

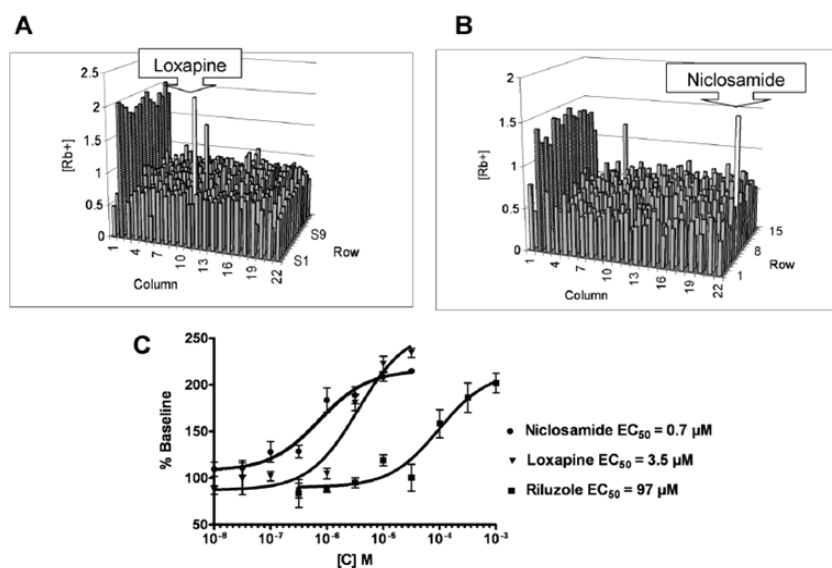
The Antipsychotic Drug Loxapine Is an Opener of the Sodium- Activated Potassium Channel Slack (Slo2.2)

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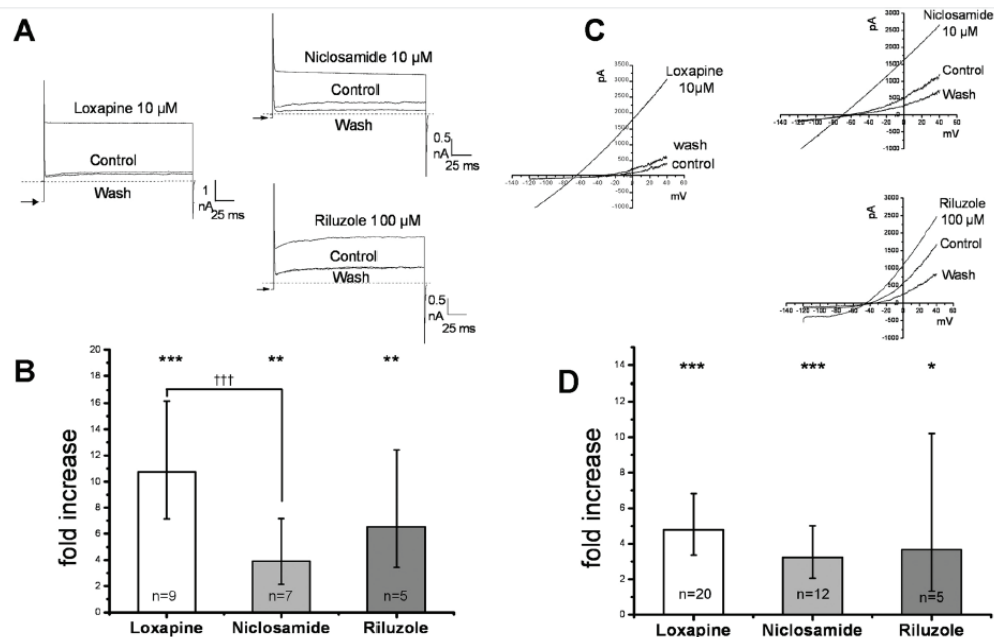
Abstract

Sodium-activated potassium (KNa) channels have been suggested to set the resting potential, to modulate slow afterhyperpolarizations, and to control bursting behavior or spike frequency adaptation (Trends Neurosci 28:422–428, 2005). One of the genes that encodes KNa channels is called Slack (Kcnt1, Slo2.2). Studies found that Slack channels were highly expressed in nociceptive dorsal root ganglion neurons and modulated their firing frequency (J Neurosci 30:14165–14172, 2010). Therefore, Slack channel openers are of significant interest as putative analgesic drugs. We screened the library of pharmacologically active compounds with recombinant human Slack channels expressed in Chinese hamster ovary cells, by using rubidium efflux measurements with atomic absorption spectrometry. Riluzole at 500 μM was used as a reference agonist. The antipsychotic drug loxapine and the anthelmintic drug niclosamide were both found to activate Slack channels, which was confirmed by using manual patch-clamp analyses ($\text{EC}_{50} = 4.4 \mu\text{M}$ and $\text{EC}_{50} = 2.9 \mu\text{M}$, respectively). Psychotropic drugs structurally related to loxapine were also evaluated in patch-clamp experiments, but none was found to be as active as loxapine. Loxapine properties were confirmed at the singlechannel level with recombinant rat Slack channels. In dorsal root ganglion neurons, loxapine was found to behave as an opener of native KNa channels and to increase the rheobase of action potential. This study identifies new KNa channel pharmacological tools, which will be useful for further Slack channel investigations.

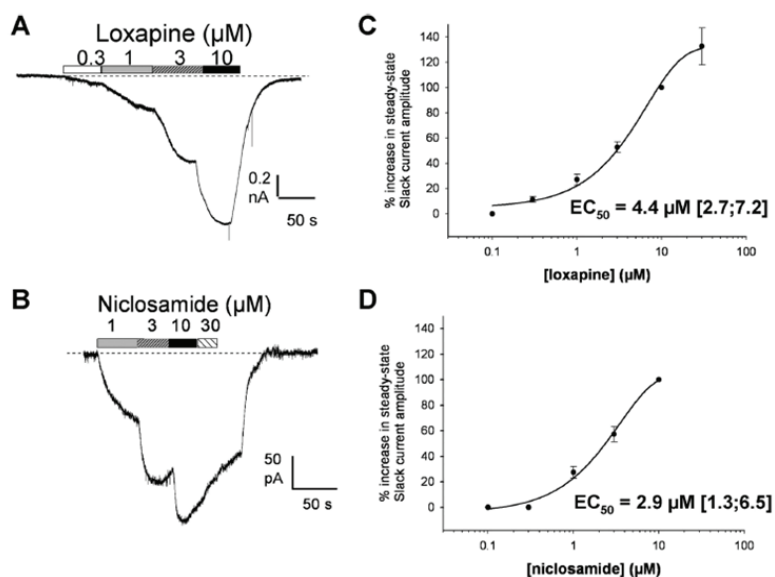
Results



Medium-throughput screening at recombinant Slack channels by using atomic absorption spectrometry. A and B, bar graph representations of typical results obtained in a 384 well-plate format, showing Rb^+ concentration variations in the supernatant in response to LOPAC compounds tested at 30 μM and the identification of loxapine (A) and niclosamide (B). The effects of riluzole at 500 μM (used as a reference Slack channel opener) can be seen as the second-highest columns. C, effects of increasing concentrations of loxapine, niclosamide, and riluzole on Rb^+ flux in CHO-hSlack cells. Data are expressed as percentages of baseline values. Data points and bars represent mean \pm S.E.M. ($n = 4$).



Loxapine, niclosamide and riluzole as Slack channels openers. A, original whole-cell current traces showing Slack current time course evoked in CHO-hSlack cells by depolarizing steps from 90 to 20 mV for 200 ms, under control conditions or after superfusion of loxapine, niclosamide, or riluzole. The inward current (black arrow) evoked by loxapine should be noted (dotted line, control leakage current). B, bar graph of Slack current amplitude variations expressed as fold increases, determined by using the same protocol as described for A. Data points are geometric mean \pm 95% confidence interval. *, Student's t test, versus constant; †, one-way analysis of variance/Newman-Keuls test, comparison between treatment groups. **, $p < 0.01$; *** or †††, $p < 0.001$. C, effects of loxapine, niclosamide, and riluzole on the time course of Slack currents evoked by a ramp protocol depolarizing the membrane from 120 to +40 mV in 2 s. The linear aspect of the current-voltage relationship during superfusion with loxapine or niclosamide at 10 μ M and the strong inward currents elicited by these compounds, as shown above, should be noted. In contrast, riluzole at 100 μ M only weakly modified the current-voltage relationship and induced moderate inward currents. D, bar graph of Slack current amplitude variations expressed as fold increase, determined by using the same protocol as described for C. *, $p < 0.05$; ***, $p < 0.001$.



Concentration-dependent effects of loxapine and niclosamide. A and B, typical recordings showing the concentration-dependent effects of loxapine (A) and niclosamide (B) on steady-state inward Slack currents evoked at a holding voltage of 100 mV. Slack steady-state current amplitude was close to 0 under control conditions (dotted line) and was strongly increased by the two compounds. C and D, concentration-response curves for loxapine (C) and niclosamide (D). For each compound, data points indicate the mean \pm S.E.M. of steady-state current amplitudes ($n = 3-7$ cells), normalized to currents evoked by 10 μ M levels of the compound. Curves are the best fit of data points with the following single-site equation: $y = \text{Max} \cdot C^n / (C^n + \text{EC}_{50}^n)$, where C is the concentration, Max the maximal effect, and n the Hill coefficient. The EC_{50} values are given with 95% confidence intervals.