch soleus expresses β1 preferentially, whereas the more glycolytic and fast-twitch EDL expresses more selectively the β2 gene. To further differentiate the segregation of these transcripts between oxidative (types I and IIa) and

glycolytic fibres (IIa and IIb) and between slow (type I) and fast (types IIa and IIb) fibres, RNA was isolated from red gastrocnemius (35% type I, 56% type IIa, 9% type IIb) and white gastrocnemius (9% type IIa, 91% type IIb) muscles. RNA from soleus muscle was run in parallel. The results of 2–3 independent experiments are given in Table I, $\beta 1$ mRNA was present at the following relative abundances: soleus > red gastrocnemius >> white gastrocnemius. The $\beta 2$ mRNA was present in soleus << red gastrocnemius < white gastrocnemius. Therefore, $\beta 1$ mRNA appears to segregate with type I and type IIa fibres (oxidative) whereas $\beta 2$ mRNA appears to segregate with type IIa and type IIb fibres (fast and glycolytic). This suggests that type I fibres express the $\beta 1$ gene, type IIb fibres the $\beta 2$ gene, and type IIa fibres both products.

Hence, $\beta 1$ expression correlates with the oxidative phenotype and $\beta 2$ with the glycolytic phenotype. The $\beta 1$ mRNA does not correlate simply with the slow-twitch phenotype since it is relatively abundant in red gastrocnemius muscle, which contains only 35% slow-twitch fibres, and is reduced but present in EDL muscle, which does not contain slow-twitch fibres. Similarly, the $\beta 2$ mRNA does not correlate simply with the fast-twitch phenotype since it is negligible in soleus muscle which contains 16% fast-twitch fibres, and does correlate with the proportion of fast-twitch fibres in the red gastrocnemius.

In contrast to the striking difference in expression of $\beta 2$ subunit transcripts in different muscles, mRNA of α subunits were found in all muscles investigated (Fig. 1B). No statistically significant differences in $\alpha 1$ mRNA (3.7 kb) abundance were found between soleus and EDL, but $\alpha 2$ mRNA (5.3 and 3.4 kb) was more abundant in the former. Densitometric scanning of 4 individual experiments revealed that $\alpha 2$ gene expression was 3.76 ± 0.82-fold higher in soleus than in EDL ($P < 0.05$, Student's t-test). The contents of $\alpha 1$ mRNA in the soleus, red gastrocnemius and white gastrocnemius are shown in Table I. The more glycolytic the muscle, the lower the content of $\alpha 1$ or $\alpha 2$ mRNA was, with no clear differences seen between slow and fast twitch muscles. However, these differences were modest compared with the striking segregation of β subunit gene expression.

Table I

Relative proportions of β and α subunit mRNA in rat skeletal muscles

Isoform	Muscle		
	Soleus	Red gastrocnemius	White gastrocnemius
$\beta 1$	1.00 ± 0.17	0.69 ± 0.09	0.13 ± 0.04
$\beta 2$	0.04 ± 0.01	0.47 ± 0.03	1.00 ± 0.50
$\alpha 1$	1.00 ± 0.16	0.95 ± 0.08	0.70 ± 0.11
$\alpha 2$	1.00 ± 0.09	0.92 ± 0.07	0.69 ± 0.01

Total RNA was extracted from individual muscles and subjected to Northern blots using cDNA probes specific for the $\beta 1$, $\beta 2$, $\alpha 1$ or $\alpha 2$ mRNAs. RNA from 2–3 independent extractions was run simultaneously. Autoradiograms were scanned and the abundance of each transcript is given in relative units, assigning a value of 1.00 for each subunit to the muscle with the highest content. Valid comparisons can be made for each subunit isoform between the different muscles, but not between subunit isoforms.

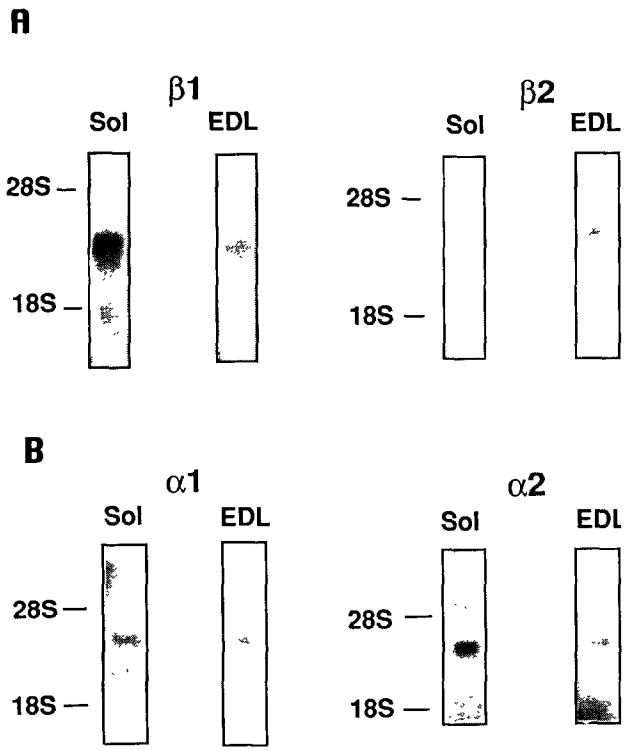

Fig. 1. Representative Northern blots of $\beta 1$ and $\beta 2$ mRNA (A) and $\alpha 1$ and $\alpha 2$ mRNA (B) in soleus and EDL muscles. Total RNA was isolated from soleus or EDL muscles and analyzed as described in section 2. The position of the 18S and 28S ribosomal RNA is indicated. Sol, soleus; EDL, extensor digitorum longus.

3.2. *Abundance of Na^+,K^+-ATPase subunit proteins in red and white muscles*

In order to investigate whether the isoform mRNA distribution was paralleled by the expression of the corresponding proteins, membrane fractions were isolated from specific muscles. Due to methodological requirements for at least 2 g of material per muscle fractionation, it was not possible to investigate separately the soleus, EDL, red or white gastrocnemius muscles. Instead, red muscles were pooled (soleus, red gastrocnemius and red quadriceps), separately from white muscles (white gastrocnemius and white quadriceps). Considering the fibre composition of each muscle and the mass contribution of each one to the mixture of muscles, the red muscle pool consisted of approximately 40% type I, 42% type IIa and 18% type IIb fibres, i.e. largely oxidative but with almost equal content of fast and slow fibres. In contrast, the white muscle pool consisted of 8% type IIa and 92% type IIb fibres, i.e. predominantly glycolytic and fast-twitch muscle fibres. Crude (unfrac-

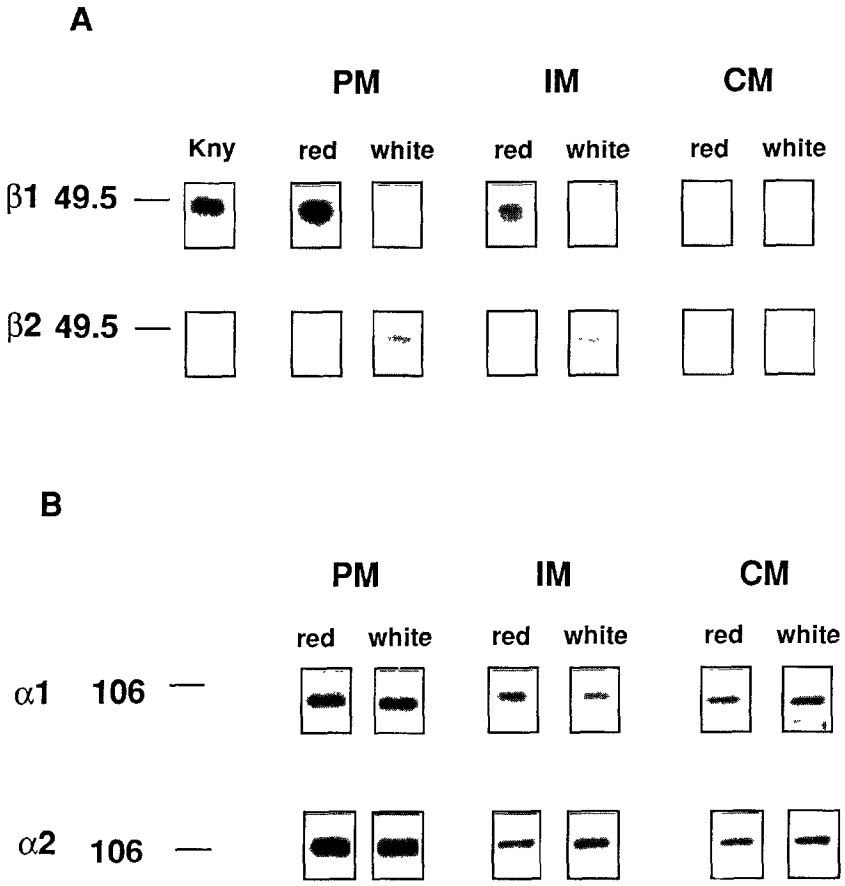

Fig. 2. Representative Western blots of $\beta 1$ and $\beta 2$ protein (A) or of $\alpha 1$ and $\alpha 2$ protein (B) in plasma membranes (PM), intracellular non-sarcoplasmic reticulum membranes (IM) and crude unfractionated membranes (CM) of pooled red or white muscles. Isolated membranes were probed as described in section 2. At the exposure time used, the β subunits are not detectable in the CM. The position of the closest molecular weight standards are indicated on the lefthand side. Kny represents a kidney microsomal sample used as standard of specificity for the anti-β antibodies.

tioned) membranes (CM), and purified PM and IM were isolated and Na^+,K^+-ATPase subunits were detected immunologically on Western blots. Fig. 2A shows a greater content of $\beta 1$ protein in the PM and IM fractions of the red than white pooled muscles, consistent with the distribution of $\beta 1$ transcripts in oxidative muscles. Quantitative analysis indicated that this protein was nearly 5-fold more abundant in the PM of red than of white muscles. In contrast, the $\beta 2$ protein was approximately 3-fold more in the PM and IM of the white than of the red muscles, consistent with the distribution of $\beta 2$ transcripts in glycolytic, fast-twitch muscles. At this level of exposure of the gels, neither β subunit is detectable in CM, highlighting the usefulness of subcellular fractionation.

Analysis of the content of $\alpha 1$ and $\alpha 2$ subunit proteins revealed no major differences between pooled red and white muscles, whether in the PM, the IM or the total CM (Fig. 2B). This observation was surprising given the higher expression of $\alpha 2$ mRNA in soleus (red, slow) than in EDL (white, fast) muscles (Fig. 1B). The difference may arise from the different muscles required for the membrane preparation. Alternatively, $\alpha 2$ mRNA may be under post-transcriptional control in red muscle. Indeed, discordant $\alpha 1$ mRNA and protein expression has been reported for other cell types [26]. Furthermore, a recent in vitro study showed that α subunit mRNA may not compete favourably with other mRNA transcripts for the translational machinery [27]. This was attributed to the complex 'loop' structures formed by the 5'-untranslated (UT) region upstream from the coding sequence. The 5'-UT region contains a GC rich segment which can potentially generate stable secondary structures disrupting scanning by the 40S initiation complex [27]. If similar post-transcriptional control occurs for $\alpha 2$ mRNA as well, it migh allow red muscle fibers to modify the synthesis of $\alpha 2$ protein in conditions affecting the contractile and metabolic status of the muscle (e.g. muscle fatigue, hypoxia).

3.3. *Implications of the selective* β *subunit expression in specific muscle fibre types*

It is not known whether the formation of a particular $\alpha\beta$ complex results in Na^+,K^+-ATPase molecules of distinct activity. Results from four separate preparations of CM from pooled red muscles revealed a modestly

higher KpNPPase activity than in CM of pooled white muscles (0.865 ± 0.051 vs. 0.618 ± 0.055 μmol/h·mg protein; $P < 0.02$). This suggests that both β isoforms can sustain functional ATPase activity. The small but significant difference between both groups of muscles may be due to either differential ability of the β isoforms to support activity or to quantitative differences in the net content of functional dimers. Present technology does not allow us to calculate the molar content of $\alpha 1$ and $\alpha 2$ isoforms in muscle membranes. In our study the abundance of either $\alpha 1$ or $\alpha 2$ proteins in isolated PM was not significantly different between pooled red and white muscles. EDL biopsies have slightly (20–30%) more ouabain binding sites than soleus biopsies [28] under conditions which measure only the $\alpha 2$ isoform, i.e. the component saturable below 1 μM ouabain. However, both muscles have comparable ouabain-suppresible Rb^+ uptake [29]. These results again support the tenet that both β isoforms are capable of sustaining pump activity.

Based on the results presented we propose that slow oxidative (type I) fibres contain $\alpha 1\beta 1$ and $\alpha 2\beta 1$ complexes, fast glycolytic (type IIb) fibres contain $\alpha 1\beta 2$ and $\alpha 2\beta 2$ heterodimers, and fast oxidative-glycolytic (type IIa) fibres can form all four combinations. Future studies should address the proportion of each complex within different fibres. Definitive proof of the exact fibre-type specific expression of $\beta 1$ and $\beta 2$ will require immunocytochemical localization of the subunits. Good antibodies compatible with the latter technique are presently unavailable. In situ hybridization may be required coupled to analysis of fibre type in serial sections.

The precise role of the β subunit in Na^+,K^+-ATPase function is presently a matter of intense investigation. Expression studies in *Xenopus* oocytes suggest that the β subunit is required for the correct assembly and export of mature Na^+,K^+-ATPase units to the PM [10,30]. Such studies have highlighted the susceptibility of unassociated α subunits to degradative enzymes and show that association with β subunits provides stability to the catalytic subunit [31].

The observation that the $\beta 2$ isoform is expressed only in fast glycolytic muscle fibres is intriguing given that this isoform has recently been identified as the adhesion molecule on glia cells (AMOG) [32]. AMOG is thought to function as a neuronal receptor recognition site facilitating neuron–astrocyte adhesion. Oocyte expression studies have shown that AMOG can interact with the $\alpha 1$ subunit to form $\alpha 1$–$\beta 2$ (AMOG) complexes capable of mediating ouabain-sensitive Rb^+ uptake [33]. However, $\alpha 1$–$\beta 2$ (AMOG) complexes expressed in oocytes were functionally less active than $\alpha 1$–$\beta 1$ complexes when identical amounts of cRNA for $\alpha 1$, AMOG and $\beta 1$ were injected into oocytes [33]. Assuming that the cRNA of AMOG and $\beta 1$ were equally expressed, these in vitro observations imply that $\beta 1$ subunits confer higher functional activity than $\beta 2$ (AMOG), a proposition consistent with our observation of higher Na^+,K^+-ATPase activity in red than white muscles. An attractive hypothesis proposes that the $\beta 2$ (AMOG) isoform may fulfill a dual role linking its receptor function to cation transport, whereby the binding of receptor ligands would induce conformational constraints in $\beta 2$ (AMOG) subunit and its associated α subunit with consequential effects on Na^+,K^+-ATPase activity [33].

In summary, red muscles rich in oxidative fibres, whether slow- or fast-twitch, express $\beta 1$ subunits, and white muscles rich in glycolytic fast-twitch fibres, express $\beta 2$ (AMOG) subunits. Both muscle groups have similar levels of $\alpha 1$ and $\alpha 2$ subunit proteins although red muscles have a higher proportion of $\alpha 2$ mRNA than white muscles do. Both $\beta 1$ and $\beta 2$ (AMOG) can support K-pNPPase activity and in isolated membranes this activity is higher for red than for white muscles.

Acknowledgements. We are grateful to Dr. Yasuhide Mitsumoto for advice and useful discussions, and to Dr. Louis Lavoie for comments to this manuscript. H.S.H. was supported by a long-term research fellowship from the International Human Frontier Science Program and is presently a Wellcome University Lecturer. A.M. was supported by a fellowship from the Medical Research Council, Canada. This work was supported by a Medical Research Council of Canada grant to A.K.

REFERENCES

[1] Schwartz, A., Lindenmayer, G.E. and Allen, J.C. (1975) Pharm. Rev. 27, 3–134.
[2] Jorgensen, P.L. (1982) Biochim. Biophy. Acta. 694, 27–68.
[3] Christensen, H.N. (1990) Physiol. Rev. 70, 43–77.
[4] Moore, R.D. (1983) Biochim. Biophys. Acta 737, 1–49.
[5] Klip, A. (1988) in: Na/H Exchange (Grinstein, S. ed.) pp. 285–303, CRC Press, FL.
[6] Clausen, T. (1985) in: Regulatory Peptides, pp. 59–67, Elsevier Science, Netherlands.
[7] Sweadner, K.J. (1989) Biochim. Biophys. Acta. 988, 185–220
[8] Horisberger, J.-D., Jaunin, P., Good, P.J., Rossier, B.C. and Geering, K. (1991) Proc. Natl. Acad. Sci. USA 88, 8397–8400.
[9] Horisberger, J.-O., Lemas, V., Kraehenbuhl, J.-P. and Rossier, B.C. (1991) Annu. Rev. Physiol. 53, 565–584.
[10] Geering, K. (1991) FEBS Lett. 285, 189–193.
[11] Hundal, H S., Marette, A., Mitsumoto, Y., Ramlal, T., Blostein, R and Klip, A. (1992) J. Biol. Chem. 267, 5040–5043.
[12] Pette, D. and Staron, R.S. (1990) Rev. Physiol. Biochem. Pharmacol. 116, 1–76.
[13] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. Pharmacol. 162, 156–159.
[14] Sambrook, J., Fritsch, E.F and Maniatis, T. (1989) Molecular Cloning· A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.
[15] Feinberg, A.P and Vogelstein, B. (1983) Anal. Biochem. 132, 6–13
[16] Klip, A., Ramlal, T., Young, D A. and Holloszy, J.O. (1987) FEBS Lett. 224, 224–230.
[17] Marette, A., Richardson, J.M., Ramlal, T., Balon, T.W., Vranic, M., Pessin, J.E. and Klip, A. (1992) Am. J. Physiol. 263, C443 C452.
[18] Douen, A., Burdett, E., Ramlal, T., Rastogi, S., Vranic, M. and Klip, A. (1991) Endocrinology 128, 611–616.
[19] Marette, A., Hundal, H.S. and Klip, A (1992) in: Diabetes Mel-

litus and Exercise (Devlin, J., Horton, E.S and Vranic, M. eds.) pp. 27–43, Smith-Johnson, London.
[20] Marette, A., Burdett, E., Douen, A.G., Vranic, M. and Klip, A. (1992) Diabetes 41, 1562–1569.
[21] Laemmli, U.K. (1970) Nature 227, 680–685.
[22] Felsenfeld, D.P. and Sweadner, K.J. (1988) J. Biol. Chem. 263, 10932–10942.
[23] Shyjan, A.W. and Levenson, R. (1989) Biochemistry 28, 4531–4535.
[24] Bers, D.M. (1979) Biochim Biophys. Acta 555, 131–146
[25] Koerker, D.J., Sweet, I.R. and Baskin, D.G. (1990) Am. J. Physiol. 259, E517–E523.
[26] Tamkun, M M. and Fambrough, D. (1986) J. Biol. Chem. 261, 1009–1019.
[27] Devarajan, P., Gilmore-Herbert, M. and Benz Jr., E J (1992) J. Biol. Chem. 267, 22435–22439.
[28] Norgaard, A., Kjeldsen, K , Hansen, O. and Clausen, T (1983) Biochem. Biophys. Res Commun. 111, 319–325.
[29] Everts, M.E and Clausen, T. (1992) Acta Physiol Scand. 145, 353–362.
[30] Ackermann, U. and Geering, K. (1990) FEBS Lett 269, 105–108
[31] Geering, K., Kraehenbuhl, J -P. and Rossier, B.C (1987) J. Cell Biol. 105, 2613–2619.
[32] Gloor, S., Antonicek, H , Sweadner, K.J., Pagliusi, S., Frank, R , Moos, M. and Schachner, M (1990) J Cell Biol. 110, 165 174.
[33] Schmalzing, G., Kroner, S., Schachner, M. and Gloor, S. (1992) J. Biol. Chem. 267, 20212–20216.