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Short communication

Altered gating and conductance of Na^+ channels in hyperkalemic periodic paralysis

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Abstract. Electrophysiological studies on muscle fibres from patients with hyperkalemic periodic paralysis with myotonia have shown that the episodes of weakness are caused by a sustained depolarization of the sarcolemma to potentials between -40 and -60 mV. In muscle fibre segments from three such patients this sustained depolarization was caused by non-inactivating Na^+ channels with reduced single-channel conductance blocked by TTX and procainamide. As the chloride conductance was normal, myotonia may be best explained with the abnormal reopenings of the Na^+ channels. The recently described genetic linkage between hyperkalemic periodic paralysis with myotonia and the gene coding for the TTX-sensitive Na^+ channel suggests an altered primary structure of this channel causing its abnormal function.

Key words: Adynamia episodica hereditaria with myotonia, Hereditary muscle disease, Sodium channel disease, Patch clamp technique

Introduction

Hyperkalemic periodic paralysis, also called adynamia episodica hereditaria, is a hereditary muscle disease with autosomal dominant transmission. Its characteristic symptom are episodic attacks of generalized muscle weakness (Gamstorp 1956). The weakness does not occur during exercice even if serum $[K^+]$ is elevated, but during rest after work. The serum $[K^+]$ of the patients is then usually elevated. Weakness can also be provoked by the oral intake of K^+. In the state of weakness, the muscle fibres are depolarized to the extent that the membrane is inexcitable.

In vitro, force of muscle preparations from such patients diminishes as the extracellular $[K^+]$ is raised. The sarcolemma is then much more depolarized than predicted by the Nernst equation (Lehmann-Horn et al. 1983). A non-inactivating Na^+ current, which can be completely blocked by tetrodotoxin (TTX), is the cause of this sustained depolarization (Lehmann-Horn et al. 1987). A consequence of this inward current is a shift of serum water from the extracellular space into the muscle fibres, resulting in an increase in extracellular $[K^+]$ (Spier et al, 1990).

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The molecular basis of hyperkalemic periodic paralysis is further described by single-channel recordings in this report and genetic linkage studies by Fontaine et al. 1990.

Methods

Three patients with hyperkalemic periodic paralysis with myotonia, who were not related, gave us informed consent for a biopsy of the vastus or the biceps bracchii muscle. A muscle specimen (fibre segments about 3 cm long) was removed under local anaesthesia. Several fibre bundles with diameters of 2-3 mm were prepared from each specimen. All procedures were in accordance with the Helsinki convention and were approved by the Ethics Commission of the Technical University of Munich.

The standard solution used for transportation, dissection, and electro-physiological experiments contained (in mM): NaCl 108, KCl 3.5, $CaCl_2$ 1.5, $MgSO_4$ 0.7, $NaHCO_3$ 26.2, NaH_2PO_4 1.7, Na-gluconate 9.6, glucose 5.5, sucrose 7.6 (315 mosmol/l). The Cl^- free solution, used in some of the voltage clamp experiments, was made by 1) replacing NaCl and KCl with respective methane sulfonate salts, 2) replacing $CaCl_2$ with Ca gluconate and 3) omitting the sugars in order to avoid hyperosmolarity. All solutions were maintained at 37 °C if not indicated otherwise. The pH was adjusted to 7.4 by gassing the solutions with 95% O_2 and 5% CO_2. Some solutions contained 1μM TTX (Roth, Karlsruhe, FRG).

For the determination of the steady-state relationship between current density and membrane potential, resealed fibre segments were impaled midway with three microelectrodes as previously described (Lehmann-Horn et al. 1990). Data were collected and analysed with an AT personal computer system.

For the measurement of single Na^+ channels we performed patch clamp experiments in the inside-out mode (Franke et al. 1990). For these, the fibre segments were superfused for up to 2 hours with standard solution containing 1-2 mg/ml collagenase (Type Ia, Sigma Chemical Co., St. Louis, MO, USA) at room temperature. After this membrane treatment, giga seals to patch pipettes were obtained. The 'intracellular' solution with which the sarcoplasmic side of an inside-out patch was perfused contained (in mM): KCl 150, $MgCl_2$ 2, $CaCl_2$ 1, EGTA 10, HEPES 10, pH 7.2.

Results

When bundles of fibre segments from the patients were kept in the standard solution they did not repolarize to the same degree as control muscles (Lehmann-Horn et al. 1990). However when the bundles were placed in a

solution containing 1 M TTX immediately after biopsy, the resting potential of the fibres recovered to normal value within 2-3 hours (-77.7 mV ± 3.4, n=40; X ± S.D.). Subsequently when TTX was removed many fibres but not all, depolarized to values between -55 and -70 mV. Reapplication of TTX resulted in repolarization.

Steady-state relationships between current density and membrane potential were determined in either normal or Cl^- free bathing solution. The slope of this curve reflects the membrane conductance as a function of the membrane potential. The steady-state relationship determined for the diseased fibre segments in standard solution showed a negative slope in the voltage range less negative than -85 mV, i.e. the 'resting' membrane has a 'negative resistance' and therefore it is electrically unstable (Fig.1). When 1 M TTX was added to the bath, the relationship was normalized, which indicates that the negative resistance was caused by the flow of Na^+ current (Fig.1). In Cl^- free solution containing TTX, the relationship reflects the K^+ conductance. This curve always had a normal slope in the whole membrane potential range (not shown); at a resting potential of -80 mV the K^+ conductance was 51 $\mu S/cm^2$. The difference between the relationships for standard and Cl^- free solution, both containing TTX, represents the voltage dependence of the Cl^- current, and its slope the Cl^- conductance. The average value determined for the total membrane conductance at -80 mV was 174.7 S/cm^2 ± 32.1 (n=20; X ± S.D.) and the fraction of g_{Cl} was 0.68 for the three patients. These values are not statistically different from normal controls.

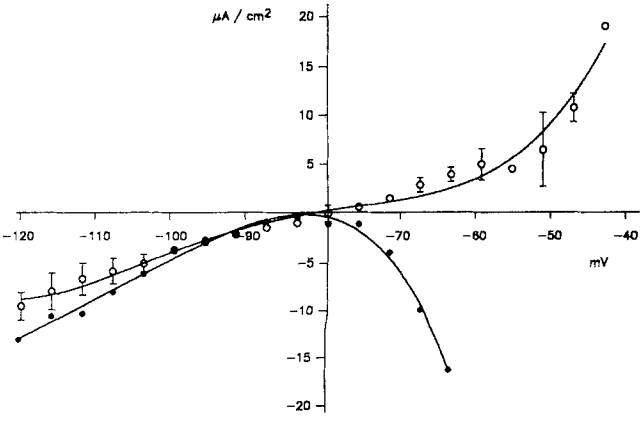

Fig. 1: Steady-state relationships between current density and membrane potential determined for muscle fibre segments from patients with hyperkalemic periodic paralysis. Filled circles: The average relationship was determined for 6 fibre segments from one of the patients in the absence of TTX. The negative slope in the potential range less negative than the resting potential indicates an increased total steady-state Na^+ conductance. Open circles: The average relationship of 20 fibre segments ($\bar{X}$ ± S.D.) bathed in TTX-containing solution (1 M) was determined for the three patients.

In the inside-out patches (n=11), immediately at the begin of a depolarization step, the opening of several Na^+ channels resulted in a large negative deflection of the current trace caused by synchronized opening of several Na^+ channels. These channels had a normal conductance of about 15 pS, their gating characteristics were as in controls (Franke et al. 1990). In contrast to normal fibres, all patches from the three patients contained also non-inactivating Na^+ channels that kept opening and closing throughout the depolarizing pulse of 10 ms (Fig.2A). After several depolarization steps early openings disappeared and the number of bursting Na^+ channels markedly increased. The activity of these channels was not potential-dependent. Even after hyperpolarization of the patches to -100 mV for several minutes we did not observe inactivation. The non-inactivating channel was reversibly blocked by 50µM procainamide added to the 'intracellular' solution. Also, the channels were never observed when 100 nM TTX was in the pipette solution. The amplitude of the single-channel currents was voltage-dependent (Fig.2B). The single-channel conductance was only 6 pS, i.e. much smaller than control. The result was the same for the three on-cell patches which were obtained from one of the patients.

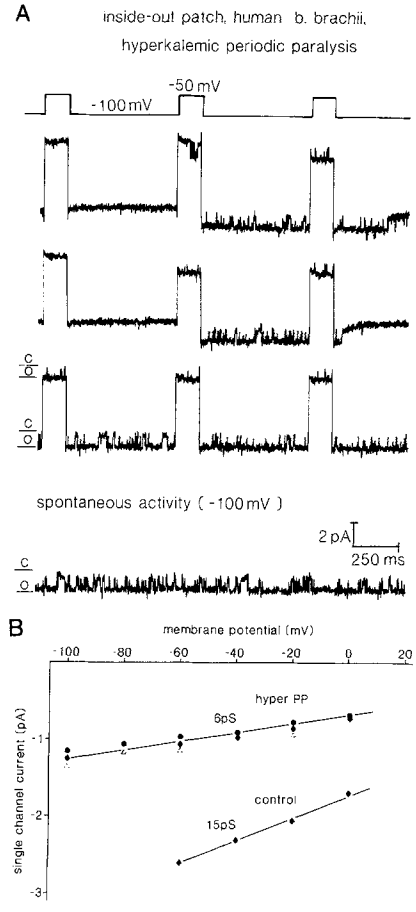

Fig. 2: (A) Recordings from a patch of a muscle fibre membrane from a patient with hyperkalemic periodic paralysis. Bursts of Na^+ channel openings were induced by repetitive depolarizing voltage pulses going from a holding potential of -100 mV to -50 mV. "Spontaneous" activity was also observed at high negative potential (-100 mV). (B) Current-voltage relationships indicate that the conductance of the non-inactivating channels of 11 patches from the three patients (hyper PP; one symbol for each patient) is only about 1/3 the normal single-channel conductance (control).

Discussion

Earlier studies on intact intercostal muscle fibres from two patients with hyperkalemic periodic paralysis with myotonia showed that the total Na^+ conductance is increased while all other component conductances seem normal (Lehmann-Horn et al. 1983, Lehmann-Horn et al. 1987). We now confirm these results in additional three patients using resealed fibre segments, which can be routinely taken under local anaesthesia. In addition, we state that this abnormal Na^+ current is due to both non-inactivation and a reduced single-

channel conductance of the TTX-sensitive Na^+ channel. We conclude that this abnormal function of the Na^+ channel is the cause of the sustained membrane depolarization and the episodic attacks of muscle weakness in vivo. As in some other human hereditary diseases associated with myotonia, the myotonia is not due to low-chloride conductance, but can be best explained with the abnormal reopenings of the sarcolemmal Na^+ channels.

Our electro-physiological findings are corroborated by the recently described genetic linkage between a family with hyperkalemic periodic paralysis with myotonia and the gene on chromosome 17 coding for the TTX-sensitive Na^+ channel (Fontaine et al. 1990). This linkage suggests an altered primary structure of the channel protein causing its abnormal function in hyperkalemic periodic paralysis with myotonia.

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