ROLE OF THE cAMP/PKA PATHWAY IN SALBUTAMOL-INDUCED FORCE RECOVERY IN HyperKPP SOLEUS

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INTRODUCTION

Hyperkalemic Periodic Paralysis (HyperKPP) is an autosomal genetic disorder that is characterized by missense mutations in the skeletal muscle Na^+ channel, NaV1.4. Patients suffer from both myotonia and flaccid paralysis. Skeletal muscles from the M1592V HyperKPP mouse model generates less tetanic force than normal muscle as many fibers have depolarized resting membrane potential (EM) because of an abnormal high Na^+ influx at rest. As a consequence of the large depolarization many fibers are totally unexcitable resulting in the lower force. When HyperKPP patients experience myotonic discharges (i.e. spontaneous action potential generation), extracellular $[K^+]$ ($[K^+]e$) increases causing further force decrease and full paralysis because HyperKPP muscle fibers are more sensitive to the K^+-induced force depression.[1]

Salbutamol, a β-2-adrenergic receptor agonist, is fully capable of counteracting muscle weakness at high $[K^+]e$ in both wild type and HyperKPP muscles[2]. However, salbutamol efficacy wears off overtime and its chronic use is harmful to the heart. Although it has long been established that salbutamol acts via the cAMP/PKA pathway, exposing HyperKPP muscles to forskolin, an adenylyl cyclase agonist, to increase cAMP levels produced variable results, and more importantly was much less efficient than salbutamol at overcoming muscle weakness (Nahas and Renaud, unpublished results). This raises the question as to whether PKA plays an important role in the increase in force observed in the presence of salbutamol. The objective of this study was to document whether or not salbutamol acts through the cAMP/PKA pathway in HyperKPP muscles as it does in normal wild-type muscles.

HYPOTHESIS

H-89, a PKA inhibitor, will be less effective at inhibiting the salbutamol-induced force recovery at elevated $[K^+]e$ in HyperKPP than in wild type muscles.

METHODS

ANIMALS AND MUSCLES. All experiments were performed using 1-2 month old HyperKPP mice (strain FVB.129S4(B6)-*Scn4a*$^{tm1.1Ljh}$/J) or wild type FVB mice. The effects of H-89 were determined using the slow twitch soleus muscle, which is primarily composed of type I and IIA fibers.

SOLUTIONS. Control solutions contained (mM) : 118.5 NaCl, 4.7 KCl, 1.3 $CaCl_2$, 3.1 $MgCl_2$, 25 $NaHCO_3$, 2 NaH_2PO_4, and 5.5 D-glucose. Solutions containing different K^+ concentrations or salbutamol were prepared by adding the appropriate amount of KCl or salbutamol, respectively. Solutions containing H-89 were prepared by dissolving H-89 in DMSO before adding H-89 into the physiological solution. In all solutions, the final DMSO concentration was 0.1% (vol/vol). Solutions were continuously bubbled with 95% O_2–5% CO_2 to maintain a pH of 7.4. Total flow of solutions in the muscle chamber was 15 ml/min. **Experimental temperature was 37°C.**

STIMULATION PROTOCOL. Muscles were placed in a glass Plexiglas chamber. One end of the muscle was attached to a stationary hook and the other end to a force transducer. Muscle length was adjusted to give maximum contractile force. Tetanic contractions were elicited every 5 minutes by field stimulation consisting of 200 msec long train of 0.3 msec, 10 V square pulses at 140 Hz.

FIGURE 1

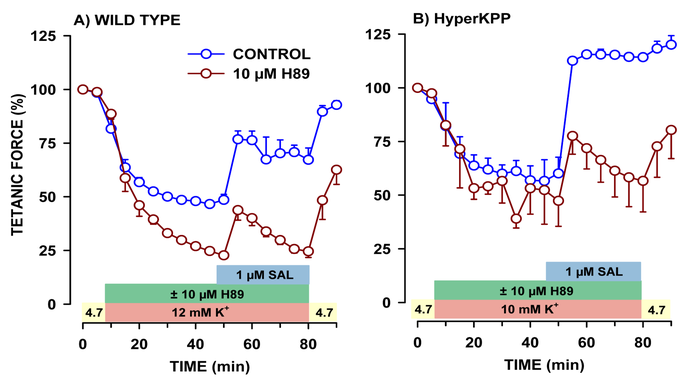

Figure 1. Extent of force recovery at high $[K^+]e$ through exposure to salbutamol (○) was reduced by H-89 (○) in A) wild-type and B) Hyper-KPP mice. Extracellular $[K^+]e$ was increased to 12 mM for wild type soleus and 10 mM for HyperKPP soleus because the latter is more sensitive to the K^+-induced force depression. H-89 was introduced when $[K^+]e$ was increased while salbutamol was added 40 min later. Tetanic force is expressed as a percent of the force at 4.7 mM K^+ (Time 0 min). Vertical bars represent the standard error of the mean (S.E.) of 3 wild type solei and 2 HyperKPP solei (analysis for statistical difference was not carried out due to the small sample size).

FIGURE 2

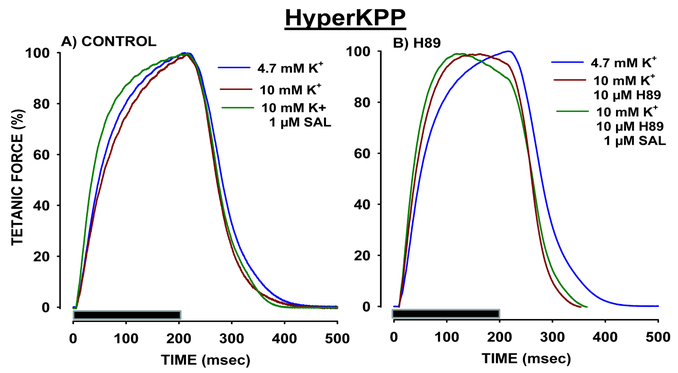

Figure 2. At 10 mM K^+, salbutamol increased the rate at which force increased while H-89-exposed HyperKPP solei experienced a decline in force while being stimulated (horizontal black bar). Tetanic force is expressed as a percent of the maximum force of each contractions.

FIGURE 3

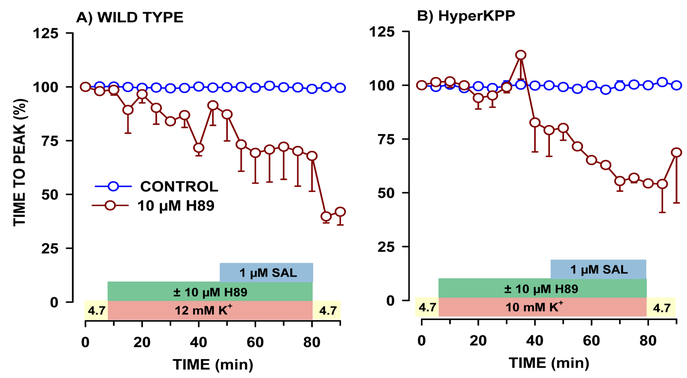

Figure 3. Salbutamol (○) has no effect on the time to peak while H-89 (○) shortened the time to peak in both A) wild type and B) HyperKPP solei. Experimental conditions are as described in Fig. 1. Time to peak is expressed as a percent of the time at 4.7 mM K^+ (Time 0 min). Vertical bars represent the standard error of the mean (S.E.) of 3 wild type solei and 2 HyperKPP solei (analysis for statistical difference was not carried out due to the small sample size).

FIGURE 4

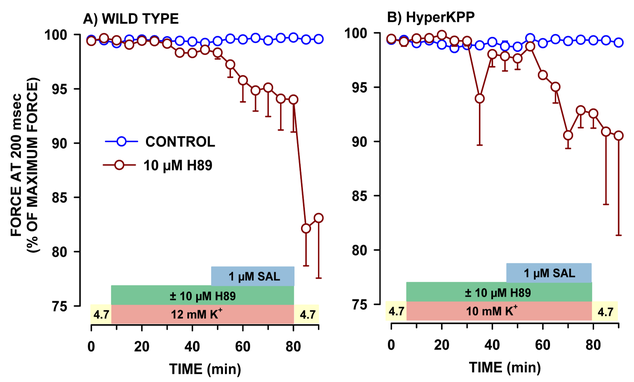

Figure 4. Salbutamol (○) does not alter force at 200 msec while H-89 (○) decreases the force at 200 msec in both A) wild type and B) HyperKPP solei. Experimental conditions are as described in Fig. 1. Muscles are stimulated for 200 msec every 5 minutes. Force at 200 msec is expressed as a percent of the maximum force attained during the contraction. Vertical bars represent the standard error of the mean (S.E.) of 3 wild type solei and 2 HyperKPP solei (analysis for statistical difference was not carried out due to the small sample size).

DISCUSSION

This study demonstrates that the salbutamol effect is reduced by the addition of H-89, a PKA inhibitor, to the same extent in wild-type and Hyper-KPP solei. These results indicate that salbutamol acts through the cAMP/PKA pathway in HyperKPP solei as it does in wild type solei; i.e., the results do not support the hypothesis.

It was also observed that H89-exposed muscles from both wild type and HyperKPP mice cannot maintain force constant while being stimulated over 200 msec (Fig. 2 and 3). The force decline is likely due to a decrease in calcium release from the sarcoplasmic reticulum as a result in the blockage of the cAMP/PKA pathway by H-89. More importantly, the effect of H-89 was the same in wild type and HyperKPP solei, further supporting the concept that salbutamol acts via the cAMP/PKA pathway in HyperKPP solei.

CONCLUSION

In conclusion, salbutamol-induced force recovery at elevated $[K^+]e$ acts via the cAMP/PKA pathway in both wild type and HyperKPP muscle.

REFERENCES

1. Ammar et al. 2015. J. Gen. Physiol, 146:509-525.
2. Clausen et al. 2011. J.Gen.Physiol. 138:117-130.