Insulin and AMPK activation mimic Fatigue pre-conditioning.

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INTRODUCTION

The K_{ATP} channel is crucial in preventing fiber damage as well as severe contractile dysfunctions in skeletal muscle during exercise and fatigue[1-3]. The contractile dysfunctions include excessive membrane depolarizations, excessive increase in intracellular resting calcium and tension, faster rate of fatigue and decreased capacity to recover force after fatigue.

Recently, a new phenomenon was discovered in which a first fatigue bout reduces the dependency of skeletal muscle to the myoprotective effect of the K_{ATP} channels during a subsequent second bout of fatigue[4]. This effect was termed fatigue pre-conditioning (FPC) and last up to 2 hrs after one fatigue bout. Interestingly, the intracellular signaling pathways involved in ischemic pre-conditioning (IPC) are not implicated in FPC. It was proposed that FPC may result from a better capacity to generate ATP during the second fatigue bout.

AMPK is an energy receptor that is activated during an energy stress and that increases glucose and fatty acid transport, glycolysis and oxidative phosphorylation. It is therefore possible that an activation of AMPK is involved in the FPC mechanism.

HYPOTHESES

1. Replacing a first fatigue bout by an activation of AMPK with AICAR will mimic FPC.
2. Replacing a first fatigue bout with an exposure to insulin to increase glucose transport and metabolism will also mimic FPC.

METHODS

SOLUTIONS. Control saline solution contained (in mM): 118.5 NaCl, 4.7 KCl, 2.4 $CaCl_2$; 3.1 $MgCl_2$; 25 $NaHCO_3$, 2 NaH_2PO_4; 5.5 D-glucose, 95% O_2: 5% CO_2, pH 7.4. Solutions containing 9 mM K^+ were prepared by adding the proper amount of KCl. **Experimental temperature was 37°C.**

STIMULATION. Tetanic contractions were elicited every 100 s with 400 ms train of 0.3 ms, 6 volt, 200 Hz square pulses. Fatigue was elicited by reducing the time interval between contractions to 1 sec.

PROTOCOL. FPC was triggered in some mouse flexor digitorum brevis (FDB) with one fatigue bout in control conditions, followed by a 60 min recovery before the second fatigue bout was elicited in the presence of 10 µM glibenclamide to block K_{ATP} channels. To test whether AMPK is implicated, the first fatigue bout was replaced by a 30 min exposure to 2 mM AICAR. The same was done with 100 µU insulin. For comparison, some FDB were fatigued only once in the presence of 10 µM glibenclamide to demonstrate the effect of a lack of K_{ATP} channels.

CONCLUSION

An activation of AMPK kinase with AICAR reduced the rate of fatigue and the increase in resting tension when K_{ATP} channels are blocked. In other words, an activation of AMPK mimics FPC. It therefore suggests that AMPK may be responsible to activate FPC. To further test this, it will now be necessary to determine if FPC can be prevented in AMPK knockout muscles.

FPC was also mimicked by an exposure to insulin. While insulin is not involved during exercise and fatigue, it probably mimicked FPC by increasing the capacity of muscle to generate ATP from glucose. To further test this, it will now be important to determine whether the glucose utilization for ATP production is increased following FPC.

REFERENCES

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2. Cifelli et al. 2007. J.Physiol.(Lond.) 582:843-857.
3. Cifelli et al. 2008. Exp.Physiol. 93:1126-1138.
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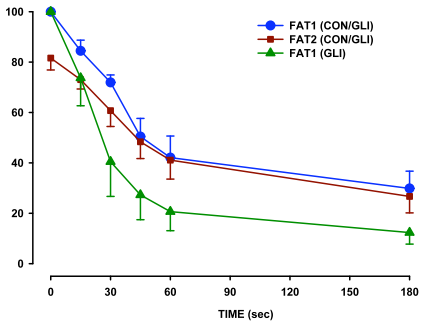
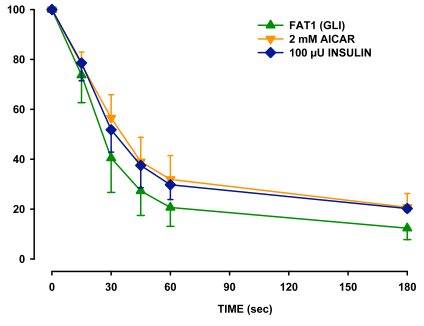
FIGURE 1



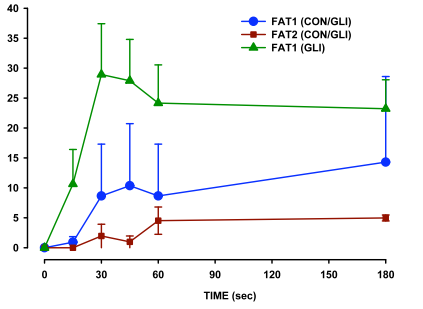
The decrease in tetanic force in the presence of glibenclamide was less if the fatigue was preceded by a fatigue bout in control conditions. One group of FDB was first fatigued under control conditions (FAT1, CON/GLI) and after a 60 min recovery was fatigued a second time in the presence of 10 µM glibenclamide (FAT2, CON/GLI) to block K_{ATP} channels. A second group of FDB was fatigued only once in the presence of 10 µM glibenclamide (FAT1, GLI). Peak tetanic force is expressed as a percent of the pre-fatigue force. Vertical bars represent the S.E. of 3-4 muscles.

FIGURE 2



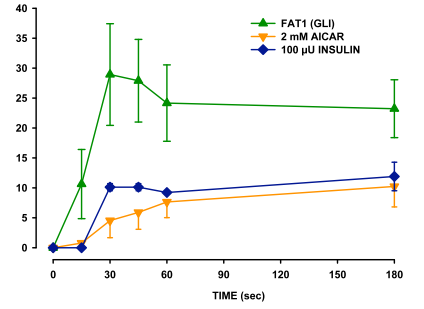
Insulin and AMPK activation with AICAR mimicked the effect of a first fatigue bout. One group of FDB was exposed 30 min to AICAR, 60 min to control solution and then fatigue in the presence of 10 µM glibenclamide (AICAR). A second group of FDB was exposed 1.5 hrs to insulin before fatigued in the presence of 10 µM glibenclamide (INSULIN). The third group of FDB were fatigued once in the presence of 10 µM glibenclamide (FAT1, GLI; this curve is the same as in Figure 1). Peak tetanic force is expressed as a percent of the pre-fatigue force. Vertical bars represent the S.E. of 3-4 muscles.

FIGURE 3



The increase in resting tension force in the presence of glibenclamide was less if the fatigue was preceded by a fatigue bout in control conditions. One group of FDB was first fatigued under control conditions (FAT1, CON/GLI) and after a 60 min recovery was fatigued a second time in the presence of 10 µM glibenclamide (FAT2, CON/GLI) to block K_{ATP} channels. A second group of FDB was fatigued only once in the presence of 10 µM glibenclamide (FAT1, GLI). Resting tension is expressed as a percent of the pre-fatigue tetanic force. Vertical bars represent the S.E. of 3-4 muscles.

FIGURE 4



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