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ORIGINAL PAPER

Ion channels and ion transporters of the transverse tubular system of skeletal muscle

Karin Jurkat-Rott · Michael Fauler · Frank Lehmann-Horn

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Abstract This review focuses on the electrical properties of the transverse (T) tubular membrane of skeletal muscle, with reference to the contribution of the T-tubular system (TTS) to the surface action potential, the radial spread of excitation and its role in excitation-contraction coupling. Particularly, the most important ion channels and ion transporters that enable proper depolarization and repolarization of the T-tubular membrane are described. Since propagation of excitation along the TTS into the depth of the fibers is a delicate balance between excitatory and inhibitory currents, the composition of channels and transporters is specific to the TTS and different from the surface membrane. The TTS normally enables the radial spread of excitation and the signal transfer to the sarcoplasmic reticulum to release calcium that activates the contractile apparatus. However, due to its structure, even slight shifts of ions may alter its volume, *Nernstian* potentials, ion permeabilities, and consequently T-tubular membrane potential and excitability.

Keywords Skeletal muscle · Transverse tubular system · T-tubular membrane · Ion channels · Ion transporters · Excitation-contraction coupling · Electrical membrane bistability · Potassium depletion · Periodic paralyses · Potassium accumulation · Muscle weakness · Muscle fatigue · Caveolae

K. Jurkat-Rott (✉) · M. Fauler · F. Lehmann-Horn
Department of Physiology, Ulm University,
D-89081 Ulm, Germany
e-mail: karin.jurkat-rott@uni-ulm.de

Introduction

The primary task performed by the electrically excitable membrane of a skeletal muscle fiber is the activation of the contractile machinery in response to signals received from the motor nerve. Endplate potentials generate the action potentials that propagate along the surface membrane. From there, excitation spreads along the T-tubular system (TTS) into the depth of the fibers thereby achieving uniform distribution of activation. This is a prerequisite for the synchronization of contraction which is initiated by intracellular calcium release brought about by direct interaction of channel proteins of TTS and sarcoplasmic reticulum (SR).

Common basis for the electrical properties are transmembrane proteins that act as ion channels or ion transporters. Ion channels are equipped with a membrane-spanning ion-conducting pathway and channel gates (Fig. 1). They activate, i.e. open, in response to ligands, transmitters or voltage changes and inactivate, i.e. close, by a usually intrinsic inactivation process. Voltage-gated channels contain additional voltage-sensing transmembrane segments and are essential for the generation and modification of the action potentials. In contrast, ligand-gated ion channels are essential for setting myoplasmic calcium concentration and establishing signal transduction pathways. Ion transporters are electrogenic or electroneutral and are driven by ATP hydrolysis or ion gradients (Fig. 2).

More detailed reviews on related topics such as expression of ion channels and ion transporters of skeletal muscle (Jurkat-Rott and Lehmann-Horn 2004), excitation-contraction coupling of skeletal muscle (Dulhunty et al. 2002), and the reversible

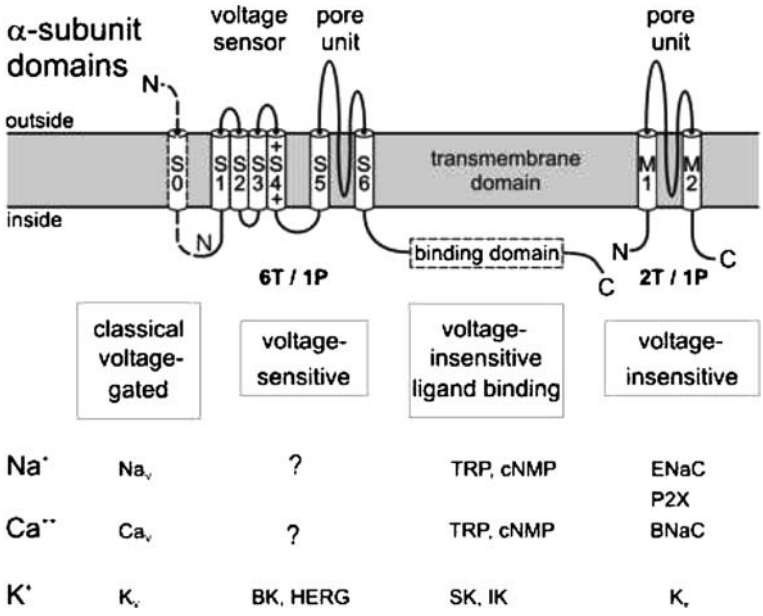

Fig. 1 Diversity of domains forming cation channel alpha subunits. The most simple domain, typically used for inward going rectifier potassium channel alpha subunits, is a pore unit (2T/1P) that consists of two transmembrane segments M1 and M2, an extracellular loop dipping into the membrane and lining the pore, and intracellular N- and C-terminals. The transmembrane segments are thought to be alpha helices. All voltage-gated alpha subunit domains are 6T/1P domains since they contain a four transmembrane segment units S1 to S4 acting as voltage sensor and the two transmembrane pore unit. S4 is the particular voltage-sensing segment that contains positive charges at each third amino acid residue. Ligand-gated cation channel alpha subunit domains usually possess a C-terminal binding site in addition to the 6T/1P domain. Although some ligand-gated channels, e.g., the calcium-activated SK potassium channel, contain a positively charged S4 segment they are not voltage-sensitive at all, maybe due to uncoupling of sensor and activation gate. BK potassium channels possess an additional S0 segment. Not expressed in skeletal muscle, but completing the classification of the alpha subunit domains are: HERG, a potassium channel encoded by the human ether-a-go-go related gene which is similar to the *drosophila* ether-a-go-go gene (eag); IK, a calcium-activated potassium channel with intermediate conductance; cNMP or CNG, sodium or calcium channels gated by cyclo-nucleotide monophosphates such as cGMP; and voltage-insensitive sodium channels of epithelial cells (ENaC) and in free nerve terminals of the brain (BNaC). BNaC was later identified to conduct calcium. Not shown is another group of alpha subunit domains, 4T/2P, which contain four transmembrane segments and two pore units but do not occur in skeletal muscle

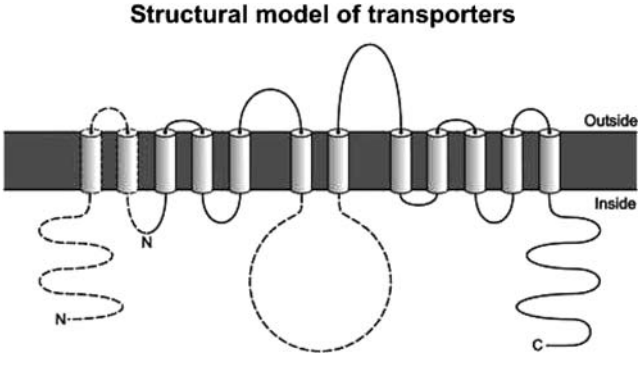

Fig. 2 Scheme of the membrane topology of the alpha subunits of ion transporters. The protein contains 10 or 12 transmembrane segments which form alpha helices. The sodium pump alpha subunits contain 10 segments, their long intracellular loop possesses the catalytic and transport units of the enzyme and the binding sites for the cations and ATP. Ouabain binds to the first extracellular loop. The cation-chloride cotransporters NKCC and KCC contain 12 transmembrane segments. They are driven by the sodium gradient established by the sodium pump. They need neither a catalytic unit nor ATP binding sites and the intracellular loop connecting segments 6 and 7 are short

vacuolation of T tubules (Krolenko and Lucy 2002) are available.

The TTS

During normal development of skeletal muscle, the surface membrane invaginates to form T tubules and caveolae. In adult skeletal muscle fibers, the T tubules have an overall transverse orientation; continuously across the fiber, completely encircling myofibrils, and located either singularly at the Z line (e.g. frog muscle) or as pair at the A-I junction level (mammalian muscle) (Franzini-Armstrong and Jorgensen 1994). A contraction initiated by local depolarization of a T tubule opening spreads transversely with increase in stimulus strength. Exposure to and subsequent withdrawal from glycerol induces swelling and vacuolization of T tubules and interruption of their continuity. The concomitant block of excitation–contraction coupling strongly confirms the role of T tubules as

important links between surface depolarization and SR Ca^{2+} release (Krolenko and Lucy 2002).

In most vertebrate muscles, the T tubules open via convoluted T tubules and/or caveolae (Franzini-Armstrong 2004). This has suggested the possibility of a high access resistance to the T-tubule lumen. Caveolae are relatively immobile membrane invaginations generated by polymerized caveolins clustered with cholesterol (Thomsen et al. 2002) and may constitute a specialized type of the more generic lipid rafts, or dynamic assemblies of proteins and lipids floating freely in the liquid bilayer and regulating cellular membrane function (Simons and Ehehalt 2002). Cholesterol depletion alters channel function by inducing a hyperpolarizing shift in the voltage dependence of activation and inactivation. Additionally, volume-regulated anion channels (Trouet et al. 1999) and sodium channels (Yarbrough et al. 2002) are localized to caveolae. In contrast, calcium channels have not been identified there, but an association with the development of calcium sparks has been described (Lohn et al. 2000).

The surface area of these two components fully accounts for the much larger electrical capacitance of a muscle fiber compared to nerve. However, the reason for the abundance of caveolae in muscle remains a mystery. The extracellular space of skeletal muscle is about 8% (Ling and Kromash 1967) including the TTS which is only 0.32% of the fiber volume itself (Mobley and Eisenberg 1975). Since the diffusion in the T tubules is slow (~85 $\mu m^2/s$ for K^+), they constitute a functional compartment (Swift et al. 2006).

The muscle fiber action potential

The condition for initiating an action potential is that the net membrane current be inward. The potential at which this condition is reached is termed the ''threshold potential'' and is, under normal conditions, always exceeded by the end plate potential in mammalians. The upstroke of the action potential is mediated by opening of the voltage-gated sodium channels that passively conduct a fast sodium inward current in a feed forward mechanism along both electrical and concentration gradient. Due to the resulting high conductance of the membrane for sodium ions, the membrane suddenly depolarizes from the resting value of –84 mV to approximately + 25 mV. As in the action potential of nerve, partial membrane repolarization is yielded by fast inactivation of the sodium channels, resulting in the usual predominance of the membrane conductance for potassium ions. In contrast to nerve, delayed rectifying potassium channels are more or less replaced by chloride channels in skeletal muscle. This high chloride conductance enforces the final repolarization of the skeletal muscle fiber membrane. Compared to the nerve action potential which shows an afterhyperpolarization, the repolarization of skeletal muscle is much slower, a phenomenon called afterdepolarization which consists of an early and late part mediated by different ionic currents.

The early part of the afterdepolarization is considered to be caused by the spreading of the spike in the depth of the TTS, the late afterdepolarization by an accumulation of potassium ions in the TTS which increases with frequency and duration of repetitive action potentials (Almers 1980).

Contribution of the TTS to the surface action potential

An experiment in which this has been first revealed was a comparison of action potentials in normal and glycerol-treated fibers (Gage and Eisenberg 1969a, b). When muscles were returned to normal Ringer's solution, the T tubules became disconnected from the surface membranes and from the extracellular solution and the membrane capacitance was drastically reduced. Although fibers so treated no longer contract in response to electrical stimulation, propagated surface action potentials can be elicited in which the afterdepolarization is absent. Therefore, the afterdepolarization reflects events taking place in the TTS. The type of radial spread along the TTS differs between species. Radial electrotonic spreading occurs in some species. In mammalian muscle, propagation is based on T-tubular action potentials as in the surface membrane as theoretical calculations earlier had demanded (Adrian and Peachey 1973).

Although longitudinal tubules during maturation of the TTS disappear, few such elements do remain and allow the spread of action potentials in adult muscle. This could be a mechanism to ensure that the TTS gets excited even if there is fibre swelling and vacuole formation (Posterino et al. 2000).

Ion channels of the TTS

The voltage-gated sodium channel, Nav1.4

The precise control of opening and closing of the sodium channel is necessary for the regulation of an action potential and the excitability of nerve and muscle cells. The sodium channel complex of adult

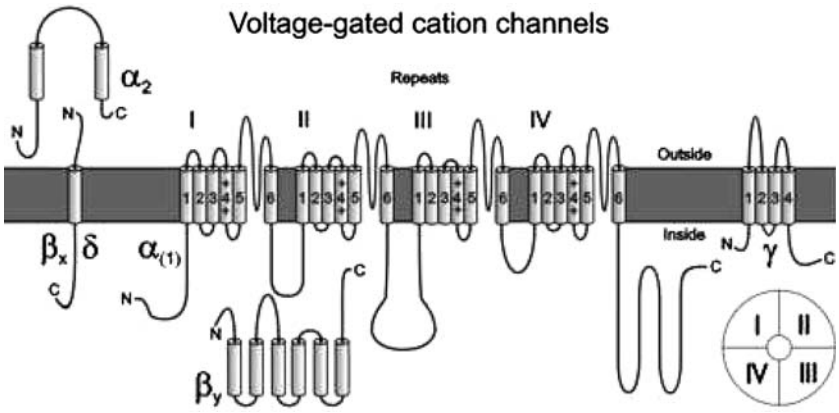

Fig. 3 Scheme of the membrane topology of the voltage-gated cation channels. The alpha1 subunit consists of four repeated domains of six transmembrane segments each including the voltage sensor unit. When inserted in the membrane, the four domains fold to generate a central pore as schematically indicated on the right-hand bottom of the figure. The four domains are encoded by a single sodium (or calcium) channel gene, whereas potassium channel genes code for only one domain so that the channel alpha subunit is a homotetramer or a heterotetramer, if the products of different channel genes assemble. Auxilliary subunits alpha2/delta, beta-1 to beta-*n*, and gamma are optional intracellular, transmembrane or extracellular proteins. Intracellular beta subunits bind to the N- or C-terminal or to an intracellular loop. Alpha2 is a calcium channel subunit that was misnamed: it was originally thought to possess an ion conducting pore as expression in cells devoid of functional calcium channels resulted in an appreciable calcium current. In the meantime, this phenomenon can be explained by the drastic increase in expression of endogenous alpha1 subunits by coexpression of the alpha2/delta subunit

(innervated) skeletal muscle consists of a tissue-specific alpha-subunit, Nav1.4, and a ubiquitously expressed beta1 subunit with modifying effects. The alpha subunit is a quadrefoil assembly of a series of six transmembrane amphipathic alpha-helical segments, numbered S1–S6, connected by both intracellular and extracellular loops, the interlinkers (Fig. 3). It contains the ion-conducting pore and determines main characteristics of the cation channel complex conveying ion selectivity, voltage sensitivity (S4 segments), pharmacology and binding characteristics for endogenous and exogenous ligands.

The distribution of sodium channels over the surface of a muscle fiber is not homogenous (Bailey et al. 2003). Channel density varies up to 3-fold over distances of 10–30 μm and is high surrounding the endplate and low at the ends of the fibers. There is no indication of a specific location either relative to the sarcomere intervals nor to the openings of T tubules, nor is there any information on how closely clustered the channels are. The lower sodium channel density in the TTS than in the surface membrane results in a smaller excitatory inward sodium current that suggests that the potassium channel population in the TTS must differ from the surface membrane as well. To reduce outward potassium current in the TTS, inwardly rectifying potassium channels which conduct almost no outward current during depolarization are predominantly expressed in the TTS (Kristensen et al. 2006). Mutations in Nav1.4 cause myotonic disorders and periodic paralyses (Jurkat-Rott et al. 2000; Lehmann-Horn and Jurkat-Rott 1999).

Kir2.1, the major inwardly rectifying potassium channel of TTS

Several of the *KCNJ* genes are expressed in the surface and T-tubular membrane of skeletal muscle (see Table 1). They encode the most simple potassium channels consisting of two transmembrane segments, the linking loop lining the pore as well as the N- and C-termini. They do not possess gates and therefore cannot activate or inactivate. The strength of the rectification is determined by specific amino acid residues in the second transmembrane segment near the cytoplasmic side of the membrane, whereby a negatively charged aspartate confers strong rectification and a neutral asparagine confers weak rectification (Wei et al. 1996). The major skeletal muscle inward-going rectifier, Kir2.1, has a highly conserved TIGYG motif in the pore region and the absence of the peculiar lysine residue in the N-terminal explains its pH insensitivity. The channel block by Mg^{2+}, spermine, spermidine, and putrescine may be linked to amino acid D172. The RRESEI motif situated in the C-terminal is a candidate for the observed modulation by phosphatases (Kamouchi et al. 1997).

To excite a cell whose resting potential is stabilized by an inward rectifier, a depolarizing stimulus must reach the threshold at which the inward rectifier is sufficiently blocked. Then the potassium current will decline while the sodium current rapidly reaches its maximum along with fast depolarization (action potential). Obviously, the inward rectification is an

Table 1 Ion channels genes expressed in the surface and/or T-tubular membrane of skeletal muscle

Gene	Gene locus	Channel protein	Channel properties	Significance for muscle function	Disease
Voltage-gated potassium channels					
KCNA4	11p14	alpha subunit Kv1.4	fast activation, fast inactivation	maybe modifies action potential	
KCNC4	1p21	alpha subunit Kv3.4	fast activation, fast inactivation	maybe modifies action potential	(dominant deafness due to hair cell dysfunction)
KCNQ5	6q14	alpha subunit Kv7.5	slow activation, slow inactivation	reduces subthreshold excitability	
Voltage-insensitive potassium channels					
KCNJ2	17q23	alpha subunit Kir2.1 (IRK1) high density in T tubules	inward going rectifier, Andersen syndrome	membrane potential stabilization, potassium reuptake, muscle endurance	
Calcium-activated potassium channels					
KCNMA1	10q22–23	alpha subunit KCa1.1 (BK) high density in T tubules	voltage sensitive	involved in muscle fatigue	(Cerebellar ataxia in -/- mice, generalized epi-lepsy at gain-of-function)
KCNN2	5q22	alpha subunit KCa2.2 (SK2)	insensitive to voltage	maybe terminating bursts of action potentials, open at high intracellular calcium concentration	
KCNN3	1q21	alpha subunit KCa2.3 (SK3)	insensitive to voltage		upregulated in denervated and myotonic dystrophy fibers
voltage-gated sodium channels					
SCN4A	17q23–25	alpha subunit $Na_v1.4$, Skm1, $\mu1$	major sodium channel of adult muscle, TTX sensitive	essential for action potential generation in plasmalemm and T-tubular membrane	hyperkalemic periodic paralysis, paramyotonia congenita, potassium-aggravated myotonia, hypokalemic periodic paralysis type 2
SCN5A	3p21	alpha subunit $Na_v1.5$, Skm2	predominant in fetal muscle, TTX insensitive	excitability of fetal muscle	(long QT syndrome 3)
SCN6A = SCN7A	2q21–23	alpha subunit $Na_v1.6$	probably in denervated muscle, TTX insensitive	excitability of denervated muscle	
SCN1B	19q13	beta subunit Na_vbeta1	increases current, accelerates fast inactivation	potential influence on action potential	(generalized epilepsy with febrile seizures plus type 1 ($GEFS^+1$))
SCN3B	11q24.1	beta subunit Na_vbeta3	facilitates fast gating mode	potential influence on action potential	
voltage-gated calcium channels					
CACNA1H	16p13	$Ca_v3.2$, T-type alpha1H subunit	rapid activation and inactivation, small conductance	transient expression during fetal development	
CACNA1S	1q31–32	$Ca_v1.1$, L-type, DHP receptor, alpha1S sub-unit	major calcium channel of adult muscle	essential for ECC, expressed on T tubules	hypokalemic periodic paralysis type 1, malignant hyperthermia 5, dysgenic mice
CACNA2D1	7q21–22	alpha2/delta subunit	increases current	unknown	
CACNB1	17q21–22	beta1 subunit	increases current	essential for ECC	-/- mice lethal

Table 1 continued

Gene	Gene locus	Channel protein	Channel properties	Significance for muscle function	Disease
CACNB3	12q13	beta3 subunit			
CACNG1	17q24	gamma subunit 1	increases inactivation	probably low (-/- mice normal)	
voltage-gated chloride channels					
CLCN1	7q32–qter	ClC1, high density in surface and T-tubular membrane	outward rectifier, deactivating at hyperpolarization	stabilizes membrane potential due to high conductance	myotonia congenita Thomsen and Becker
CLCN2	3q27–28	ClC2	slowly activating inward rectifier	ubiquitous, reduces fiber swelling	(idiopathic generalized epilepsy)

Diseases other than those affecting the skeletal muscle are given in brackets. ECC = excitation–contraction coupling

important channel property preventing large potassium losses from active muscle and it also facilitates fast cell activation. It improves muscle endurance, since it reduces potassium accumulation in the TTS (Kristensen et al. 2006; Wallinga et al. 1999).

K_{ATP}, the inwardly rectifying potassium channel activated at energy depletion

Two skeletal muscle inward going rectifiers are activated by depletion of intracellular ATP: $K_{ir}6.1$ (or KATP1) and $K_{ir}6.2$ (or KATP2). $K_{ir}6.1$ is ubiquitous, whereas $K_{ir}6.2$ is relatively specific for pancreatic islet cells, brain, and skeletal muscle. The density of these channels in muscle is about as high as that of the voltage-gated potassium channels. Both channels interact in the skeletal muscle with the sulfhydrylurea receptor SUR2B forming hetero-octameric K_{ATP} channel complexes with a $(SUR2B\text{-}K_{ir}6.x)_4$ stoichiometry and a tetrameric pore of 76 pS in the fully open state (Ämmälа et al. 1996). The distribution of the K_{ATP} channels is similar to the sodium pump, i.e. is higher in the surface membrane and in vesicles than in the T tubules and, in contrast to in-vitro conditions, the channels are active in vivo at rest (Nielsen et al. 2003).

$K_{Ca}1.1$, the calcium-activated potassium channel of adult muscle TTS

$K_{Ca}1.1$ is known as the large conductance (>150 pS with symmetrical high potassium concentrations) calcium-activated channel or B_K (B for big), maxi K, or SLO channel. In contrast to all other K_{Ca} channels, $K_{Ca}1.1$ possesses an additional transmembrane segment, called S0, resulting in an extracellular N-terminus. In contrast to the other K_{Ca} channels, it shows voltage-dependence in addition to the calcium sensitivity (Lerche et al. 1995). It has been suggested that the binding of Ca^{2+} activates the channel by attenuating the inhibitory activity of the C-terminal (Schreiber et al. 1999). Western Blotting revealed a markedly higher expression level in T tubules than in the surface membrane (Nielsen et al. 2003). $K_{Ca}1.1$ requires a large depolarization and a high intracellular calcium concentration for activation since its beta subunit which markedly increases the sensitivity to intracellular calcium (Tseng-Crank et al. 1996) is not present in skeletal muscle. Therefore, activation of $K_{Ca}1.1$ in TTS might only occur during a burst of action potentials and its hyperpolarizing effect may terminate the burst and thus reduce tetanic muscle strength protecting the muscle from overexertion.

$K_{Ca}2.1$, $K_{Ca}2.2$ and $K_{Ca}2.3$

These are calcium-sensitive potassium channels with small potassium conductance (SK1-3; approximately 10 pS at symmetrical potassium concentrations). They possess a typical 6T/1P alpha subunit structure with positive residues in S4 at almost every third amino acid, but do not show voltage-dependent gating. $K_{Ca}2.2$ and $K_{Ca}2.3$ are present in mature skeletal muscle, the latter at low level which is markedly increased in denervated and myotonic dystrophy muscle (Renaud et al. 1986) and involved in the generation of the myotonia that can be blocked by apamin (Behrens et al. 1994). According to a model, SK channel activity in the T tubules of denervated skeletal muscle causes a local increase in potassium ion concentration that results in hyperexcitability (Neelands et al. 2001).

Kv channels

Some voltage-gated potassium channels operate in the subthreshold range of an action potential and are thought to assist in maintaining the resting potential and to modulate electrical excitability. These are

mainly the fast and slowly inactivating Kv channels some of which are expressed in skeletal muscle (see Table 1). Therefore, at least some of these channels are necessary for the repolarization phase of action potentials in the TTS as well.

Fast inactivating Kv channels

These ''A-type'' currents with fast ''N-type inactivation'' operate in the subthreshold range of an action potential. Most of these channels are non-functional at low extracellular potassium ion concentration; at 1–2 mM there is a ~50% current reduction (Pardo et al. 1992; Lopez-Barneo et al. 1993; Jäger et al. 1998). Potassium is bound to a ''pocket'' at or in the vicinity of the TEA binding site. The two skeletal muscle channels show the potassium dependency: Kv1.4 which is present in skeletal muscle at only low expression level shows the highest potassium sensitivity; and Kv3.4, with mRNA levels being five-to-six-fold lower in the soleus muscle than in fast muscles (Vullhorst et al. 1998). Differential splicing and alternative transcription start sites are utilized to generate a set of Kv3.4 variants in murine skeletal muscle, presumably involved in the regulation of excitability (Vullhorst et al. 2001).

Slowly activating and inactivating Kv channels

Of the *KCNQ* gene family, a splice variant of the *KCNQ5* mRNA is clearly expressed in skeletal muscle (Schroeder et al. 2000). The C-terminal of all Kv7.x channels is long compared to other K^+ channels and is endowed with distinctive structural domains. There is evidence that the C-terminal contains a channel assembly domain and binds calmodulin. Therefore, it is likely that Kv7.3 is also expressed in muscle because it forms a heterotetramer with several other Kv7.x alpha subunits and is important for trafficking (Cooper et al. 2000).

All Kv7.x channels modify subthreshold membrane excitability. In contrast to all other Kv channels which reach current peaks within 10–20 ms following depolarization, only those of the Kv7.x family show slow activation. The most likely function of these channels in the TTS is therefore to lower the excitability of muscle membrane during long stimulation which protects the muscle from overexertion.

The voltage-gated calcium channel of the TTS, Cav1.1

Of the many genes (*CACNx*) encoding alpha-subunits, *CACNA1S* is predominantly expressed in the T-tubular membrane of adult skeletal muscle. It encodes the L-type calcium channel alpha1 subunit, Cav1.1 (Fig. 3). The term L-type means long lasting currents according to their inactivation properties in contrast to the transient (T-type) currents. L-type channels reveal high thresholds for activation. All L-type channels are very sensitive to dihydropyridines (DHP, e.g. nifedipine), phenylalkylamines (PAA, e.g. verapamil), and benzothiazepines (BTZ, e.g. diltiazem) which has led to the term dihydropyridine receptor, a misnomer as it suggests ligand-activation when, in fact, the channel is activated by voltage.

The alpha1 subunit is structurally homologous to the alpha subunit of sodium channels, being 6T/1P, however, recent biochemical and 3D structural data indicate that functional channels could contain two alpha1 subunits (Wang et al. 2002). As functional studies have not yet been performed, it is not clear whether there are one or two pores in the whole channel complex.

The high selectivity for calcium over sodium is conferred by a group of conserved glutamate residues forming a high-affinity calcium binding site in the pore exhibiting an apparent dissociation constant of about 700 nM (Yang et al. 1993). Nevertheless, the channel conducts a reasonably high calcium current, probably because of the vicinity of a second binding site. When only one of the sites is occupied, which is the case at low calcium concentration, calcium is bound tightly. However, as soon as the probability of double occupancy increases at higher calcium concentration, electrostatic repulsion drastically reduces the time which the ions spend at the site and calcium flows through the channel along its electrochemical gradient. Therefore, monovalent cations (e.g. sodium) pass the channel in the absence of divalents, micromolar calcium blocks the monovalent current and mM external calcium leads to an almost pure calcium inward current (Almers et al. 1984). This binding site is conserved through all alpha1 subunits of the calcium channel family.

Physiologically, at least two Cav1.1 isoforms are expressed in muscle, the rare 212 kD complete protein and a similar 190 kD truncated form. The 190 kD subunit, comprising 95% of the total channel population, results by posttranslational proteolysis at amino acid 1690 (De Jongh et al. 1991; Beam et al. 1992). An additional variant has been suggested to exist, at least in postnatal skeletal muscle. As L-type channels can be modulated by cAMP-dependent protein kinase A via certain G_S proteins (Yatani et al. 1995), further functional alterations have also been demonstrated. The physiological importance of these alterations is acknowledged for the cardiac channel, but seems questionable for skeletal muscle (Fleig and Penner 1996). Mutations in Cav1.1 cause hypokalemic periodic paralysis and malignant hyperthermia susceptibility

type 5 in humans, and muscular dysgenesis in mice (Jurkat-Rott et al. 1994; Monnier et al. 1997). In hypokalemic periodic paralysis, all mutations are situated in the voltage sensors and reduce channel availability. It is still a mystery how the mutations can produce, at low potassium, the sustained membrane depolarization which causes the weakness (Rüdel et al. 1984; Jurkat-Rott and Lehmann-Horn 2005).

Kinetics and potential dependency of $Ca_v1.1$ channel states

As in voltage-gated sodium channels, depolarization causes $Ca_v1.1$ channel activation in a positive feedback mechanism along the concentration gradient and the electric field. The time-dependent channel inactivation reduces the current, a process that is not intrinsically voltage-dependent. As the time constants for activation are ~60 ms, the current cannot substantially contribute to the action potential which lasts only 1–2 ms. Additionally, at the peak of the action potential, only a small fraction of the channels can be activated as indicated by the Boltzmann curves for activation and inactivation (Fig. 4). It has not been unambiguously clarified whether $Ca_v1.1$ channels conduct calcium during high frequency discharges of action potentials under physiological conditions at all or whether the sole function of the channels consists of

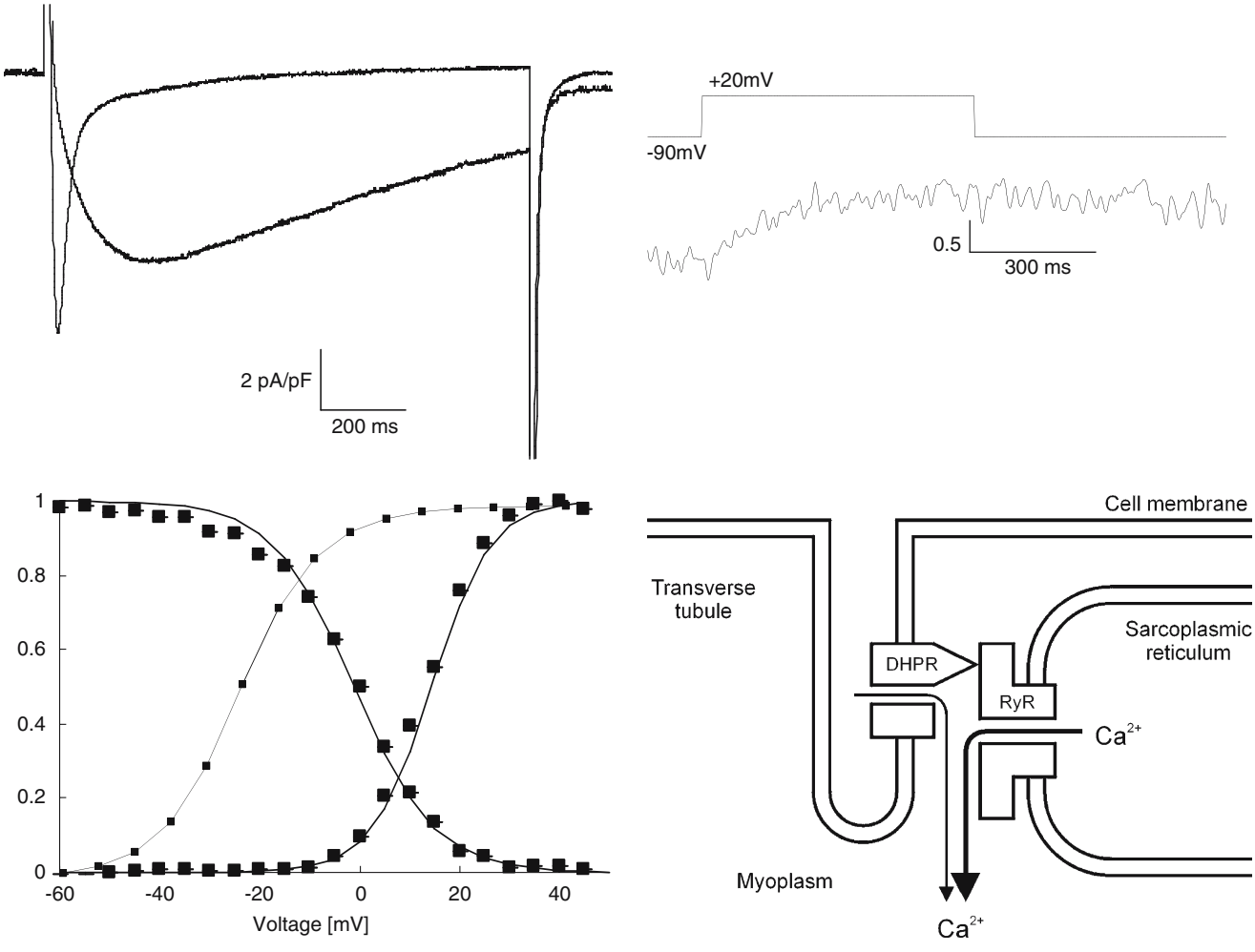

Fig. 4 Whole-cell calcium currents and calcium transient of human myotubes. Upper left panel: the myotubes revealed a rapidly activating and inactivating calcium current with a voltage threshold at –20 mV (T-type current) and a slowly activating and inactivating L-type current at +25 mV. Upper right panel: depolarization-induced calcium transient as normalized fluorescent intensity increases elicited from a holding potential of –90 mV. Lower left panel: Steady-state activation and inactivation curves of the L-type current (large squares) and steady-state activation of calcium transients (small squares). Note that the calcium transients occur at much smaller cell depolarizations than the current. Lower right panel: the triadic junction between the TTS and the SR—note the position of the two calcium channels of skeletal muscle, the L-type calcium channel, also called dihydropyridine (DHP) receptor, and the calcium release channel, also called ryanodine receptor, that interact directly during excitation-contraction coupling

coupling to the ryanodine receptor to initiate calcium release.

The $Ca_v1.1$ channel as voltage sensor for the ryanodine receptor

The $Ca_v1.1$ channel has been shown to interact with the ryanodine receptor of skeletal muscle (RYR1) by the interlinker between domains II and III, for mediating EC coupling (Tanabe et al. 1990; Paolini et al. 2004). Corresponding regions of RYR1 binding to the DHPR are residues 1303–1406 whose deletion preserved the function of the channel but led to loss of EC coupling (Yamazawa et al. 1997). RYR1 not only receives an activating signal from DHPR, but also gives a retrograde signal enhancing DHPR activity, which is mediated by the RYR1 residues 2659–3720 (Nakai et al. 1998).

The calcium release function is also voltage dependent, but the midpoint of half-maximal activation is shifted towards hyperpolarizing potentials by 40 mV compared to the midpoint of current activation (Fig. 4). Therefore, Cav1.1 functions as voltage-sensor of excitation contraction coupling and activator of the ryanodine receptor (RYR1) which releases calcium from the SR initiating contraction.

Modifying subunits

While voltage-gated sodium and potassium channels consist of alpha and optional beta subunits, only voltage-gated calcium channels consist of additional proteins, alpha2, beta, gamma and delta. The extracellularly located alpha2 protein is anchored by disulfide bonds to the membrane spanning alpha1-subunit (Jay et al. 1991; Gurnett and Campbell 1996a) and the two proteins are encoded by a single gene. The originally assumed existence of an alpha2 associated ion conducting pore can be explained by the drastic increase in expression of endogenous alpha1 subunits by coexpression of the alpha2/delta subunit (Gurnett et al. 1996b). The alpha2/delta subunit which can bind the anticonvulsant drug gabapentin, not only increases alpha1 expression rates and current density, but also accelerates inactivation kinetics and slightly shifts both steady-state inactivation and activation curves in hyperpolarising directions (Singer et al. 1991).

Coexpression of any of the four beta subunits with alpha1A markedly increases the number of channel complexes inserted into the membrane and the current amplitude (Brice et al. 1997). For $alpha_{1S}$, the skeletal muscle variant, beta coexpression increased the number of DHP-binding sites and accelerated current activation kinetics, however, without increasing current density (Lacerda et al. 1991; Varadi et al. 1991). Of the so far four identified beta subunits, beta1 is an intracellular acidic protein and binds to the loop connecting domains I and II of Cav1.1, distinct from the consensus site for the G protein beta-gamma complex (De Waard et al. 1997). Besides the intracellular I/II loop, the C-terminal also seems to act as a binding site for beta (Walker et al. 1998).

The gamma subunits consist of four transmembrane segments and at least one of them, gamma1, is expressed in skeletal muscle. Coexpression of gamma1 with the cardiac alpha1 subunit in amphibian and mammalian cell systems moderately increased calcium current amplitude and inactivation rate. The main effect is a marked shift of the voltage dependence of inactivation in the hyperpolarizing direction (Singer et al. 1991; Sipos et al. 2000) also in mature skeletal muscle (Ursu et al. 2004).

ClC1, the major chloride channel of skeletal muscle

The encoding gene for this chloride channel, *CLCN1*, is almost exclusively expressed in skeletal muscle (Steinmeyer et al. 1991). The electrophysiological identification and characterization of ClC1 at the single-channel level was difficult because its conductance is very low, i.e. near 1 pS as estimated from noise analysis (Pusch and Jentsch 1994). The large macroscopic chloride conductance of the skeletal muscle fiber membrane, i.e. 80% of the total conductance of the surface and the T-tubular membrane, must therefore result from an extremely high channel density. The channel is functional as homodimer without any other subunits and conducts over the whole physiological voltage range, showing inward rectification in the negative potential range. It is activated upon depolarization, and with hyperpolarizing voltage steps it is deactivated to a non-zero steady-state level (Fig. 5). The dimeric channel complex possesses two independent ion-conducting pores each with a fast opening mechanism of its own, two selectivity filters, and two voltage sensors (Fahlke 2001). Recent cryo-electron microscopy and X-ray measurements have elucidated the structure of a similar channel (Mindell et al. 2001; Dutzler et al. 2002) and confirmed the conclusions derived from electrophysiologic results.

The large chloride membrane conductance reduces fast potential deviations and thus stabilizes the resting potential. Due to this large conductance, chloride transporters can cause deviations from the membrane potential that is given by potassium and sodium

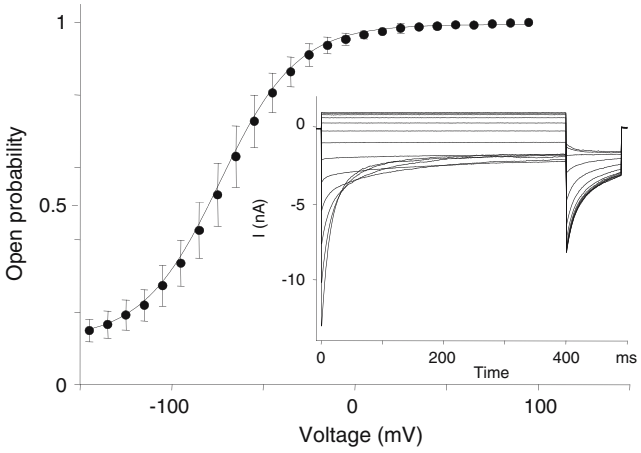

Fig. 5 Behavior of the human ClC1, the major chloride channel of skeletal muscle, expressed in a mammalian cell line and measured in a symmetrical chloride solution. The channel open probability depends on the voltage and is high in the physiological potential range. It is about 40% at the resting potential of –80 mV. The inset shows macroscopic currents recorded in the whole-cell mode. They are activated by steps going from a holding potential of 0 mV to potentials over a range of –145 to +95 mV, and deactivated after 400 ms by polarization to –105 mV. Adapted from Wagner et al. (1998)

reversal potentials and corresponding conductances. Vice versa, fast deviations of the membrane potential will be slowed by the voltage-stabilizing effect of the chloride ''buffer'' capacity as long as the chloride conductance is large compared to the potassium or sodium conductance. During tetanic muscle excitation, the high chloride conductance is thought to be necessary for a fast repolarization of the T-tubular membrane which becomes depolarized by potassium accumulation in the T tubules. Then, the repolarization is more negative than the potassium reversal potential. This stimulates an inward potassium flux through Kir channels and thereby reduces the potassium accumulation.

Although many experiments with various techniques such as potentiometry, detubulation, chloride withdrawal, and skinned fibers have shown that the chloride permeability is high in the T-tubular membrane of mammalian skeletal muscle (Palade and Barchi 1977; Dulhunty 1979; Coonan and Lamb 1998; Pedersen et al. 2004), immunofluorescence has not yet been successful to prove it (Gurnett et al. 1995).

Ion transporters of the TTS

The sodium pump

The sodium-potassium pump extrudes three sodium ions in exchange for the uptake of two potassium ions by using the energy from the hydrolysis of one molecule of ATP. Under physiological conditions, the resting membrane potential is about 3 mV more negative due to pump activity than it would be without. Under pathologic conditions, e.g. an increased sodium permeability with P_{Na}/P_K approaching 1, the pump contributes up to 11 mV to the membrane potential.

The sodium-potassium ATPase is an oligomer composed of two major polypeptides, the alpha- and beta-subunits (Blanco and Mercer 1998). The former, a protein containing 10 transmembrane segments, is responsible for the catalytic and transport properties of the enzyme and contains binding sites for cations, ATP, and the inhibitor ouabain (See Fig. 2). The beta-subunit, a small polypeptide that crosses the membrane once, is essential for the normal activity of the enzyme and conveys the sodium affinity to the enzyme. In addition, in vertebrate cells, the beta-subunit may act as a chaperone, stabilizing the correct folding of the alpha-polypeptide to facilitate its delivery to the plasma membrane. A third protein, a single membrane domain-possessing gamma subunit, seems to modify the voltage dependence of potassium activation, is the substrate for kinases, and is tissue-specifically spliced (Crambert et al. 2002).

Of the many isoforms, alpha1 in association with beta1-subunit is found in nearly every tissue including skeletal muscle (Table 2). The alpha2/beta2-complex predominates in skeletal muscle (Hundal et al. 1992; Lavoie et al. 1997). A subunit specifically expressed in skeletal muscle and at a much lower level in heart is beta4 (Pestov et al. 1999). Its significance has not been yet clarified.

As extracellular potassium is required for pump cycling, T-tubular potassium depletion blocks the sodium pump whereas intracellular sodium ions activate it. Exercise translocates preexisting alpha2/beta1 from intracellular stores to the plasma membrane in skeletal muscle within 20 s (Hundal et al. 1992). This results in the recruitment of additional functional sodium pumps to the cell surface and increased Na-K-ATPase activity, an effect with a half-life of approximately 20 min (Juel et al. 2001). Hormones can elicit their action by directly affecting the pump rate and the number of pump sites and also by modulating the expression of a particular isoform (Ewart and Klip 1995) e.g., insulin leads to the same rapid translocation as described for exercise.

Long-term alteration of thyroid hormone levels change the amount of alpha2 mRNA and protein, with free T4 index giving the highest positive correlation. Hypothyroidism decreases and hyperthyroidism increases 3H-ouabain binding sites (Kjeldsen et al. 1984)

Table 2 Ion transporter genes expressed in the surface and/or T-tubular membrane of skeletal muscle

Gene	Gene locus	Transporter protein	Transporter properties	Significance for muscle function	Disease
ATPases					
ATP1A1	1p13	Na^+/K^+ pump alpha1 subunit	Transport and catalytic units, bind cations, ATP and oubain	ubiquitous	Ouabain binding sites reduced in McArdle's disease, not specified if alpha1 or alpha2
ATP1A2	1q21–23	Na^+/K^+ pump alpha2 subunit		predominates in brain and skeletal muscle	(loss-of-function mutations cause familial hemiplegic migraine type 2)
ATP1B1	1q22–25	Na^+/K^+ pump beta1 subunit	Na^+ affinity, chaperon	ubiquitous	
ATP1B2	17p13	Na^+/K^+ pump beta2 subunit		predominates in skeletal muscle	
ATP1B4	Xq24	Na^+/K^+ pump beta4 subunit		muscle specific, unknown function	
FXYD1	19q13	PLM, phospholemman precursor, reacts to insulin, adrenalin	Induces chloride current at hyperpolarization	predominates in muscle and heart, contraction	
FXYD2	11q23	ATP1G1, Na^+/K^+ pump gamma1 subunit	Involved in ouabain binding	higly expressed in kidney, muscle	(renal hypomagnesemia type 2)
Anion-anion exchanger					
SLC4A2	7q35–36	B3RP2	Cl^-/HCO_3^- exchange	wide distribution	
SLC4A3, int CGG	2q36	B3RP3		cardiac and brain splice variants	
Cation-anion exchanger					
SLC4A7	4q21	NBC3	Na^+/HCO_3^- exchange	muscle specific	
Cation-cation exchanger/transporter					
SLC8A1	2p23–22	NCX1	Na^+/Ca^{++} exchange, extrudes calcium from myoplasm	in oxidative fibers	
SLC8A2	19q13.3	NCX2		prevents calcium stores overload	
SLC8A3	14q24.1	NCX3		in fast glycolytic (type 2B) fibers	
SLC9A2	2q11.2	NHE2	Na^+/H^+ exchange	pH regulation	
SLC9A5	16q22.1	NHE5		low in muscle	
SLC12A2	5q23.3	NKCC1	$Na^+/K^+/2$ Cl^- transport	highest density in sarcolemma, improves muscle endurance	
SLC12A4	16q22	KCC1	K^+/Cl^- cotransport	volume regulation	
SLC12A6	15q13–14	KCC3			
SLC12A7	5p15.33	KCC4		high expression in muscle	(renal tubular acidosis)

Diseases other than those affecting the skeletal muscle are given in brackets

and these changes may account for the variations in digitalis sensitivity associated with thyroid disorders. Long-term administration of glucocorticoids increases the amount of alpha2 and beta1 mRNA and protein. Chronic hypokalemia, e.g. due to potassium-wasting diuretics administered to human individuals, reduces the number of 3H-ouabain binding sites (Dorup et al. 1988), probably by alpha2 and beta1 downregulation. In rat muscle, potassium deprivation over 10 days reduces alpha2 and beta2 proteins, particularly in glycolytic fibers (Thompson and McDonough 1996).

As sodium pump activity facilitates potassium ion clearance from the T tubules within seconds, the pump is expected to reside in the TTS (Drost et al. 2001).

The sodium-calcium exchangers, NCX

NCX1 is the primary cardiac mechanism by which the calcium is extruded from the cell into the TTS during relaxation (Table 2). The exchange of three Na^+ for one Ca^{2+} is powered to the sodium pump. Sodium/calcium exchange rate depends on extracellular sodium and intracellular calcium concentrations. The direction of the exchange is reversible (extracellular Ca^{2+} exchanging for intracellular Na^+). NCX proteins consist of 12 membrane-spanning segments, but disulfide bond analysis and cysteine mutagenesis coupled with accessibility studies indicate that nine transmembrane segments are alpha helices while the other structures are alpha repeat regions forming non-helical re-entrant loops (Nicoll et al. 2002). NCX1 and NCX3 expression is developmentally regulated as studied in rat muscle (Fraysse et al. 2001). NCX1 peaks shortly after birth while NCX3 is expressed at low quantities at birth but then increases rapidly during the first weeks of life. NCX2 is abundantly expressed in skeletal muscle (Li et al. 1994).

The exchanger is expressed in the T-tubular membrane (Dosnoso and Hidalgo 1989; Sacchetto et al. 1996). At least in amphibian skeletal muscle, it assists in reducing the sarcoplasmic calcium concentration after stimulation (Cifuentes et al. 2000).

The cation-chloride cotransporters

Cation-chloride cotransporters are putative homodimers (Moore-Hoon and Turner 2000) each monomer consisting of 10 to 12 transmembrane segments (Fig. 2). They mediate the translocation of ions (Na^+, K^+, Cl^-, and others) and the majority is driven by the sodium gradient established by the sodium pump (Table 2). Transmembrane domains T3-T12 are homologous to the large amino acid permease domain. Amino- and carboxyl-terminal domains are located intracellularly, are subject to phosphorylation by protein kinases, and are involved in the regulation of cotransport activity. T2 may possess several splice variants and contributes to ion affinity (Isenring et al. 1998).

$Na^+/K^+/2Cl^-$ cotransporters mediate the electroneutral transport of 1 Na^+, 1 K^+, and 2 Cl^- ions across the membrane. While NKCC2 is kidney-specific, NKCC1 is widely expressed including skeletal muscle (Delpire et al. 1994), particularly the sarcolemma, but also in the TTS although less abundantly (Kristensen 2006). Glycolytic fibers show a higher expression level. The NKCC helps to prevent early muscle fatigue. Its effects are threefold: it reduces interstitial potassium accumulation and intracellular potassium depletion (Kristensen 2006), it avoids fiber-shrinkage induced hyperpolarization in adjacent resting fibers (Ferenczi 2003; Gosmanov 2003) and it is involved in the maintenance of fiber volume. Although the NKCC is only slightly active at resting conditions, it participates in the restitution and preservation of muscle's potassium homeostasis (Lindinger et al. 2002).

Hypertonicity is supposed to support membrane depolarization by enhancing chloride import through the $Na^+/K^+/2Cl^-$ cotransporter (Van Mil et al. 1997; Dulhunty 1978) and to alter the bistable behavior of mammalian muscle fibers (Geukes Foppen et al. 2002). Due to the large chloride conductance, the inwardly transported chloride ions will leave and thereby depolarize the cell. Addition of bumetanide, a potent inhibitor of the $Na^+/K^+/2Cl^-$ (and perhaps of chloride channels) and of anthracene-9-carboxylic acid, a blocker of chloride channels, leads to membrane hyperpolarization particularly under hypertonic conditions. $BaCl_2$ or temperature reduction from 35°C to 27°C induces depolarization of fibers that are originally hyperpolarized.

KCC1, KCC4 and to a lower extent KCC3 are expressed in skeletal muscle (Gillen et al. 1996; Mount et al. 1999; Race et al. 1999). These transporters consist of 12 membrane-spanning segments, a large extracellular loop with potential N-glycosylation sites, and cytoplasmic N- and C-terminal regions with phoypshorylation sites. The chloride gradient is important for the activity (Lytle and McManus 2002) and also an increased extracellular potassium level stimulates the electroneutral cotransport. Dephosphorylation activates KCC1 cotransporters while phosphorylation inactivates them under isotonic conditions (Delpire and Mount 2002). KCC is activated by cell swelling at hypotonic conditions (Mount and Gamba 2001). This

suggests that the TTS as an area which is very liable to volume changes may well express these transporters.

Consequences of TTS structure and its composition of channels and transporters

The TTS is equipped with ion channels and ion transporters which permit synchronous excitation. Since its volume is only 0.32% of the fiber volume, certain ions can reach extreme concentration levels which severely alter *Nernstian* potential or ion permeability, thereby resulting in an unstable membrane potential. Accumulation of potassium which would result in muscle fatigue is managed by the coordinated action of inwardly rectifying potassium and of chloride channels. An immanent problem is a severe reduction of the T-tubular potassium concentration that can result in either hyperpolarization or depolarization of the fiber as the current-voltage relationship of Kir2.1 is extremely flat under this condition. Transitions between two polarization levels can occur (Rüdel et al. 1984; Lehmann-Horn and Jurkat-Rott 1999)—a phenomenon termed *bistability of the resting membrane potential* (van Heukelom 1991; Nilius and Droogmans 2001). The more negative polarization state depends on the reversal potential E_K that is large at low extracellular potassium, the less negative polarization level follows the potassium conductance that is small at low extracellular potassium. If depolarized, the membrane will respond to an increase in extracellular potassium with repolarization because of an increase in potassium conductance. This happens although the reversal potential simultaneously shifts toward less negative values.

The question arises whether a larger volume of the TTS could solve the problem of low-potassium-induced depolarization and, if so, other disadvantages would be raised. Under normal circumstances in skeletal muscle, action potentials propagating within the TTS are not able to generate a recurrent action potential on the surface membrane. This is an advantageous electrical transmission block that occurs where a narrow tubule opens into a larger space (Lamb 2005), such as where a dendrite opens to the soma of a neuron (Jack et al. 1975). Therefore, the narrow TTS might guarantee that action potentials spread from the surface into the TTS and not vice versa.

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