



Title	Epidermal Growth Factor Stimulates Cell Proliferation by Activating Voltage-Gated Potassium Channels in Rat Bone Marrow-Derived Mesenchymal Stem Cells
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EPIDERMAL GROWTH FACTOR STIMULATES CELL PROLIFERATION BY ACTIVATING VOLTAGE-GATED POTASSIUM CHANNELS IN RAT BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

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Background and objective: We have previously found that voltage-gated delayed rectifier potassium current ($I_{K_{DR}}$, encoded by Kv1.2 and Kv2.1) participated in regulation of cell cycling progression in rat mesenchymal stem cells (MSCs) from bone marrow. The present study was designed to investigate whether epidermal growth factor (EGF) regulates cell growth is mediated by activating $I_{K_{DR}}$.

Methods: Whole-cell patch voltage-clamp, RT-PCR, Western blots, siRNA, cell proliferation assay were employed in the present study

Results: EGF increased cell proliferation in a concentration-dependent manner, and the effect was countered by the broad spectrum protein tyrosine (PTK) inhibitor genistein and the EGFR kinase inhibitor AG556. We found that genistein and AG556 inhibited $I_{K_{DR}}$ in a concentration-dependent manner, The protein tyrosine phosphatase (PTP) inhibitor orthovanadate enhanced $I_{K_{DR}}$, and counted the inhibitory effect of $I_{K_{DR}}$ by genistein or AG556, suggesting the PTK-mediating modulation of $I_{K_{DR}}$. Interestingly EGF also increased $I_{K_{DR}}$, Downregulation of $I_{K_{DR}}$ with siRNA targeting to Kv1.2 or Kv2.1 channels inhibited basal proliferation, and prevented EGF-stimulated proliferation in rat MSCs.

Conclusion: These results demonstrate for the first time that EGF stimulates cell proliferation activating $I_{K_{DR}}$, and silencing Kv1.2 or Kv2.1 channels prevents the augmentation of proliferation by EGF, indicating that Kv1.2 and Kv2.1 channels mediate EGF effect in regulating cell growth in rat MSCs.

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THE NATURAL FLAVONE ACACETIN BLOCKS KV4.3 CURRENT BY INTERACTING WITH P-LOOP FILTER HELIX OF THE CHANNEL

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Background and objective: We have recently demonstrated that the natural flavone acacetin is an atrial-selective compound that inhibits ultra-rapid delayed rectifier potassium current (I_{Kur}) and transient outward potassium current (I_{to}) in human atrial myocytes, and also acetylcholine-activated potassium current ($I_{K_{ACh}}$). It increased atrial effective refractory period and effectively prevented atrial fibrillation (AF) in anesthetized dogs without prolonging QT interval of ECG. The present study was designed to investigate the potential molecular determinants of hKv4.3 channels that encode human cardiac I_{to} .

Methods: Cell culture, mutagenesis and whole-cell patch voltage-clamp techniques were used in the present study.

Results: It was found acacetin inhibited hKv4.3 current in HEK 293 cells stably expressing Kv4.3 gene (*KCND3*) in a concentration-dependent manner. The current inhibition with an increase of time-to-peak and inactivation time constant of the current, suggesting an open channel blockade. However, the stimulation pause during drug administration revealed a strong tonic blocking property. This effect induced a use- or frequency-dependent inhibition at lower concentrations (1 and 3 μ M), but not at high concentrations. The IC_{50} of acacetin for inhibiting hKv4.3 was reduced from 6.09 μ M at 0.2 Hz to 5.80, 4.55, 3.96, and 3.65 μ M respectively at 1, 2, 3, and 4 Hz. The mutagenesis study showed that the channel blockade by acacetin was dramatically reduced in hKv4.3 mutant T366A and T367A (IC_{50} , 197.8 μ M for T366A and 166.1 μ M for T367A) of the P-loop helix, and IC_{50} was also reduced in V392A, I395A, and V399A (IC_{50} : 25.9 μ M, 24.1 μ M, and 9.5 μ M) of the S6 domain.

Conclusion: These results demonstrate the novel information that acacetin is a tonic and open channel blocker of hKv4.3 by binding to T365 and T366 of the P-loop helix, and also interacts with V392, I395, and V399 of the S6 domain of hKv4.3 channels. The use- and rate-dependent blocking property of hKv4.3 by acacetin indicates that this natural compound could exert a strong suppressive effect in the treatment of tachycardiac arrhythmia diseases.