Reduction of Calcium Influx through L-type Calcium Channel by Shakuyaku-kanzo-to (SKT) and one of its Chemical Components, Glycyrrhetic acid (GA)

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INTRODUCTION

Our previous work has shown that SKT, a standardized 1:1 combination of root extracts from paeony and licorice, can rapidly relax smooth muscle within 1-2 mins, and slowly relax skeletal muscle within about 30 minutes (Sam, Terrar, Tasaki & Noble, 2015a). We have also shown that increasing extracellular potassium from 5.4 to 10.8 mM is a powerful facilitator of the relaxing action of SKT (Sam, Terrar, Noble, Tasaki & Noble, 2015b).

METHODS

To investigate possible molecular mechanisms we recorded calcium influx through the alpha1 pore-forming subunit of the L-Type calcium channel, expressed in HEK293 cells. Influx was estimated by measuring the difference in Fura-2 fluorescence before and after activating the channels with high (100 mM) potassium. Calcium current was measured using whole cell patch clamp.

RESULTS

SKT produced up to 50% inhibition of calcium influx through the pore-forming subunit of the L-type calcium channel (Figure 3). The results also show considerable variability that may be consistent with opposing actions of some of the individual chemical components.

One of the pure chemical components, GA, however produces a consistent and statistically significant reduction of calcium influx, which is up to 80% (Figure 4). There was, however, no statistically significant reduction in calcium current under voltage clamp conditions (Figure 5).

FIGURE LEGENDS

Figure 1. Relaxing effect of SKT 10mg/ml on ileum muscle preparation in response to repeated nerve stimulation. The vertical line shows the time at which TJ-68 was added. Both resting and active tension are reduced within 1-2 minutes (Sam et al, 2015a).
Figure 2. Relaxing effect of SKT 10mg/ml on diaphragm muscle preparation in response to repeated nerve stimulation. The vertical line shows the time at which TJ-68 was added. The twitch tension is reduced within 30 minutes (Sam et al, 2015a).
Figure 3. Reduction of calcium influx by SKT. Error bars indicate SEM.
Figure 4. Reduction of calcium influx by GA. Error bars indicate SEM.
Figure 5. No significant effect on ICaL in patch clamp conditions (left: 0.38mg/ml SKT. Right: 0.01 mg/ml GA) .
Figure 6. Left: direct action hypothesis. Right: indirect action hypothesis.

CONCLUSION

In muscle cells, such reductions in influx of calcium would be expected to deplete cell calcium and so reduce the phasic contractile response and relax tonic resting tension. Since ICaL plays an immediate role in smooth muscle but only a long term role in skeletal muscle, this would be consistent with our previous observation of different time courses between SKT's actions on smooth and skeletal muscles.

Since there was no statistically significant reduction in calcium current under voltage clamp conditions, the action on reduction in flux through ICaL could be indirect (Figure 6, right).

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References

Sam, C, Terrar, D, Tasaki, K, Noble, D. 2015a. Actions of a multi-component medication, SKT, in skeletal and smooth muscle. *Proceedings of The Physiological Society*, **34**, PC024.
Sam, C, Terrar, D, Noble, P, Tasaki, K, Noble, D. 2015b. Extracellular potassium may potentiate the action of a multi-component medication, SKT, in skeletal muscle. *Proceedings of The Physiological Society*, **34**, PC025.

Figure 1

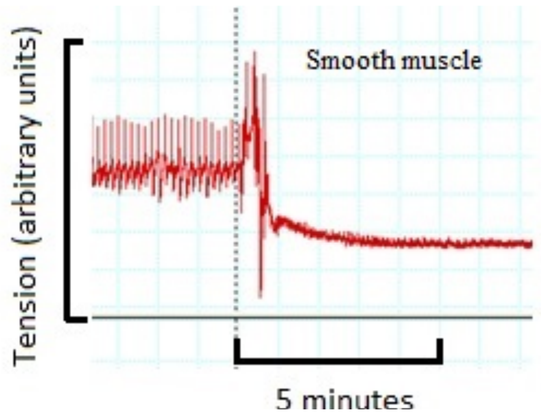
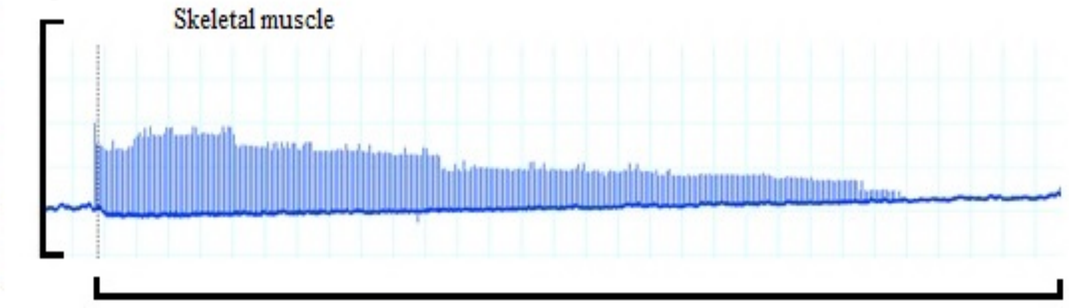


Figure 2



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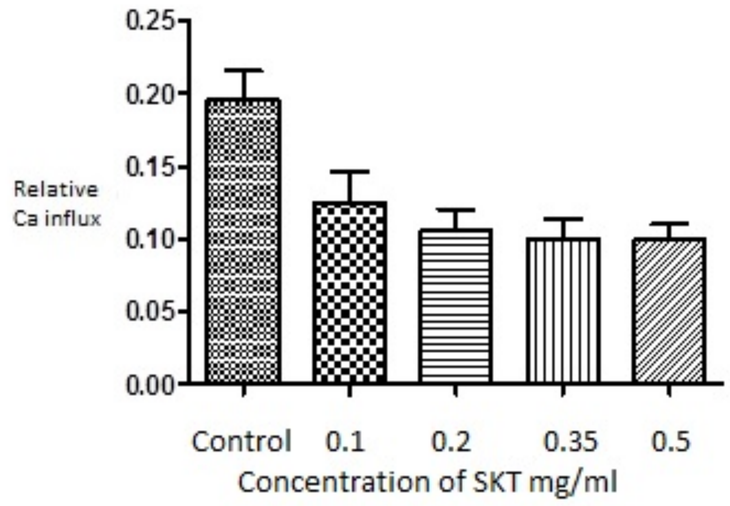

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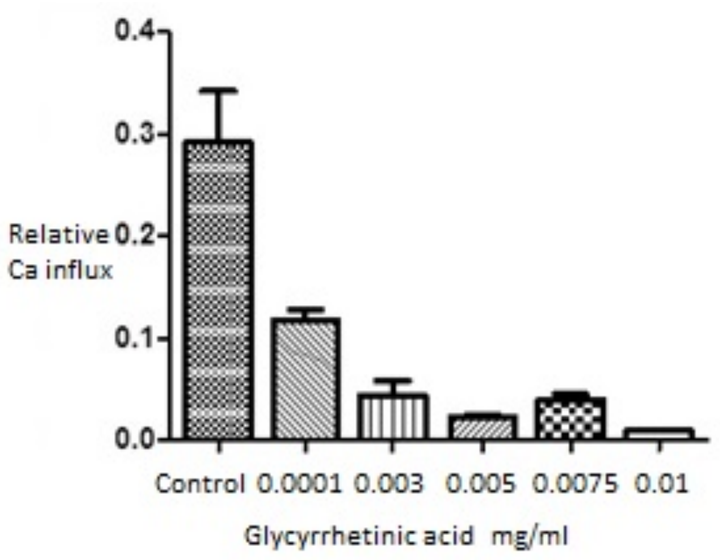

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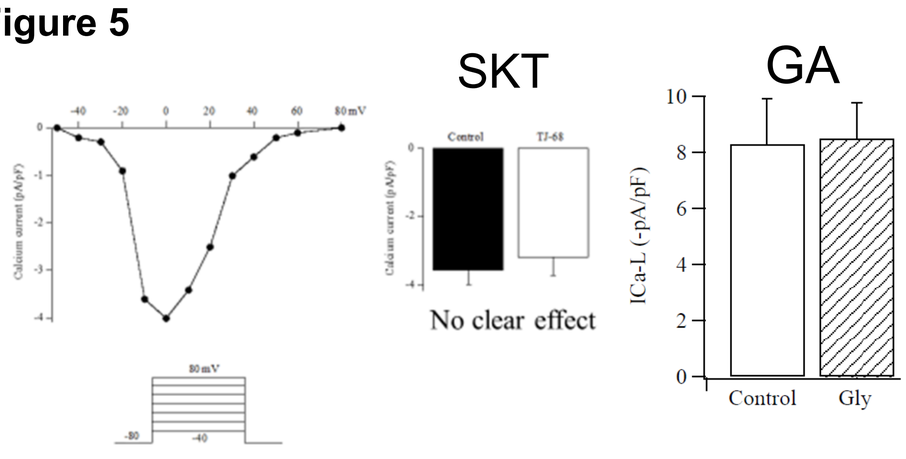

Figure 6