N15 Osthol is a Potent and Use-dependent Blocker of Voltage-gated Na+ Channels in Mouse Neuroblastoma N2A Cells

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Aim of investigation: Osthol has been shown to possess vasorelaxant and neuroprotective actions. Not much is known about the effects of osthol on ionic channels. The aim of this study is to exam the effects of osthol on ionic channels activities in mouse neuroblastoma N2A Cells of which are implicated in vasorelaxation and neuroprotection.

Methods: Electrophysiological experiments were performed. N2A cells were voltage-clamped in the whole-cell configuration. Currents were recorded using an EPC-10 amplifier with Pulse 8.60 acquisition software and analyzed by Pulsefit 8.60 software (HEKA Electronik, Lambrecht, Germany). Data were filtered at 2 kHz and sampled at 10 kHz. After a whole-cell configuration was established, the cells were held at -70 mV (or otherwise stated) and subject to various protocols as detailed in the Results section and the legends. A holding potential of 70 mV was chosen as this value was close to the resting membrane potential of N2A cells (using current-clamp method). $P<0.05$ were considered significant. (Unpaired or paired student's t test).

Results: Osthol could potently inhibit voltage-gated Na+ currents with state-dependence in mouse N2A cells (IC50 = 12.3 M and 31.5 M at holding potentials of -70 mV and -100 mV, respectively). Current blockade was equally effective in both extracellular and intracellular application of osthol. Osthol did not significantly affect the kinetics and voltage-dependence of Na+ channel activation, but left-shifted the steady-state inactivation curve (V1/2 =60.5 mV and -78.7 mV in the absence and presence of osthol, respectively). Osthol also mildly but significantly retarded channel recovery from inactivation (recovery time constant = 19.9 ms and 35.6 ms in the absence and presence of osthol, respectively). Osthol also blocked Na+ currents in a frequency-dependent fashion: blockade of 17%, 34 % and 49 % when currents were triggered at 0.33 Hz, 1 Hz and 3.33 Hz, respectively.

Conclusions: Our results suggest that osthol potently blocked voltage-gated Na+ channels intracellularly with state- and frequency-dependence. The antagonistic effects of osthol on neuronal ion channels may in part account for its protective effects on neuronal tissues.