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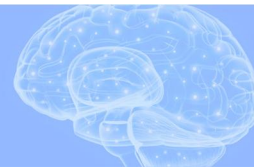
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Retigabine suppresses loss of force in mouse models of hypokalaemic periodic paralysis

Marbella Quiñonez,¹ Marino DiFranco,¹ Fenfen Wu¹ and  Stephen C. Cannon^{1,2}

Recurrent episodes of weakness in periodic paralysis are caused by intermittent loss of muscle fibre excitability, as a consequence of sustained depolarization of the resting potential. Repolarization is favoured by increasing the fibre permeability to potassium. Based on this principle, we tested the efficacy of retigabine, a potassium channel opener, to suppress the loss of force induced by a low-K⁺ challenge in hypokalaemic periodic paralysis (HypoPP). Retigabine can prevent the episodic loss of force in HypoPP. Knock-in mutant mouse models of HypoPP (*Cacna1s* p.R528H and *Scn4a* p.R669H) were used to determine whether pre-treatment with retigabine prevented the loss of force, or post-treatment hastened recovery of force for a low-K⁺ challenge in an *ex vivo* contraction assay. Retigabine completely prevents the loss of force induced by a 2 mM K⁺ challenge (protection) in our mouse models of HypoPP, with 50% inhibitory concentrations of $0.8 \pm 0.13 \mu\text{M}$ and $2.2 \pm 0.42 \mu\text{M}$ for Na_v1.4-R669H and Ca_v1.1-R528H, respectively. In comparison, the effective concentration for the K_{ATP} channel opener pinacidil was 10-fold higher. Application of retigabine also reversed the loss of force (rescue) for HypoPP muscle maintained in 2 mM K⁺. Our findings show that retigabine, a selective agonist of the K_v7 family of potassium channels, is effective for the prevention of low-K⁺ induced attacks of weakness and to enhance recovery from an ongoing loss of force in mouse models of type 1 (*Cacna1s*) and type 2 (*Scn4a*) HypoPP. Substantial protection from the loss of force occurred in the low micromolar range, well within the therapeutic window for retigabine.

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Introduction

Hypokalaemic periodic paralysis (HypoPP) presents with recurrent episodes weakness, often in association with low serum potassium ($[\text{K}^+] < 3.5 \text{ mEq/l}$).^{1,2} Attacks of weakness are variable, both among affected members in a family or for a single individual over time, with regard to affected muscle groups, severity, frequency and

duration. Trigger factors that increase the risk of experiencing an attack of weakness are a prominent feature in all forms of periodic paralysis, with the events in HypoPP being carbohydrate-rich meals, rest after exercise, or stress. Consequently, the first approach to disease management is lifestyle changes to minimize the occurrence of trigger events.³ When these measures are insufficient, then pharmacological interventions are used with oral K

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supplements, K-sparing diuretics and carbonic anhydrase inhibitors. While benefit has been established in double-blind placebo-controlled trials,^{4,5} there is an important unmet need with about 50% of patients on carbonic anhydrase inhibitors receiving inadequate control of episodic weakness or unable to tolerate these medications.⁶

The search for more effective pharmacological intervention has been driven by our understanding of the pathomechanism for episodic weakness. HypoPP may be caused by missense mutations in either the *CACNA1S* gene encoding the $Ca_v1.1$ calcium channel (type 1 HypoPP, accounting for about 60% of kindreds) or less commonly in the *SCN4A* gene encoding the $Na_v1.4$ skeletal muscle isoform of the sodium channel (type 2 HypoPP in about 20% of kindreds).^{1,7} In periodic paralysis, the transient weakness is caused by anomalous depolarization of the muscle resting potential,¹ from a normal value of about -90 mV to the ictal range of -50 to -60 mV. Muscle sodium channels are inactivated by this sustained depolarization, which reduces fibre excitability and may lead to failure of action potential initiation or propagation. Interventions that favour the normal hyperpolarized resting potential of muscle are expected to be effective for reducing the risk of episodic weakness in periodic paralysis. Increasing the membrane conductance to K^+ will hyperpolarize the resting potential. Many K^+ channels are closed at the resting potential and drugs that promote the opening of these resting channels have been shown to hyperpolarize a wide variety of cells including neurons, skeletal muscle, smooth muscle, heart and pancreatic beta cells.^{8,9} Two classes of so-called 'K-channel openers' are in clinical use. Openers of K_{ATP} channels, such as pinacidil and diazoxide, are used to treat hypertension and hypoglycaemia, respectively. Studies on biopsied muscle from patients with periodic paralysis showed pinacidil can prevent or hasten recovery from a loss of muscle force,^{10,11} but in clinical practice intolerable hypotension occurred before a meaningful improvement of muscle function was obtained. The other clinically used class of K^+ channel openers promotes the activation of K_v7 channels (also known as *KCNQ* channels). Retigabine (ezogabine) is a potent agonist of $K_v7.2$ – $K_v7.5$ channels, with the insensitive $K_v7.1$ isoform expressed primarily in heart, and was available in the US as a first-in-class antiepileptic drug.¹² There have been no case reports or clinical trials of retigabine use in muscle channelopathies, but experimental studies have shown the drug reduces myotonia in mouse models of myotonia congenita.^{13,14} Neither retigabine nor any other K_v7 agonist has previously been studied experimentally as a mechanism to ameliorate the attacks of weakness in periodic paralysis.

In this preclinical study, we tested the efficacy of retigabine to prevent the loss of force or to hasten recovery of force in mouse models of HypoPP. Our knock-in mutant mouse models of HypoPP (type 1 *Cacna1S* p.R528H15 and type 2 *Scn4a* p.R669H16) have a robust phenotype with paradoxical fibre depolarization and loss of contractile force in low K^+ . Susceptibility to HypoPP was determined by an *ex vivo* assay that measured the loss of force in response to a reduction of extracellular $[K^+]$ from 4.7 to 2 mM. Substantial protection from low- K^+ induced loss of force was observed after pre-treatment with 1 μ M retigabine, and 10 μ M completely suppressed any detectable loss of force. By comparison with this same assay, pinacidil was an order of magnitude less potent than retigabine for the prevention of weakness. The K_v7 -channel opener also hastened recovery. For HypoPP muscle maintained in 2 mM K^+ the initial loss of force was reverse by application of retigabine. These data show retigabine, or other K_v7 agonists, has the potential to reduce the severity of episodic weakness

in HypoPP and is superior to previously studied K channel openers acting on K_{ATP} channels.

Materials and methods

Mouse model of HypoPP

We previously generated knock-in mutant mouse models of HypoPP; Type 1 with a missense mutation of the $Ca_v1.1$ calcium channel (*Cacna1s* p.R528H¹⁵) and Type 2 with a missense mutation of the $Na_v1.4$ sodium channel (*Scn4a* p.R663H¹⁶) homologous to the p.R669H missense mutation in patients with HypoPP Type 2.¹⁷ Mutant mice have a robust HypoPP phenotype with loss of force and paradoxical depolarization of skeletal muscle in response to a challenge in low extracellular $[K^+]$ (<3 mM) or to intravenous administration of insulin plus glucose.^{15,16} Consistent with the dominant inheritance in patients, heterozygous mutant mice exhibit all the features of HypoPP. Mouse studies have revealed a gene dosage effect of the mutant allele,^{15,16} and homozygous mutant mice were used in this study to improve the sensitivity for detecting a beneficial effect of retigabine on the outcome measure of muscle force.

All procedures performed on mice were in accordance with animal protocols approved by the David Geffen School of Medicine Institutional Animal Care and Use Committee.

Ex vivo contraction studies

The outcome measure for susceptibility to HypoPP was the peak isometric force of the soleus muscle in response to a tetanic stimulation, as previously described.^{16,18} In brief, after euthanizing the animal, the extensor digitorum longus (EDL) muscle or the soleus muscle was dissected free and suspended in a tissue bath held at 37 °C. Similar responses were observed for either muscle, but the EDL muscle was preferentially used for $Ca_v1.1$ -R528H mice because the HypoPP phenotype is more pronounced¹⁵ and the soleus muscle was used for $Na_v1.4$ -R669H mice because the dissection is easier. Muscles were suspended in a bicarbonate-buffered bath that was bubbled continuously with 5% $CO_2/95\%$ O_2 to maintain a pH of 7.4. The standard bath consisted of (in mM): 118 NaCl, 4.75 KCl, 1.18 $MgSO_4$, 2.54 $CaCl_2$, 1.18 NaH_2PO_4 , 10 glucose and 24.8 $NaHCO_3$. The low- K^+ solution was identical, except for the reduction of KCl to 2 mM by replacement with NaCl. The osmolality of all solutions was 290 mOsm. Retigabine (a gift from Prof. M. Tagliatela, University of Naples) and pinacidil (Sigma-Aldrich) were prepared as 100 mM stock solutions in dimethyl sulfoxide (DMSO) and diluted into the bath solution for a working concentration of 0.1–100 μ M. The K_v7 inhibitor, XE991 (Sigma-Aldrich), was prepared as a 100 mM DMSO stock solution and applied to muscle in a 10 μ M bath solution.

Electrical stimulation was applied by a pair of platinum wires, oriented perpendicular to the long axis of the muscle. Suprathreshold stimulation (80 mA) was applied as a tetanic burst (40 pulses, 0.4 ms, at 100 Hz), under computer control. All bath solutions contained 0.25 μ M D-tubocurarine (Sigma-Aldrich) to prevent any contribution to muscle excitation from motor nerve endings. Muscle force was measured with a stiff strain gain (Forte 25, World Precision Instruments) and digitally sampled at 5 kHz. Muscle contractility was monitored by measuring the peak isometric force every 2 min, and test solutions were applied by complete exchange of the bath solution.

Membrane potential measurements

The membrane potential was recorded by impalement of superficial fibres from an *ex vivo* whole mount of the EDL muscle.

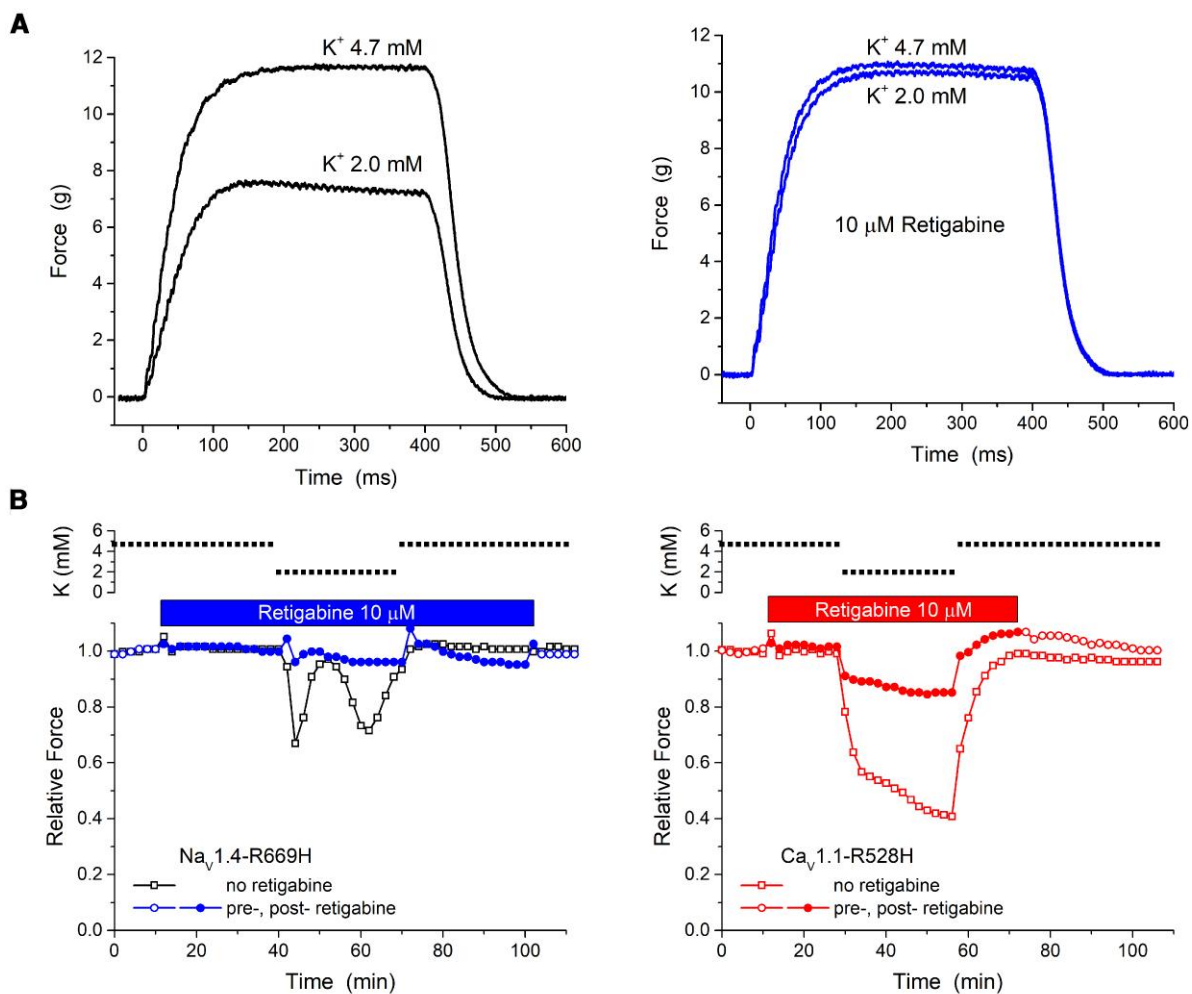


Figure 1 Ex vivo contraction test for susceptibility to HypoPP. (A) Isometric force measured in response to tetanic stimulation of the soleus muscle from the $\text{Na}_v1.4\text{-R669H}$ mouse. The left panel shows the baseline force during an isometric contraction in 4.7 mM K^+ , and then a decrease in the peak force 4 min after bath exchange to 2 mM K^+ . In a bath containing $10\text{ }\mu\text{M}$ retigabine (right), the baseline force in 4.7 mM K^+ was unchanged and the peak force in preserved during a 2 mM K^+ challenge. (B) The time course for the change in relative peak force in response to a 2 mM K^+ challenge is shown in control (open symbols, no drug) and after pre-treatment with $10\text{ }\mu\text{M}$ retigabine (filled symbols). These are representative responses from single trials for the soleus muscle in $\text{Na}_v1.4\text{-R669H}$ (left) and the EDL in $\text{Ca}_v1.1\text{-R528H}$ (right). For each trial, the internal control (no drug) is the response measured in the contralateral muscle from the same animal.

Long-duration stable recording of the resting potential was achieved by using an orthogonal approach to the fibre with fine-tipped microelectrodes (resistance of $15\text{--}20\text{ M}\Omega$, filled with 3 M KCl). The bath solution was the same as that used for the ex vivo contraction studies, continuously bubbled with $5\%\text{ CO}_2/95\%\text{ O}_2$, with a flow rate of $\sim 3\text{ ml/min}$ of the 0.3 ml recording chamber.

Data availability

The raw data for the outcome measures of this study are digitized traces of muscle force or fibre membrane potential, stored in a custom 16-bit binary format. Example traces, converted to Excel file format, are available upon request from the corresponding author.

Results

The efficacy of potassium channel openers to prevent the loss of force in HypoPP muscle was assessed by measuring isometric contractions, ex vivo, in both control (4.7 mM) and low extracellular $[\text{K}^+]$

(2.0 mM). The HypoPP phenotype is shown for the drug-free responses of the soleus muscle from an $\text{Na}_v1.4\text{-R669H}$ mouse in the left panel of Fig. 1A. The peak isometric force during the tetanic contraction (100 Hz stimulation) was initially 11.7 g in control $[\text{K}^+]$ and then decreased to 7.6 g (35% decrease) after 4 min in 2 mM K^+ . The loss of force is reversible, upon return to 4.7 mM K^+ (Fig. 1B). For the soleus muscle from the other hindlimb of the same animal, control and low- K^+ solutions containing $10\text{ }\mu\text{M}$ retigabine were used and the loss of force from a 2 mM K^+ challenge was completely prevented (Fig. 1A, right).

The time course of the relative force in response to a 30 min low- K^+ challenge, with and without pre-treatment with retigabine, is shown in Fig. 1B. The relative force was stable before the low- K^+ challenge and was not affected by the addition of retigabine for either the $\text{Na}_v1.4\text{-R669H}$ soleus muscle or the $\text{Ca}_v1.1\text{-R528H}$ EDL muscle (time = 10 min in Fig. 1B) over the range of concentrations used ($0.1\text{--}20\text{ }\mu\text{M}$). As we previously reported,¹⁶ the loss of force during a low- K^+ challenge in the $\text{Na}_v1.4\text{-R669H}$ HypoPP mouse in drug-free conditions may exhibit oscillations with spontaneous periods of

recovery for the soleus muscle (Fig. 1B, left). While this oscillatory response is variable across different mice, the concordance is high between the left and right soleus muscles of a single mouse, as shown by the in-phase oscillations for control and retigabine in Fig. 2 or for paired drug-free recordings in Wu et al.¹⁶ The representative responses in Fig. 1B reaffirm that pre-treatment with 10 μM retigabine completely suppressed the HypoPP phenotype in the 2 mM $[\text{K}^+]$ challenge, such that the relative force was >0.85 (filled symbols) as occurs for wild-type (WT) muscle.^{15,16}

Our method to quantify the extent of protection from loss of force by retigabine is illustrated in Fig. 2. This example was selected to illustrate the maximum variability in the extent of protection for the oscillations of soleus muscle force within a single trial (compare the responses at 24 min to those at 40 min). Qualitatively, even 1 μM retigabine was sufficient to provide substantial protection. The relative protection was calculated from the responses for each pair of soleus muscles from the same mouse serving as an internal control. In almost every trial (22 of 24), two clear minima occurred during the 30 min low- K^+ challenge. The relative protection for the retigabine response, defined as $1 - (\text{force loss in retigabine})/(\text{force loss in control})$, was calculated for each of the two minima, and the average value was used as the quantitative estimate for the retigabine protection in that trial. Each soleus muscle was used for only a single trial (i.e. control or retigabine) because cumulative effects and run-down may occur if this long protocol (typically 70 min) was repeated. Oscillations in force did not occur in the low K^+ challenge for EDL muscle in $\text{Ca}_v1.1\text{-R528H}$ mice, and so the time at which the nadir occurred was used to calculate the relative protection.

The dose–response relation for the relative protection from loss of force by pre-treatment with retigabine is shown in Fig. 3. The concentration-dependent protection had an equivalent $K_d = 0.82 \pm 0.13 \mu\text{M}$ and $2.2 \pm 0.42 \mu\text{M}$ for $\text{Na}_v1.4\text{-R669H}$ and $\text{Ca}_v1.1\text{-R528H}$, respectively, based on the best-fit by a single binding-site model. A limited series of measurements were also performed with pinacidil, a K_{ATP} channel opener, which has previously been shown to protect against loss of force for an *ex vivo* contraction assay of human HypoPP muscle at a concentration of 100 μM .¹⁰ Using our $\text{Na}_v1.4\text{-R669H}$ mouse model, we confirmed that pre-treatment with pinacidil protects HypoPP muscle from a loss of force in a low- K^+ challenge (Fig. 3); however, the potency was about 10-fold lower ($K_d = 8.1 \pm 0.5 \mu\text{M}$) compared to retigabine.

We also tested whether application of retigabine would hasten the recovery from an ongoing low- K^+ induced loss of force in HypoPP muscle. The paired response from the contralateral muscle, without drug exposure, was used as an internal control. Figure 4A shows two examples from soleus muscles of separate $\text{Na}_v1.4\text{-R669H}$ HypoPP mice. A 2 mM K^+ challenge was applied first, and then after confirming a loss of peak force, the bath was exchanged by a new 2 mM K^+ solution that also contained 10 μM retigabine. Application of retigabine at 10 min after the onset of reduced force (Fig. 4A, left) or even after 30 min when two loss-of-force cycles were complete (Fig. 4A, right), produced a sustained improvement in force and prevented any further cyclical reduction of force. The improved level of tetanic force in retigabine, while still in a 2 mM K^+ bath, was on average $83.2\% \pm 0.021$ ($n=3$) of baseline, which is comparable to the performance of WT soleus in 2 mM K^+ .¹⁶ Rescue by retigabine from an on-going loss of force in low K^+ was also observed for the EDL muscle of $\text{Ca}_v1.1\text{-R528H}$ mice (Fig. 4B).

The impairment of contraction during an episode of HypoPP is caused by sustained depolarization, which inactivates sodium channels and reduces muscle fibre excitability.¹ In HypoPP, ictal

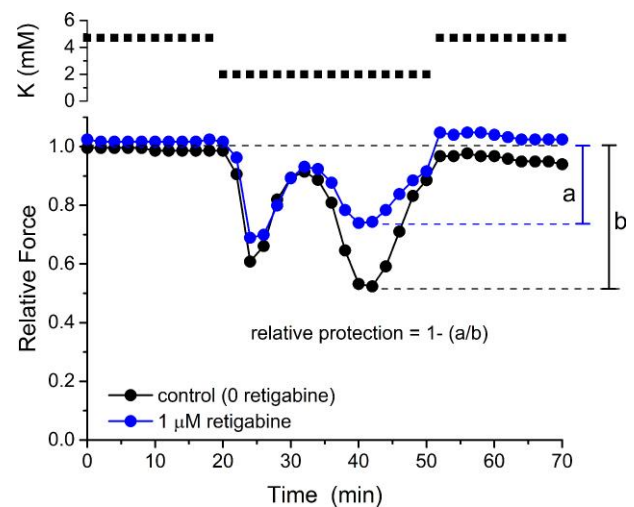


Figure 2 Protocol to quantify protection from loss of force in a low K^+ challenge, when the response waxes and wanes in an oscillatory pattern for the soleus muscle from $\text{Na}_v1.4\text{-R669H}$. This representative contraction test shows variability in the extent of protection by retigabine; compare the small difference in control to retigabine nadirs at 24 min versus the larger difference at 42 min. In general, the protection in 1 μM retigabine was less than for 10 μM (Fig. 1). The extent of protection was measured at each nadir (generally two within the 30 min low- K^+ challenge) as the ratio of the loss of force in retigabine (distance 'a') to the loss in control with no drug (distance 'b'). The average of these two measurements was used as the quantitative estimate for the extent of protection for that animal. The low- K^+ challenge (2 mM) was applied from 20 to 50 min.

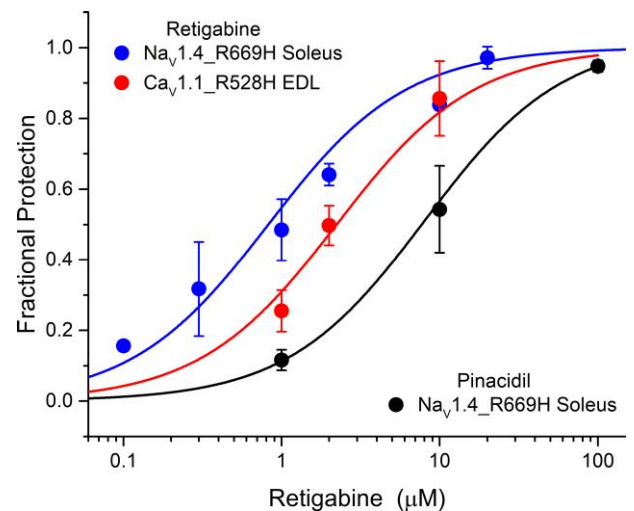


Figure 3 Dose–response relation for the protection by retigabine and by pinacidil. Each symbol is a mean value \pm SEM ($n=2$ to 4) for the relative protection of force in pair-wise comparisons of drug to no drug for a challenge with 2 mM K^+ (same mouse, two different muscles from either hindlimb). The curves show fitted estimates for a single binding-site model with K_d values of $0.82 \pm 0.13 \mu\text{M}$ ($\text{Na}_v1.4\text{-R669H}$) and $1.9 \pm 0.48 \mu\text{M}$ ($\text{Ca}_v1.1\text{-R528H}$) for retigabine and $8.1 \pm 0.49 \mu\text{M}$ for pinacidil ($\text{Na}_v1.4\text{-R669H}$). The therapeutic range for unbound drug concentration is shown by the shaded region.

depolarization of the resting potential (V_{rest}) paradoxically occurs in low K^+ that would normally hyperpolarize the membrane from a negative shift of the K^+ Nernst potential (E_{K}). We tested whether retigabine prevents or reverses this paradoxical depolarization of

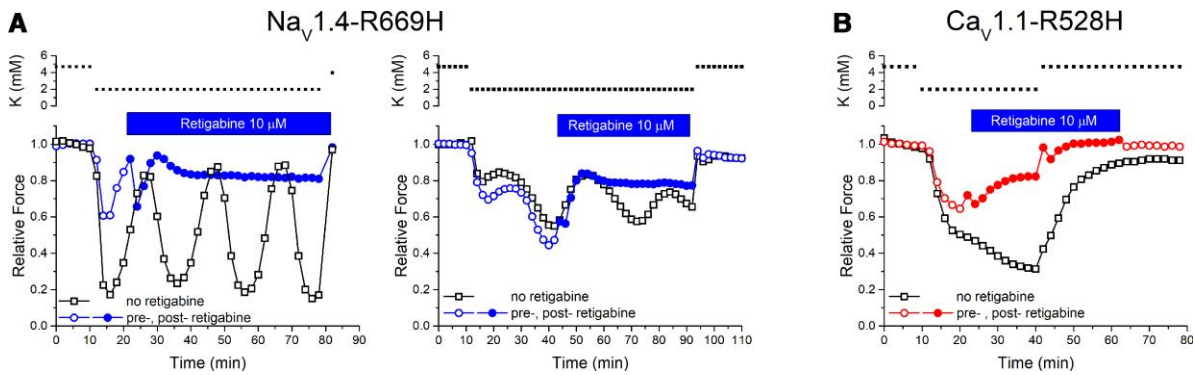


Figure 4 Retigabine promotes recovery from the loss of force for HypoPP muscle while in low K^+ . Examples of retigabine-induced rescue from loss of force are shown by comparison to the control response in the contralateral muscle. (A) For the soleus muscle from the $Na_v1.4$ -R669H mouse, application of $10 \mu M$ retigabine promptly aborted the oscillatory loss of force when applied either 10 min (left) or 30 min (right) after the onset of $2 \text{ mM } K^+$. Similar responses were observed in 5 of 6 trials to test enhanced recovery by $10 \mu M$ retigabine. (B) For the EDL muscle from the $Ca_v1.1$ -R528H mouse, $10 \mu M$ retigabine also induced a recovery of relative peak force compared to the response from the contralateral control. For both the soleus and the EDL muscles, recovery of relative peak force to the sustained value of 0.83 is comparable to the relative force for WT soleus in $2 \text{ mM } K^+$.

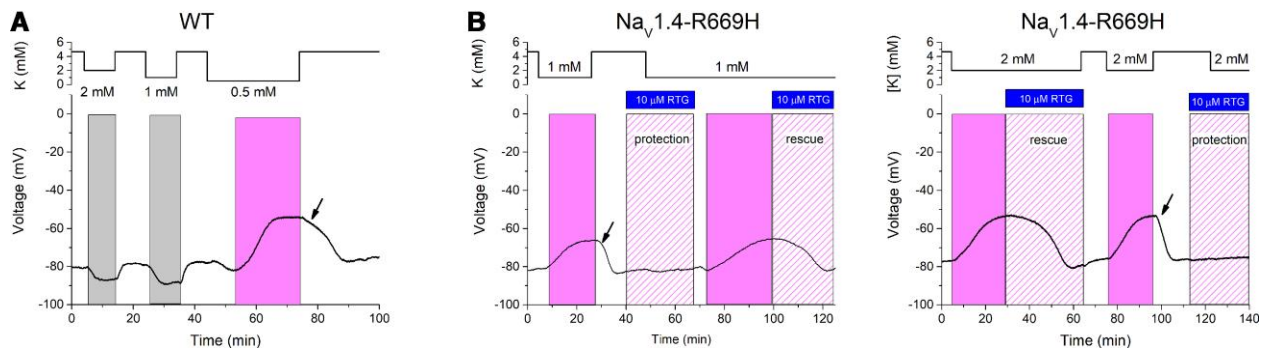


Figure 5 Retigabine prevents the paradoxical depolarization of HypoPP fibres during a low K^+ challenge. Traces show continuous recordings of the resting potential, measured by microelectrode impalement of a fibre in an *ex vivo* mount of the EDL muscle. (A) In WT EDL, low K^+ challenges of 2 mM or 1 mM elicited hyperpolarization (shaded regions) and an extremely low K^+ of 0.5 mM was required to induce paradoxical depolarization. Repolarization occurred upon turn to $4.7 \text{ mM } K^+$ (arrow). (B) For $Na_v1.4$ -R669H fibres, paradoxical depolarization occurred for more modest reduction of K^+ to 1.0 mM or 2.0 mM . Application of retigabine before the low K^+ challenges prevented the paradoxical depolarization (hatched regions labelled 'protection'). After paradoxical depolarization was established with a low K^+ challenge, application of retigabine induced fibre repolarization (hatched regions labelled 'rescue').

HypoPP fibres exposed to low K^+ . Figure 5 shows the K^+ -induced changes of V_{rest} for EDL fibres impaled with a sharp microelectrode. Reduction of K^+ to 2 mM or even 1 mM caused hyperpolarization of WT fibres, as expected from the negative shift of E_K , (Fig. 5A, grey-shaded regions). At an extremely low K^+ of 0.5 mM , even WT fibres paradoxically depolarized (magenta region) as previously described.¹⁹ For EDL fibres from the $Na_v1.4$ -R669H mouse, the more modest K^+ reductions to 2 mM or 1 mM elicited paradoxical depolarization, which is the membrane potential equivalent of ictal HypoPP. Paradoxical depolarization of WT or of HypoPP fibres was reversible upon return to $4.7 \text{ mM } K^+$ (Fig. 5, arrows), which shows this behaviour was not caused by fibre damage. Addition of retigabine before the low K^+ challenge prevented the paradoxical depolarization of HypoPP fibres (Fig. 5B, hatched regions labelled 'protection'). Moreover, HypoPP fibres that were paradoxically depolarized in low K^+ repolarized to the normal V_{rest} when retigabine was added to the bath, even though the low K^+ condition was unchanged (Fig. 5B, hatched regions labelled 'rescue'). These observations support the view that the beneficial effects of retigabine occur by a stabilizing effect on the normally polarized value of V_{rest} .

To confirm the beneficial effects of retigabine were dependent upon currents conducted by K_v7 -type K^+ channels, we assessed the effect of a K_v7 inhibitor, XE991.²⁰ As shown in Fig. 6, exposure to $10 \mu M$ XE991 did not alter the peak tetanic force in $4.7 \text{ mM } K^+$ (10 – 20 min , triangles). The other soleus muscle from the same HypoPP mouse was not exposed to XE991. Retigabine was then applied to both soleus muscle preparations, followed by a low- K^+ challenge. A marked loss of force in low K^+ occurred for the XE991 + retigabine exposed soleus, whereas the paired HypoPP muscle was protected by retigabine. These data show the beneficial effect of retigabine is blocked by XE991 and suggest that basal activity of K_v7 channels limits the severity of the loss of force in low K^+ .

Discussion

The therapeutic potential of K^+ channel openers to reduce the frequency and severity for the attacks of weakness in periodic paralysis has been recognized for over 30 years.¹¹ The fundamental principle is that because increasing the membrane conductance

to K^+ will hyperpolarize the membrane potential towards $E_K \approx -95$ mV, this class of drugs will counteract the sustained anomalous depolarization of the resting potential that causes transient weakness in periodic paralysis by inactivation of sodium channels. Many criteria must be met, however, for this strategy to be safe and effective. For example, many K^+ channels are activated by depolarization, and the drug-induced augmentation of channel opening must occur over an operational voltage range that is relevant for stabilization of a normal resting potential (-80 to -95 mV) and extend to the anomalous depolarization during paralysis (typically -50 to -60 mV). This voltage-dependent property will determine whether the increased K^+ conductance will primarily affect V_{rest} , the action potential waveform (e.g. amplitude and duration) or both. Another concern is the expression pattern across different tissues for the K^+ channels that are activated by the drug. High expression in skeletal muscle is desired, with lower expression in other tissues to minimize side effects. The drug potency (small dissociation constant, K_d) and magnitude of the K^+ conductance increase are critical as well. If the conductance increase is very small, then the drug may not produce a meaningful reduction in the susceptibility to attacks of weakness. Conversely, a very large K^+ conductance increase may impair contractility by reducing muscle fibre excitability.

The effectiveness of retigabine at low μM concentrations to protect HypoPP muscle from a loss of force in a low- K^+ challenge (2 mM) demonstrates the therapeutic potential of $K_V7.x$ channel openers in the symptomatic management of HypoPP. The $K_V7.1$ – $K_V7.5$ family of voltage-gated K^+ channels is encoded by *KCNQ1*–*KCNQ5* genes, so-named because the founding member, *KCNQ1*, was identified as a disease locus for type 1 long QT syndrome.²¹ The predominant K_V7 channel in the heart is $K_V7.1$, and importantly, this isoform is 100-fold less sensitive to retigabine than $K_V7.2$ – $K_V7.5$,²² which explains the absence of cardiac side effects. The $K_V7.2$ – $K_V7.5$ isoforms were initially characterized as ‘neuronal’, with $K_V7.2$, $K_V7.3$, $K_V7.5$ expressed in the central nervous system, $K_V7.4$ in the cochlea and $K_V7.2$ in the peripheral nervous system. Skeletal muscle was not initially considered to be a site of significant $K_V7.x$ expression, but subsequent studies using mouse and human skeletal muscle have detected $K_V7.1$ – $K_V7.5$ transcripts by RT-PCR and $K_V7.2$ – $K_V7.4$ subunit protein by both immunoblot and immunohistochemistry.²³ Changes in the expression pattern of $K_V7.x$ subunits with muscle proliferation and differentiation have implicated a role in development.²³

A physiological role has not yet been established for the voltage-gated K^+ current conducted by K_V7 channels in skeletal muscle. Voltage-clamp studies to characterize $K_V7.x$ currents in muscle have not been reported, most likely because of the technical difficulty in isolating this component of the K^+ current from the contributions by a multitude of K^+ channels in this tissue (over 20 described). The beneficial effect of retigabine is completely prevented by XE991 (Fig. 6), which at 10 μM is a specific inhibitor of K_V7 channels²⁴ and supports our interpretation that the K^+ channel-opening action of retigabine is the mechanism of drug action. Because low-dose retigabine protects HypoPP muscle from a low- K^+ induced loss of force and also suppresses after-discharges in mouse models of myotonia (using pharmacological block of the chloride conductance¹⁴ or by disruption of the *Cln1* gene¹³), the $K_V7.x$ K^+ current clearly has an important role in regulating muscle excitability. Prior experiments (using current-clamp) to explore the mechanism for retigabine-induced suppression of myotonia did not detect a change in fibre input resistance, resting potential or action potential properties of mouse muscle upon exposure to

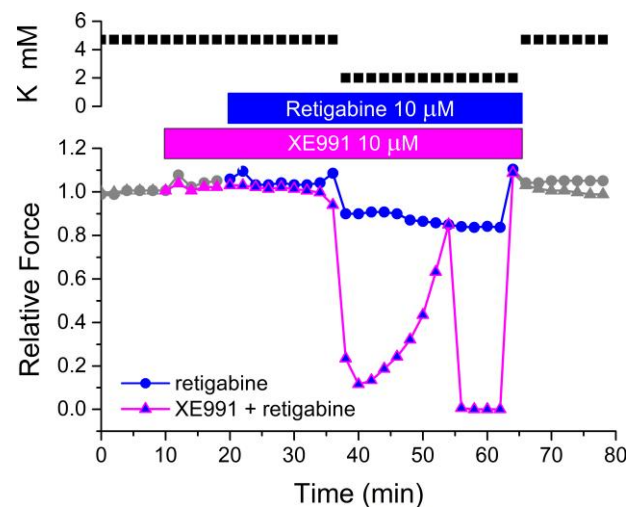


Figure 6 The protective effect of retigabine was prevented by the K_V7 channel inhibitor XE991. Paired soleus muscles from the same HypoPP mouse were used to test for an effect of XE991. Application of XE991 to one muscle did not alter the baseline peak tetanic force (10 min). Pre-treatment with retigabine at 20 min (circles) prevented the loss of force during a low- K^+ challenge (40 to 60 min). In contrast, an exceptionally large loss of force occurred for the XE991-exposed soleus muscle, despite the same pre-treatment with retigabine.

20 μM retigabine.¹³ Our data corroborate the observation that retigabine does not affect V_{rest} of normally polarized muscle fibres (Fig. 5B, hatched regions of protection where V_{rest} in 4.7 K^+ did not change when retigabine was added). Most likely, the absence of an observable drug-induced shift of V_{rest} is because in normally polarized fibres this resting voltage is nearly equal to E_K . Electrophysiological approaches with greater sensitivity and specificity will be required to elucidate the normal role of $K_V7.x$ K^+ currents in muscle and the beneficial effect of activation by retigabine.

While the chronic use of retigabine as a prophylactic antiepileptic drug for refractory partial-onset seizures was discontinued in the US in 2017 because of adverse effects (primarily skin discoloration²⁵ and also CNS effects²⁶ with dose-dependent dizziness, somnolence and headache), the enthusiasm for K_V7 channel activators remains strong for management of epilepsy (especially *KCNQ2* developmental and epileptic encephalopathies), pain management, for neuroprotection in degenerative diseases and for mood stabilization. Derivatives of retigabine with reduced dimer formation and therefore lack of skin discoloration are in clinical trials for *KCNQ2*-DDE (XEN496 in children; NCT04912856) and for focal epilepsy in adults (XEN1101; NCT03796962). Another K_V7 opener, GRT-X, which is chemically unrelated to retigabine, is in development. These pipeline drugs also act by activation of K_V7 channels, and are therefore likely to be effective in ameliorating attacks of periodic paralysis.

Moreover, our data showing rescue of both contractility (Fig. 4) and of V_{rest} (Fig. 5B) demonstrate the potential for short-term administration of retigabine, or newer K_V7 openers, as abortive therapy for an acute attack of weakness in HypoPP. After a single 100 mg oral dose, a C_{max} of 390 ng/ml occurs within 1.5 h and the half-life is 8 h.²⁷ An unbound retigabine fraction of 0.9 μM will be achieved with a single 400 mg dose (assumes 80% protein binding). Based on this observation, a clinical trial for efficacy of K_V7 openers could be performed by assessing the rate of recovery of the compound muscle action potential in the long exercise test for periodic paralysis, as was done to studies of bumetanide.³ The functional

defect in periodic paralysis operates like a binary switch, with a sustained shift between the normal V_{rest} and an anomalously depolarized value. Consequently, a single dose of retigabine may be sufficient to ‘reset’ V_{rest} to the normal range and suppress a recurrent attack of weakness over the minutes to hours required for correction of extracellular $[K^+]$.

An adverse effect of retigabine on skeletal muscle function is unlikely. One report described a dose-dependent reduction of contractile force during a 3 s tetanic stimulation of rat muscle, with a 50% inhibitory concentrations (IC_{50}) of 1 μ M, presumably from a reduced fibre excitability.²⁸ Our studies with up to 20 μ M retigabine show no impairment of peak isometric force during a 400 ms tetanic contraction, applied once every 2 min for 2 h. Similarly, the prior two studies on the effect of retigabine in mouse models of myotonia did not report a reduction of *ex vivo* tetanic force with 20 or 30 μ M retigabine.^{13,14} *In vivo* mouse and rat studies that used a very high dose of retigabine (30 mg/kg, which is 5.7 times larger than the maximal oral dose of 400 mg for a 70 kg human) showed a loss of force with tetanic contractions lasting seconds,^{13,28} but this result is not likely to be relevant to patient care. In our opinion, the ‘fatigue’ described as an adverse effect of retigabine in anti-epileptic drug trials is more likely to be of central origin rather than muscle fibre dysfunction.

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Competing interests

The authors report no competing interests.

References

- Cannon SC. Channelopathies of skeletal muscle excitability. *Compr Physiol*. 2015;5:761-790.
- Statland JM, Fontaine B, Hanna MG, et al. Review of the diagnosis and treatment of periodic paralysis. *Muscle Nerve*. 2018;57:522-530.
- Jitpimolmard N, Matthews E, Fialho D. Treatment updates for neuromuscular channelopathies. *Curr Treat Options Neurol*. 2020;22:34.
- Sansone VA, Burge J, McDermott MP, et al. Randomized, placebo-controlled trials of dichlorphenamide in periodic paralysis. *Neurology*. 2016;86:1408-1416.
- Tawil R, McDermott MP, Brown R Jr, et al. Randomized trials of dichlorphenamide in the periodic paralyses. Working group on periodic paralysis. *Ann Neurol*. 2000;47:46-53.
- Matthews E, Portaro S, Ke Q, et al. Acetazolamide efficacy in hypokalemic periodic paralysis and the predictive role of genotype. *Neurology*. 2011;77:1960-1964.
- Venance SL, Cannon SC, Fialho D, et al. The primary periodic paralyses: Diagnosis, pathogenesis and treatment. *Brain*. 2006;129:8-17.
- Quasthoff S, Spuler A, Lehmann-Horn F, Grafe P. Cromakalim, pinacidil and RP 49356 activate a tolbutamide-sensitive K^+ conductance in human skeletal muscle fibres. *Pflugers Archiv: Eur J Physiol*. 1989;414(Suppl 1):S179-S180.
- Lawson K. Potassium channel openers as potential therapeutic weapons in ion channel disease. *Kidney Int*. 2000;57:838-845.
- Iaizzo PA, Quasthoff S, Lehmann-Horn F. Differential diagnosis of periodic paralysis aided by *in vitro* myography. *Neuromuscul Disord*. 1995;5:115-124.
- Grafe P, Quasthoff S, Strupp M, Lehmann-Horn F. Enhancement of K^+ conductance improves *in vitro* the contraction force of skeletal muscle in hypokalemic periodic paralysis. *Muscle Nerve*. 1990;13:451-457.
- Orhan G, Wuttke TV, Nies AT, Schwab M, Lerche H. Retigabine/ezogabine, a KCNQ/K(V)7 channel opener: Pharmacological and clinical data. *Expert Opin Pharmacother*. 2012;13:1807-1816.
- Dupont C, Denman KS, Hawash AA, Voss AA, Rich MM. Treatment of myotonia congenita with retigabine in mice. *Exp Neurol*. 2019;315:52-59.
- Su TR, Zei WS, Su CC, Hsiao G, Lin MJ. The effects of the KCNQ openers retigabine and flupirtine on myotonia in mammalian skeletal muscle induced by a chloride channel blocker. *Evid Based Complement Alternat Med*. 2012;2012:803082.
- Wu F, Mi W, Hernandez-Ochoa EO, et al. A calcium channel mutant mouse model of hypokalemic periodic paralysis. *J Clin Invest*. 2012;122:4580-4591.
- Wu F, Mi W, Burns DK, et al. A sodium channel knockin mutant (NaV1.4-R669H) mouse model of hypokalemic periodic paralysis. *J Clin Invest*. 2011;121:4082-4094.
- Bulman DE, Scoggan KA, van Oene MD, et al. A novel sodium channel mutation in a family with hypokalemic periodic paralysis. *Neurology*. 1999;53:1932-1936.
- Wu F, Mi W, Cannon SC. Bumetanide prevents transient decreases in muscle force in murine hypokalemic periodic paralysis. *Neurology*. 2013;80:1110-1116.
- van Heukelum JS. Role of the anomalous rectifier in determining membrane potentials of mouse muscle fibres at low extracellular K^+ . *J Physiol*. 1991;434:549-560.
- Wang HS, Pan Z, Shi W, et al. KCNQ2 and KCNQ3 potassium channel subunits: Molecular correlates of the M-channel. *Science*. 1998;282:1890-1893.
- Keating M, Atkinson D, Dunn C, Timothy K, Vincent GM, Leppert M. Linkage of a cardiac arrhythmia, the long QT syndrome, and the Harvey ras-1 gene. *Science*. 1991;252:704-706.
- Tatulian L, Delmas P, Abogadie FC, Brown DA. Activation of expressed KCNQ potassium currents and native neuronal M-type potassium currents by the anti-convulsant drug retigabine. *J Neurosci*. 2001;21:5535-5545.
- Iannotti FA, Panza E, Barrese V, Viggiano D, Soldovieri MV, Tagliatalata M. Expression, localization, and pharmacological role of Kv7 potassium channels in skeletal muscle proliferation, differentiation, and survival after myotoxic insults. *J Pharmacol Exp Ther*. 2010;332:811-820.
- Elmedy P, Calloe K, Schmitt N, Hansen RS, Grunnet M, Olesen SP. Modulation of ERG channels by XE991. *Basic Clin Pharmacol Toxicol*. 2007;100:316-322.
- Shkolnik T G, Feuerman H, Didkovsky E, et al. Blue-gray mucocutaneous discoloration: A new adverse effect of ezogabine. *JAMA Dermatol*. 2014;150:984-989.
- Brodie MJ, Lerche H, Gil-Nagel A, et al. Efficacy and safety of adjunctive ezogabine (retigabine) in refractory partial epilepsy. *Neurology*. 2010;75:1817-1824.
- Ferron GM, Paul J, Fruncillo R, et al. Multiple-dose, linear, dose-proportional pharmacokinetics of retigabine in healthy volunteers. *J Clin Pharmacol*. 2002;42:175-182.
- Zagorchev P, Apostolova E, Kokova V, Peychev L. Activation of KCNQ channels located on the skeletal muscle membrane by retigabine and its influence on the maximal muscle force in rat muscle strips. *Naunyn Schmiedeberg Arch Pharmacol*. 2016;389:439-446.