ClC-2-Targeted siRNA Eliminates Lubiprostone Activation of Cl$^-$ Currents in T84 Cells

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Abstract

Introduction

Lubiprostone stimulates electrogenic Cl$^-$ transport in human intestinal T84 cells and Cl$^-$ currents in HEK293 cells stably transfected with recombinant human ClC-2 (hClC-2).[1] The purpose of this study was to determine whether ClC-2 is the Cl$^-$ ion channel in T84 cells responsible for lubiprostone's effects by employing siRNA (small interfering RNA) to knockdown hClC-2. siRNA targeting hClC-2 was expressed in T84 cells and HEK293 cells stably transfected with hClC-2 and the effect of lubiprostone on Cl$^-$ currents was determined. Loss of lubiprostone-activated Cl$^-$ currents was expected if ClC-2 is the target of lubiprostone.

Methods

Short hairpin sequences (shRNAs) producing siRNAs targeting hClC-2 (bp1302-1322), control vector (V) and a non-target (NT) sequence in lentiviral vector pLKO.1 were used. HEK293 cells stably expressing hClC-2 were transfected with the shRNAs using lipofectamine. T84 cells were transduced with lentiviral particles containing the shRNAs using ViroMag R/L. Puromycin was used for selection. Cl$^-$ currents were measured by whole cell patch clamp.

Results

In hClC-2-expressing HEK293 cells, control Cl$^-$ currents at -140 mV (n=6) of -27.2 ± 3.5 pA/pF, increased significantly to -100.9 ± 6.5 pA/pF (P<0.0005) with 20 nM lubiprostone and then were reduced by 500 μM $CdCl_2$ (-29.9 ± 5.6 pA/pF, P<0.0005). When expressing siRNA targeting hClC-2 in this HEK cell line, lubiprostone-activated Cl$^-$ currents were absent (n=6): control, -25.5 ± 3.1 pA/pF; with lubiprostone, -26.4 ± 3.3 pA/pF; with $CdCl_2$, -19.5.0 ± 4.8 pA/pF. In contrast, in hClC-2 HEK293 cells expressing vector alone (V) or non-target (NT) siRNA, 20 nM lubiprostone continued to stimulate Cd^{2+}-inhibitable Cl$^-$ currents: in V (n=6) and NT (n=7) cells respectively: control, -31.3 ± 3.4; -24.34 ± 4.2 pA/pF, with lubiprostone, -83.9 ± 4.1 (P<0.0005); -66.3 ± 9.1 (P<0.0025) pA/pF; with $CdCl_2$, -34.8 ± 4.2; 24.8 ± 4.3 pA/pF. In T84 cells (n=6), Cl$^-$ currents were significantly activated (P<0.0025) with 20 nM lubiprostone (control, -34.2 ± 3.4 pA/pF; with lubiprostone, 78.2 ± 9.0 pA/pF), and inhibited by $CdCl_2$ (P<0.001) to -25.8 ± 3.5 pA/pF. Upon expressing siRNA targeting hClC-2 in T84 cells, lubiprostone-activated Cl$^-$ currents (n=7) were absent: control, -48.9 ± 5.7 pA/pF, with lubiprostone, -50.2 ± 7.6 pA/pF, with $CdCl_2$, -25.8 ± 6.0 pA/pF.

Conclusion

Activation of Cl$^-$ currents by lubiprostone in human T84 cells and in HEK293 expressing recombinant hClC-2 is due to the opening of hClC-2 Cl$^-$ channels.

Supported by Sucampo Pharmaceuticals, Inc.

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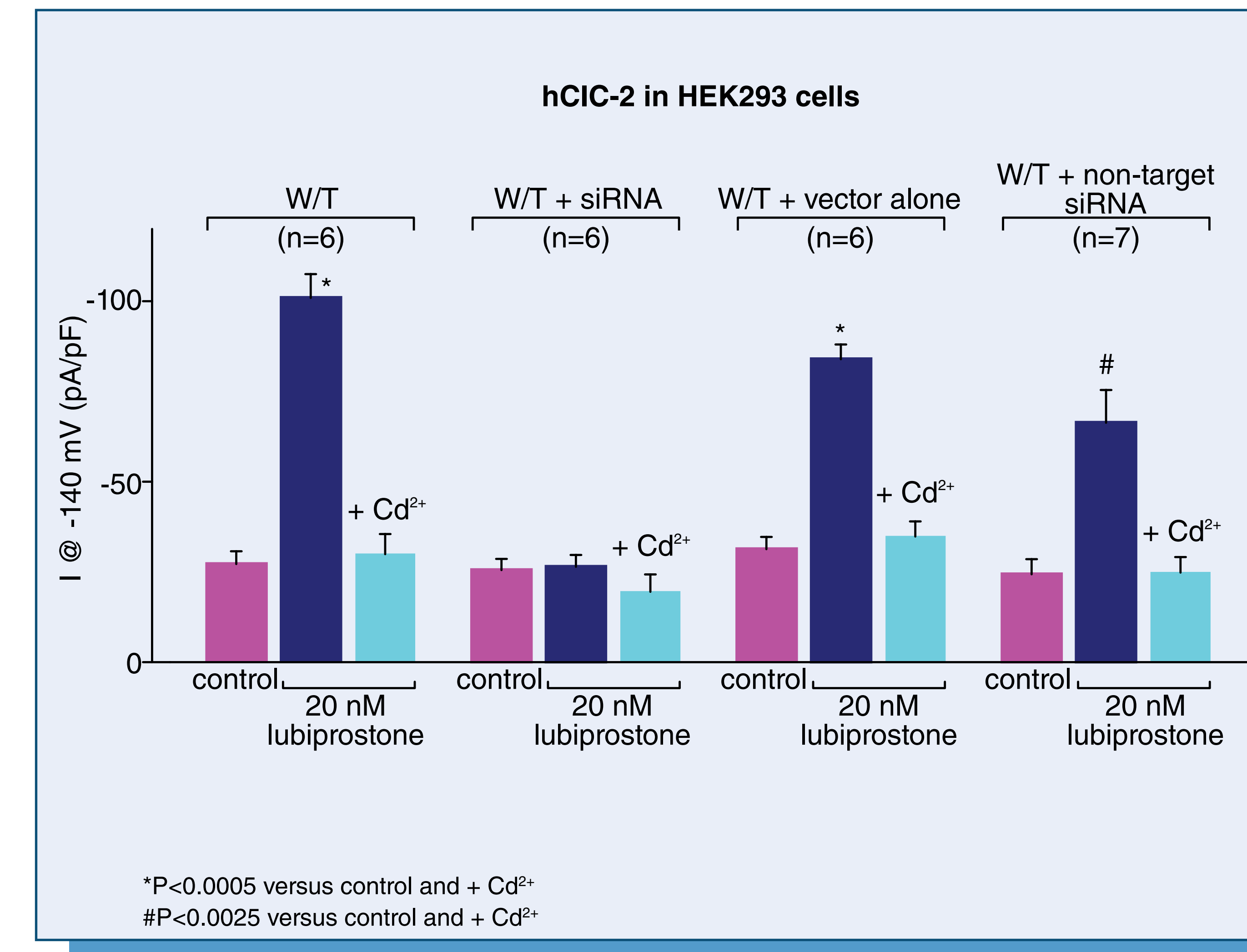
Results

HEK293 cells stably expressing recombinant hClC-2 were transfected using lipofectamine with the shRNAs producing siRNAs targeting hClC-2 (bp1302-1322), control vector and a non-target sequence in lentiviral vector pLKO.1. After puromycin selection, Cl$^-$ currents were measured for 30 s by patch clamp before and after 20 nM lubiprostone and after addition of 500 μM $CdCl_2$. Data are expressed as current at -140 mV normalized to cell capacitance (pA/pF) and plotted as mean ± SEM (Figure 1).

In hClC-2-expressing HEK293 cells, control normalized Cl$^-$ currents at -140 mV (n=6) of -27.2 ± 3.5 pA/pF, increased significantly to -100.9 ± 6.5 pA/pF (P<0.0005) with 20 nM lubiprostone and then were reduced by 500 μM $CdCl_2$ (-29.9 ± 5.6 pA/pF, P<0.0005). When expressing siRNA targeting hClC-2 in this HEK cell line, lubiprostone-activated Cl$^-$ currents were absent (n=6): control, -25.5 ± 3.1 pA/pF; with lubiprostone, -26.4 ± 3.3 pA/pF; with $CdCl_2$, -19.5.0 ± 4.8 pA/pF. In contrast, in hClC-2 HEK293 cells expressing vector alone (V) or non-target (NT) siRNA, 20 nM lubiprostone continued to stimulate Cd^{2+}-inhibitable Cl$^-$ currents: in V (n=6) and NT (n=7) cells respectively: control, -31.3 ± 3.4; -24.34 ± 4.2 pA/pF, with lubiprostone, -83.9 ± 4.1 (P<0.0005); -66.3 ± 9.1 (P<0.0025) pA/pF; with $CdCl_2$, -34.8 ± 4.2; 24.8 ± 4.3 pA/pF.

siRNA targeting hClC-2 (but not vector alone or non-target siRNA) abolished lubiprostone-activated Cd^{2+}-inhibitable Cl$^-$ currents in HEK293 cells stably expressing recombinant hClC-2. Therefore lubiprostone activated Cl$^-$ currents in recombinant hClC-2 expressing HEK293 cells are due to the opening of hClC-2 Cl$^-$ channels.

Figure 1. Effect of siRNA targeting hClC-2, vector alone and non-target siRNA on lubiprostone-activated Cd^{2+}- sensitive Cl$^-$ currents in recombinant hClC-2 expressing HEK293 cells

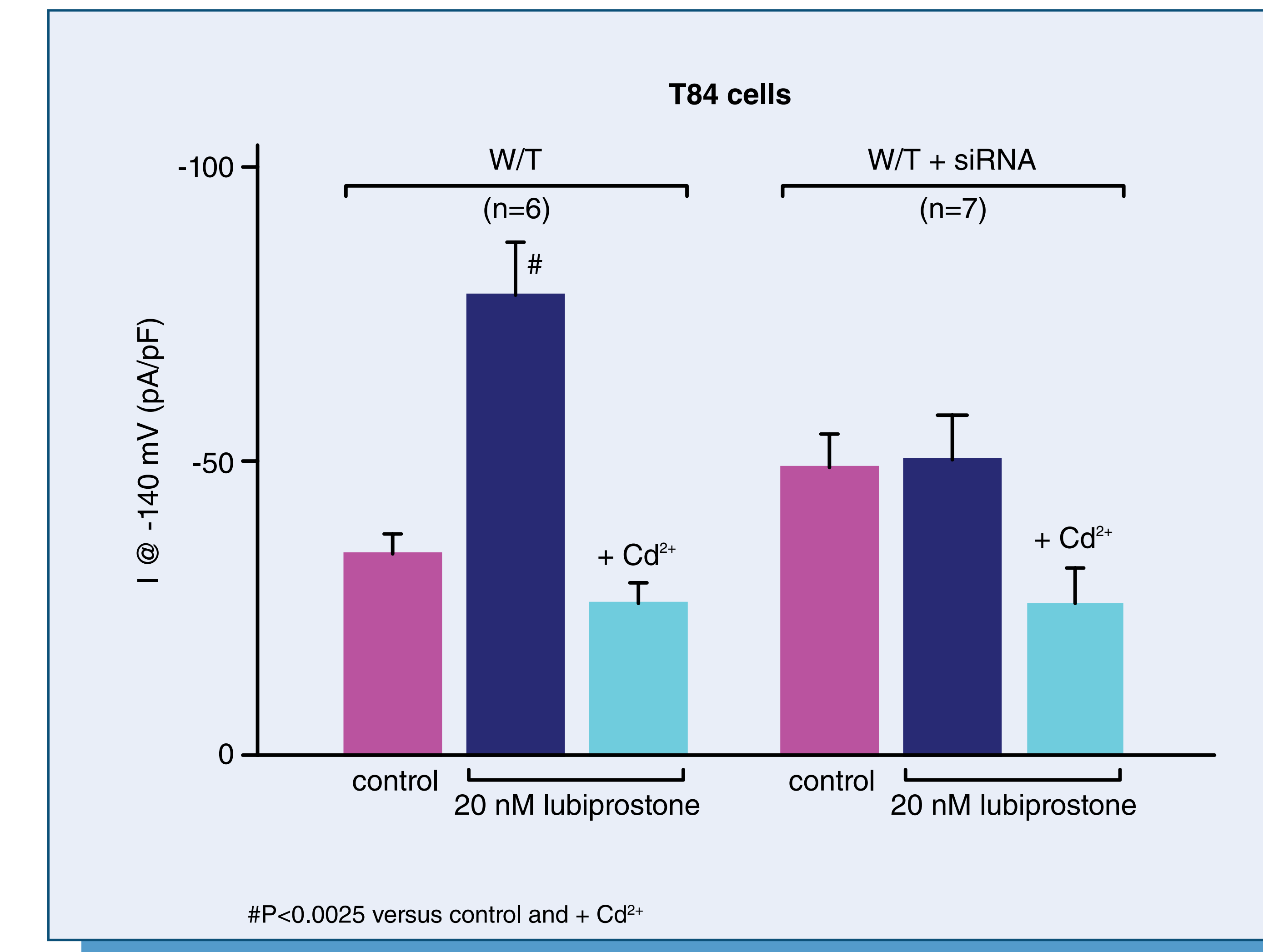


T84 cells were transduced with lentiviral particles containing the shRNA that produces siRNA targeting hClC-2 (bp1302-1322) in lentiviral vector pLKO.1 using ViroMag R/L. Puromycin was used for selection. Cl$^-$ currents were measured for 30 s by whole cell patch clamp before and after 20 nM lubiprostone and after addition of 500 μM $CdCl_2$. Data are expressed as current at -140 mV normalized to cell capacitance (pA/pF) and plotted as mean ± SEM (Figure 2).

In T84 cells (n=6), Cl$^-$ currents were significantly activated (P<0.0025) with 20 nM lubiprostone (control, -34.2 ± 3.4 pA/pF; with lubiprostone 78.2 ± 9.0 pA/pF), and inhibited by $CdCl_2$ (P<0.001) to -25.8 ± 3.5 pA/pF. Upon expressing siRNA targeting hClC-2 in T84 cells, lubiprostone-activated Cl$^-$ currents (n=7) were absent: control, -48.9 ± 5.7 pA/pF, with lubiprostone, -50.2 ± 7.6 pA/pF, with $CdCl_2$, -25.8 ± 6.0 pA/pF.

Human ClC-2 targeted siRNA abolished lubiprostone-activated Cd^{2+}-sensitive Cl$^-$ currents in T84 cells. Together with the findings in Fig. 1, these results support the view that the lubiprostone activated Cl$^-$ currents in T84 cells are due to the opening of hClC-2 Cl$^-$ channels.

Figure 2. Effect of siRNA targeting hClC-2 on lubiprostone-activated Cd^{2+}-inhibitable Cl$^-$ currents in T84 cells



Conclusion

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Acknowledgment

Supported by Sucampo Pharmaceuticals, Inc.

Reference

1. Cuppoletti J, Malinowska DH, Tewari KP, Li QJ, Sherry AM, Patchen ML, Ueno R (2004) SPI-0211 activates T84 cell chloride transport and recombinant human ClC-2 chloride currents. Am J Physiol Cell Physiol 287: C1173-83.