Beneficial effects of bumetanide in a CaV1.1-R528H mouse model of hypokalaemic periodic paralysis

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Transient attacks of weakness in hypokalaemic periodic paralysis are caused by reduced fibre excitability from paradoxical depolarization of the resting potential in low potassium. Mutations of calcium channel and sodium channel genes have been identified as the underlying molecular defects that cause instability of the resting potential. Despite these scientific advances, therapeutic options remain limited. In a mouse model of hypokalaemic periodic paralysis from a sodium channel mutation (NaV1.4-R669H), we recently showed that inhibition of chloride influx with bumetanide reduced the susceptibility to attacks of weakness, in vitro. The R528H mutation in the calcium channel gene (CACNA1S encoding CaV1.1) is the most common cause of hypokalaemic periodic paralysis. We developed a CaV1.1-R528H knock-in mouse model of hypokalaemic periodic paralysis and show herein that bumetanide protects against both muscle weakness from low K+ challenge in vitro and loss of muscle excitability in vivo from a glucose plus insulin infusion. This work demonstrates the critical role of the chloride gradient in modulating the susceptibility to ictal weakness and establishes bumetanide as a potential therapy for hypokalaemic periodic paralysis arising from either NaV1.4 or CaV1.1 mutations.

Keywords: calcium channel; skeletal muscle; NKCC transporter; acetazolamide

Abbreviations: CaV1.1 = voltage-gated calcium channel of skeletal muscle; CMAP = compound muscle action potential; HypoPP = hypokalaemic periodic paralysis; NaV1.4 = voltage-gated sodium channel of skeletal muscle; NKCC = sodium potassium two chloride transporter

Introduction

Hypokalaemic periodic paralysis (HypoPP) is a dominantly inherited channelopathy of skeletal muscle that presents with transient episodes of weakness in association with low serum potassium (Venance et al., 2006). HypoPP is caused by missense mutations in CACNA1S encoding the pore-forming z-subunit of the CaV1.1 calcium channel, or in SCN4A encoding the z-subunit of the NaV1.4 sodium channel (Ptacek et al., 1994; Elbaz et al., 1995; Bulman et al., 1999). We recently developed knock-in mutant mouse models of HypoPP with the CaV1.1-R528H mutation (Wu et al., 2012), which is the most common cause of HypoPP in humans, and the NaV1.4-R669H mutation (Wu et al., 2011). These animal models have a robust HypoPP phenotype with a severe loss of contractile force in low K+, a marked reduction of muscle excitability with glucose plus insulin challenge,
and for CaV1.1-R528H, a vacuolar myopathy. This model system provides a unique opportunity to explore therapeutic interventions aimed at reducing or eliminating the loss of muscle excitability and force triggered by provocative manoeuvres.

The carbonic anhydrase inhibitor, acetazolamide, has been used for decades to prophylactically reduce attack frequency and severity (Resnick et al., 1968), but only ~50% of patients have a favourable response (Matthews et al., 2011), adverse effects may occur, and in some patients the attacks of paralysis are worsened (Torres et al., 1981; Steinberg et al., 2001). Recent advances in understanding the mechanistic basis for loss of fibre excitability during an attack of weakness have provided a new therapeutic strategy (Geukes Poppen et al., 2002; Jurkat-Rott et al., 2009; Cannon, 2010). In an acute attack of HypoPP, affected fibres are paradoxically depolarized, despite low external K+, which reduces fibre excitability and may cause flaccid paralysis (Rüdel et al., 1984). Studies in the past 5 years have identified a common functional defect in mutant CaV1.1 or NaV1.4 channels associated with HypoPP (Sokolov et al., 2007; Struyk and Cannon, 2007; Struyk et al., 2008; Wu et al., 2012). In both channels, missense mutations occur at arginine residues in the voltage-sensors and cause an anomalous inward ‘gating pore’ current. This leakage current increases the susceptibility to paradoxical depolarization, and loss of fibre excitability, in low external K+. The propensity for the ictal depolarization is also dependent on the transmembrane chloride gradient, and therein lies the opportunity for therapeutic intervention (Geukes Poppen et al., 2002). Higher concentrations of intramuscular Cl− promote depolarization in low K+. Chloride accumulation in muscle is driven by a cotransporter of sodium–potassium–and two chloride ions (NKCC) that facilitates influx of these ions (Russell, 2000). The NKCC transporter is potently inhibited by the loop diuretic bumetanide. Although the use of bumetanide to treat HypoPP has never appeared in the clinical literature, we recently showed that micromolar bumetanide preserves muscle force and excitability during an in vitro challenge with 2 mM K+ in the murine NaV1.4-R669H model of HypoPP (Wu et al., 2013). In the present study, we have extended this work to show that bumetanide is also effective in the CaV1.1-R528H model of HypoPP, and that the drug works in vivo to protect against the loss of muscle excitability triggered by a glucose plus insulin infusion.

Materials and methods

CaV1.1 hypokalaemic periodic paralysis mice

We have previously developed and characterized a murine model for HypoPP in which the R528H mutation was introduced into exon 13 of CACNA1S that codes for the α-subunit of the CaV1.1 calcium channel (Wu et al., 2012). These knock-in mutant HypoPP mice were bred in the 129/Sv strain as heterozygous (CACNA1S<sup>−/+R528H</sup>), denoted herein as R528H<sup>−/+</sup> or homozygous (CACNA1S<sup>−/−R528H</sup> or R528H<sup>−/−</sup>) animals with wild-type littermates (CACNA1S<sup>−/+</sup>) serving as controls. All procedures performed on mice were in accordance with animal protocols approved by the UT Southwestern Medical Centre Institutional Animal Care and Use Committee.

In vitro force measurement

Isometric contractile force of the soleus muscle was measured in response to tetanic stimulation with a pair of platinum wire electrodes, as described previously (Wu et al., 2012). In brief, the soleus muscle from each hindlimb was rapidly dissected free and suspended vertically in a separate 25 ml organ bath maintained at 37°C. Tetanic stimulation (40 pulses, 1 ms, 80 mA at 100 Hz) was applied under computer control, and the force was measured with a semiconductor strain gauge (Forte25 WPI). The bicarbonate-buffered bath was continuously gassed with a 95% / 5% mixture of O<sub>2</sub> / CO<sub>2</sub> (pH 7.4) and contained 118 mM NaCl, 4.75 mM KCl, 1.18 mM MgSO<sub>4</sub>, 2.54 mM CaCl<sub>2</sub>, 1.18 mM NaH<sub>2</sub>P<sub>O</sub><sub>4</sub>, 10 mM glucose, 24.8 mM NaHCO<sub>3</sub>, 0.02 U/ml insulin (Eli Lilly), and 0.25 μM α-tubocurarine (Sigma-Aldrich). Bath solutions containing drugs under study were made by addition of concentrated stock solutions in ethanol (bumetanide or acetazolamide) or dimethylsulphoxide (furosemide). Final dilution of solvent was 1:1000 or greater, and controls with solvent alone had no effect. For studies on the effects of bath osmolality under conditions of constant ionic strength (Fig. 2), a low-sodium solution (70 mM) was used as the hypotonic standard (190 mOsm), and the hypertonic solution (235 mOsm) was produced by adding sucrose. During an experimental trial, the soleus contractility was monitored every 2 min with tetanic stimulation, and test solutions were applied by complete exchange with eight times the volume of the organ bath over 1 min.

In vivo compound muscle action potential measurement

Muscle excitability was measured as the peak-to-peak amplitude of the compound muscle action potential (CMAP), elicited by sciatic nerve stimulation in the anaesthetized mouse (Wu et al., 2012). One day before testing, sodium polystyrene sulphonate (Kayexalate, KVK-TECK Inc.) was administered by gavage to reduce the baseline extracellular K+. Anaesthesia was maintained by isoflurane inhalation, and mice were instrumented with an internal jugular venous catheter, a monopolar needle EMG electrode in the gastrocnemius or soleus, and a stimulating electrode on the sciatic nerve. The CMAP response to a single shock (0.1 ms) was recorded once per min, over a 2-h observation period. A glucose plus insulin challenge was administered by continuous intravenous infusion (0.5 ml/h with 0.175 mg/ml glucose and 0.2 U/ml insulin).

Results

Loss of force from low-K<sup>+</sup> challenge in vitro was attenuated by bumetanide

For the in vitro contraction assay, a 2 mM K<sup>+</sup> challenge consistently produced a reduction of peak tetanic force in R528H soleus muscle, and this deficit was partially reversed or could be prevented by application of bumetanide. Figure 1A shows force transients recorded from the soleus isolated from a heterozygous R528H<sup>−/+</sup> male. The control response was in 4.75 mM K<sup>+</sup>, and the series of plots shows tetanic contractions recorded from the
In vitro contraction assay demonstrates a beneficial effect of bumetanide (BMT) during a hypokalaemic challenge. Tetanic contractions were elicited by 100 Hz stimulation of the excised soleus muscle maintained at 37°C. (A) Force responses are shown for contractions in control conditions (4.75 mM K⁺), and 20 min after bath exchange to 2 mM K⁺, then 2 mM K⁺ plus bumetanide (75 μM), and then back to control. (B) Normalized peak tetanic force is shown for soleus from wild-type (left, black), R528H⁺/m (middle, blue), and R528H⁻/m (right, pink) mice. The trials were designed to test recovery after low-K⁺ induced loss of force (top row) or prevention by co-administration of bumetanide with the onset of hypokalemia (bottom row). Squares denote muscle harvested from males and circles from females. Symbols are means from three to eight animals and error bars show SEM. WT = wild-type.
same muscle at the end of a 30 min equilibration in 2 mM K+, 2 mM K+ plus 75 μM bumetanide, and then return to 4.75 mM K+ with no drug. The loss of force in 2 mM K+ was partially reversed by addition of bumetanide, even in the continued presence of severe hypokalaemia, and full recovery of force occurred upon return to normokalaemic conditions.

The time course for the onset and recovery of the force deficit in low-K+ and the efficacy of bumetanide are shown in Fig. 1B for muscles isolated from wild-type, R528H+/− and R528H/C24 mice. Tetanic contractions were performed every 2 min, the peak force for each muscle was normalized to the amplitude before the low-K+ challenge, and the symbols represent average responses from six to eight muscles. The top row in Fig. 1 shows trials for which the 2 mM K+ exposure preceded the application of bumetanide. The tetanic force was reduced in 2 mM K+ for all genotypes, but the decrease was much less for wild-type, ~30%, than for muscle with the R528H mutation, ~70%. As we reported previously (Wu et al., 2012), the HypoPP phenotype is less severe in heterozygous females compared with males (shown in Fig. 1B by the delay in the loss of force), similar to the reduced penetrance observed in female humans with the R528H mutation (Elbaz et al., 1995). Application of 75 μM bumetanide reversed ~50% of the low-K+ induced reduction in force for wild-type and R528H+/− muscle (P < 0.02, n = 8; P < 0.005, n = 6, respectively) but caused only a modest effect for R528H/C24 muscle (12%, not significant, P = 0.28, n = 7). When the muscle was returned to 4.75 mM K+ (90 min in Fig. 1B), the force fully recovered for all genotypes and even had an overshoot above the initial control response. The overshoot was attributed to the effect of bumetanide, as the recovery after a 2 mM K+ challenge alone with no drug did not increase above baseline [Fig. 3B in Wu et al. (2012)].

The bottom row of Fig. 1B shows normalized force responses when bumetanide was co-administered at the onset of the 2 mM K+ challenge. No loss of force occurred in low-K+ for wild-type or R528H+/− females, and the R528H+/− males and R528H/C24 had only a modest reduction in force by 10–20%. Interestingly, the beneficial effect of bumetanide persisted, even when the drug was washed out and the muscle remained in 2 mM K+ (60 min in Fig. 1B). This prolonged effect of bumetanide may be a reflection of the time required for myoplasmic Cl− to increase back to basal levels after washout of inhibition for the NKCC transporter (see ‘Discussion’ section).

Bumetanide protected hypokalaemic periodic paralysis muscle from loss of force in hypertonic conditions

Hypertonic conditions cause cell shrinkage and stimulate a compensatory ‘regulatory volume increase’ by activation of the NKCC transporter that promotes solute influx (Russell, 2000). One consequence of these events is an increase in myoplasmic [Cl−], which increases the susceptibility to paradoxical depolarization and loss of force in low K+ (Geukes Foppen et al., 2002), and thereby may impact the phenotypic expression of HypoPP. This sequence of events was the basis for investigating the NKCC inhibitor bumetanide as a potential therapeutic agent for HypoPP (Wu et al., 2013). If this mechanism is correct, then hypertonic solutions should exacerbate the risk of weakness in HypoPP and bumetanide should be protective.

We investigated the impact of osmolarity on susceptibility to HypoPP with the in vitro contraction assay in which one soleus was maintained in 75 μM bumetanide throughout the protocol and the paired muscle from the other limb was in drug-free conditions. Figure 2 shows that a hypertonic challenge of 325 mOsm produced a 60% reduction of force in R528H+/− drug-free soleus from males. Superposition of a coincident low-K+ challenge further reduced the peak force to 5% of control (95% loss). Pretreatment with 75 μM bumetanide (10 min in Fig. 2) caused a 10% increase in force at baseline and maintenance of the drug in all subsequent solution exchanges protected the muscle from loss of force by hypertonic solution and hypokalaemia. Conversely, a hypotonic bath (190 mOsm) produced a transient increase in force (Fig. 2) and protected R528H+/− soleus from loss of force in a 2 mM K+ challenge even without bumetanide. Return to isotonic conditions in the continued presence of 2 mM K+ promptly triggered a loss of force (black circles). Again, the continued presence of 75 μM bumetanide (red squares) protected the muscle from loss of force. We propose that hypertonic solutions activated the NKCC transporter and thereby increased susceptibility to HypoPP, whereas hypotonic conditions reduced NKCC activity below basal levels and protected R528H muscle from hypokalaemia-induced loss of force. Inhibition of NKCC by bumetanide abrogated the effects of solution osmolarity.

Bumetanide was superior to acetazolamide for the in vitro contraction test

Acetazolamide, a carbonic anhydrase inhibitor, is often used prophylactically to reduce the frequency and severity of attacks of weakness in HypoPP (Resnick et al., 1968), although not all R528H patients have a favourable response (Torres et al., 1981; Sternberg et al., 2001). We compared the efficacy of bumetanide and acetazolamide at therapeutically attainable concentrations for protection against loss of force in low-K+ with the in vitro contraction test in heterozygous R528H+/− muscle. Responses were segregated by sex of the mouse, as females had a milder HypoPP phenotype (Fig. 1B). Paired muscles from the same animal were tested in two separate organ baths. For the control bath, no drugs were applied and the force response to hypokalaemic challenge was measured for two 20-min exposures (Fig. 3, black circles). The other soleus was pretreated with acetazolamide (100 μM) and the first 2 mM K+ challenge was performed (blue squares). After return to 4.75 mM K+, the acetazolamide was washed out, bumetanide (0.5 μM) was applied (red squares), and a second 2 mM K+ challenge was performed. Acetazolamide had a modest protective effect in soleus from both males (Fig. 3A) and females (Fig. 3B), with the loss of force reduced by a ~30% compared with the responses in drug-free controls. In contrast, pretreatment with bumetanide was highly effective in preventing a loss of force from a 2 mM K+ challenge.
Furosemide also attenuated the loss of force with the in vitro Hypokalemic challenge

Furosemide is structurally similar to bumetanide and also inhibits the NKCC transporter, but at ~10-fold lower potency (Russell, 2000). Another difference is that furosemide is less specific for NKCC and inhibits other chloride transporters and chloride channels. We tested whether furosemide at a therapeutic concentration of 15 μM would have a beneficial effect on the preservation of force during a hypokalaemic challenge in vitro. Figure 4 shows that addition of furosemide after a 30 min exposure to 2 mM K⁺ did not produce a recovery of force, although further decrement appeared to have been prevented. Application of furosemide coincident with the onset of hypokalaemia did attenuate the loss of force (Fig. 4), but the benefit was quickly lost upon washout. We conclude that furosemide does provide some protection from loss of force in R528H⁺/⁻ muscle during hypokalaemia, probably

Figure 2 Hypertonicity exacerbated the susceptibility to loss of force in R528H soleus and was prevented by bumetanide (BMT). Pairs of soleus muscles dissected from the same R528H⁺/⁻ animal were tested in parallel. One was exposed continuously to bumetanide (75 μM) starting at 10 min whereas the other remained drug-free. Hypertonic challenge (left) with a sucrose containing bath (30 min) caused 60% loss of force that was further exacerbated by reduction of K⁺ to 2 mM (60 min). Bumetanide greatly reduced the loss of force from either challenge. A hypotonic challenge (right) transiently increased the force and protected the muscle from loss of force in 2 mM K⁺ (60–90 min). Return to normotonic conditions while in low K⁺ produced a marked loss of force.

Figure 3 Bumetanide (BMT) was superior to acetazolamide (ACTZ) in preventing loss of force in vitro, during a 2 mM K⁺ challenge. The soleus muscle from heterozygous R528H⁺/⁻ males (A, n = 3) or females (B, n = 4) were challenged with sequential 20 min exposures to 2 mM K⁺. Controls with no drug showed two episodes of reduced force (black circles). Pretreatment with acetazolamide (100 μM, blue circles) produced only modest benefit, whereas bumetanide (0.5 μM) completely prevented the loss of force.
through inhibition of the NKCC transporter, but that the efficacy is lower than that of bumetanide (compare with Figs 1B and 3).

**Bumetanide and acetazolamide were both efficacious in preserving muscle excitability in vivo**

The efficacy of bumetanide and acetazolamide to protect against a transient loss of muscle excitability in vivo was tested by monitoring the CMAP during a challenge with a continuous infusion of glucose plus insulin. The peak-to-peak CMAP amplitude was measured at 1 min intervals during the 2-h observation period in isoflurane-anaesthetized mice. In wild-type mice, the CMAP amplitude is stable and varies by <10% (Wu et al., 2012). The relative CMAP amplitude recorded from R528H<sup>+/m</sup> mice is shown in Fig. 5A. The continuous infusion of glucose plus insulin started at 10 min, and the CMAP had a precipitous decrease by 80% within 30 min for untreated mice (Fig. 5, black circles). For the treatment trials, a single intravenous bolus of bumetanide (0.08 mg/kg) or acetazolamide (4 mg/kg) was administered at time 0 min, and the glucose plus insulin infusion started at 10 min. For four of five mice treated with bumetanide and five of eight mice treated with acetazolamide, a protective effect was clearly evident, and the average of the relative CMAP is shown for these positive responders in Fig. 5A. The responses for the non-responders were comparable to those observed when no drug was administered, as shown by distribution of CMAP values, averaged over the interval from 100-120 min in the scatter plot of Figure 5B. A time-averaged CMAP amplitude of <0.5 was categorized as a non-responder.

Our prior study of bumetanide and acetazolamide in a sodium channel mouse model of HypoPP (NaV1.4-R669H) only used the *in vitro* contraction assay (Wu et al., 2013). We extended this work by performing the *in vivo* CMAP test of muscle excitability for NaV1.4-R669H<sup>+/m</sup> HypoPP mice, pretreated with bumetanide or acetazolamide. Both drugs had a beneficial effect on muscle excitability, with the CMAP amplitude maintained over 2 h at 70% of baseline for responders (Supplementary Fig. 1). However, only four of six mice treated with acetazolamide had a positive response, whereas all five mice treated with bumetanide had a preservation of CMAP amplitude.

The discrepancy between the lack of acetazolamide benefit *in vitro* (Fig. 3) and the protective effect *in vivo* (Fig. 5) was not anticipated. We explored the possibility that this difference may have resulted from the differences in the methods to provoke an attack of weakness for the two assays. In particular, the glucose plus insulin infusion may have produced a hypertonic state that stimulated the NKCC transporter in addition to inducing hypokalaemia, whereas the *in vitro* hypokalaemic challenge was under normotonic conditions. This hypertonic effect on NKCC would be completely blocked by bumetanide (Fig. 2) but may not be acetazolamide responsive. Therefore we tested whether the osmotic stress of doubling the glucose *in vitro* would trigger a loss of force in R528H<sup>+/m</sup> soleus. Increasing the bath glucose to 360 mg/dl (11.8 mOsm increase) did not elicit a significant loss of force, whereas when this glucose challenge was paired with hypokalaemia (2 mM K<sup>+</sup>) then the force decreased by 70% (Fig. 6). Even when the glucose concentration was increased to 540 mg/dl, the *in vitro* contractile force was >85% of control (data not shown). We conclude the *in vivo* loss of muscle excitability during glucose plus insulin infusion is not caused by hypertonic stress and most likely results from the well-known hypokalaemia that accompanies uptake of glucose by muscle.

**Discussion**

The beneficial effect of bumetanide in our CaV1.1-R528H mouse model of HypoPP provides experimental proof of principle that inhibition of the NKCC transporter is a tenable therapeutic
The efficacy of bumetanide was much stronger when the drug was administered coincident with the onset of hypokalaemia, and only partial recovery occurred if application was delayed to the nadir in muscle force (Fig. 1). Pretreatment by minutes was able to completely abort the loss of force in a 2 mM K⁺ challenge (Fig. 3). These observations imply bumetanide may be more effective as a prophylactic agent in patients with CaV1.1-HypoPP than as abortive therapy. Chronic administration of bumetanide will promote urinary K⁺ loss, which may limit clinical usage by inducing hypokalaemia. The significance of this potential adverse effect is not yet known in patients as there have not been any clinical trials nor anecdotal reports of bumetanide usage in HypoPP, and compensation with oral K⁺ supplementation may be possible. There are two isoforms of the transporter in the human genome, NKCC1 and NKCC2 (Russell, 2000). The NKCC1 isoform is expressed ubiquitously and is the target for the beneficial effects in skeletal muscle and the diuretic effect in kidney. Consequently, it is not likely that a muscle-specific derivative of bumetanide could be developed to avoid urinary K⁺ loss.

In clinical practice, acetazolamide is the most commonly used prophylactic agent to reduce the frequency and severity of periodic paralysis (Griggs et al., 1970), but several limitations have been recognized. Only ~50% of patients have a beneficial response (Matthews et al., 2011), and patients with HypoPP with NaV1.4 mutations may have worsening of symptoms on acetazolamide (Torres et al., 1981; Sternberg et al., 2001). Moreover, chronic administration of acetazolamide carries a 15% risk of developing nephrolithiasis (Tawil et al., 1993). Our comparative studies of acetazolamide and bumetanide in mouse models of HypoPP suggest bumetanide is as effective (Fig. 5) or may even be superior to acetazolamide (Fig. 3). In particular, bumetanide may be the preferred treatment in NaV1.4-HypoPP. The mechanism of action for acetazolamide in ameliorating attacks of weakness in HypoPP and hyperkalaemic periodic paralysis is not known.

Figure 5 Bumetanide (BMT) and acetazolamide (ACTZ) both prevented loss of muscle excitability in vivo. (A) Continuous infusion of glucose plus insulin caused a marked drop in CMAP amplitude for R528H⁺/²⁺ mice (black). Pretreatment with intravenous bolus injection of bumetanide prevented the CMAP decrement for four of five mice (red), while acetazolamide was effective in five of eight (blue). The mean CMAP amplitudes shown in A are for the subset of positive responders, defined as those mice with a relative CMAP > 0.5 over the interval from 100 to 120 min. (B) The distribution of late CMAP amplitudes, time-averaged from 100 to 120 min, is shown for all R528H⁺/²⁺ mice tested. The dashed line shows the threshold for distinguishing responders (> 0.5) from non-responders (< 0.5).

Figure 6 Glucose challenge in vitro did not induce weakness in R528H⁺/²⁺ soleus. Peak amplitudes of tetanic contractions elicited every 2 min were monitored during challenges with high glucose or low K⁺. Doubling the bath glucose to 360 mg/dl (20–40 min) increased the osmolarity by 11.8 mOsm, but did not elicit a substantial loss of force. Coincident exposure to 2 mM K⁺ and high glucose produced a 70% loss of force that was comparable to the decrease produced by 2 mM K⁺ alone (Fig. 1B, top row).
although proposals have included activation of Ca-activated K channels (Tricarico et al., 2000) or metabolic acidosis secondary to renal loss of bicarbonate (Matthews and Hanna, 2010). Curiously, acetazolamide had only a modest effect (CaV1.1-R528H) or no benefit (NaV1.4-R669H) for the in vitro contraction test, but was clearly beneficial for the in vivo CMAP assay (Fig. 5). This difference was not accounted for by an osmotic effect of hyperglycaemia from the in vivo glucose infusion (Fig. 6). We suggest this observation implies that systemic effects of acetazolamide, possibly on interstitial pH or ion concentration, have an important role in the mechanism of action for preventing attacks of HypoPP.

The efficacy of bumetanide in reducing the susceptibility to loss of force upon exposure to low-K+ for mouse models of HypoPP, based on both CaV1.1-R528H and NaV1.4-R669H (Wu et al., 2013), provides additional evidence that these allelic disorders share a common pathomechanism for depolarization-induced attacks of weakness. Molecular genetic analyses on cohorts of patients with HypoPP revealed a profound clustering of missense mutations with 14 of 15 reported at arginine residues in the voltage-sensor domains of CaV1.1 or NaV1.4 (Ptacek et al., 1994; Elbaz et al., 1995; Sternberg et al., 2001; Matthews et al., 2009). Functionally, these mutations in either channel produce an inward leakage current that is active at the resting potential and shuts off with depolarization, as shown in oocyte expression studies (Sokolov et al., 2007; Struyk and Cannon, 2007) and voltage-clamp recordings from knock-in mutant mice (Wu et al., 2011, 2012). This leakage current depolarizes the resting potential of muscle by only a few mV in normal K+, but promotes a large paradoxical depolarization and attendant loss of excitability from sodium channel inactivation when K+ is reduced to a range of 2 to 3 mM (Cannon, 2010). In contrast, normal skeletal muscle undergoes this depolarized shift only at extremely low K+ values of 1.5 mM or less. Computational models (Geukes Foppen et al., 2001) and studies in muscle from wild-type mice (Geukes Foppen et al., 2002) showed this bistable behaviour of the resting potential is modified by the sarcolemmal chloride gradient. High myoplasmic Cl− favours the anomalous depolarized resting potential, whereas low internal Cl− promotes hyperpolarization. The NKCC transporter harnesses the energy of the sodium gradient to drive myoplasmic accumulation of Cl− (van Mil et al., 1997), leading to the prediction that bumetanide might reduce the risk of depolarization-induced weakness in HypoPP (Geukes Foppen et al., 2002). We have now shown a beneficial effect of bumetanide in mouse models of HypoPP using CaV1.1-R528H, the most common cause of HypoPP in humans, and the sodium channel mutation NaV1.4-R669H. The beneficial effect of bumetanide on muscle force in low K+ was sustained for up to 30 min after washout (Fig. 1B) and was also associated with an overshoot upon return to normal K+ (Figs 1B and 3). We attribute these sustained effects to the slow rate of myoplasmic Cl− increase upon removal of NKCC inhibition. Conversely, bumetanide was of no benefit in our mouse model of HyperPP (NaV1.4-M1592V; Wu et al., 2013), which has a completely different pathomechanism arising from a disruption of channel inactivation (Cannon and Strittmatter, 1993). Taken together, these studies of bumetanide on mouse models of periodic paralysis add to the growing body of evidence that HypoPP arising from mutations of CaV1.1 and NaV1.4 share a common pathomechanism for paradoxical depolarization with hypokalaemia, driven by an anomalous leakage current through the voltage-sensor and modified by the Cl− gradient.

Although bumetanide was effective in preventing the loss of force in murine HypoPP caused by mutations in either CaV1.1 or NaV1.4, there were consistent differences that may impact the clinical use of this drug. The recovery of contractile force in vitro, when bumetanide was added 20 min after the onset of weakness in 2 mM K+, was only partial for CaV1.1-R528H+/− (Fig. 1B) whereas full recovery occurred for NaV1.4-R669H+/−. This suggests the use of bumetanide to abort an established attack of weakness may have greater potential for success in NaV1.4-HypoPP than CaV1.1-HypoPP.

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Supplementary material

Supplementary material is available at Brain online.

References


