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Cell-attached recordings of responses evoked by photorelease of GABA in the immature cortical neurons

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Abstract

We present a novel non-invasive technique to measure the polarity of GABAergic responses based on cell-attached recordings of currents activated by laser-uncaging of GABA. For these recordings, a patch pipette was filled with a solution containing RuBi-GABA, and GABA was released from this complex by a laser beam conducted to the tip of the patch pipette via an optic fiber. In cell-attached recordings from neocortical and hippocampal neurons in postnatal days P2-5 rat brain slices in vitro, we found that laser-uncaging of GABA activates integral cell-attached currents mediated by tens of GABA(A) channels. The initial response was inwardly directed, indicating a depolarizing response to GABA. The direction of the initial response was dependent on the pipette potential and analysis of its slope-voltage relationships revealed a depolarizing driving force of +11 mV for the currents through G. Current-voltage relationships of the currents evoked by Rubi-GABA uncaging using voltage-ramps at the peak of responses not only revealed a bumetanide-sensitive depolarizing reversal potential of the GABA(A) receptor mediated responses, but also showed a strong voltage-dependent hysteresis. Upon desensitization of the uncaged-GABA response, current-voltage relationships of the currents through single GABA(A) channels revealed depolarizing responses with the driving force values similar to those obtained for the initial response. Thus, cell-attached recordings of the responses evoked by local intrapipette GABA uncaging are suitable to assess the polarity of the GABA(A)-Rs mediated signals in small cell compartments. © 2013 Minlebaev, Valeeva, Tcheremiskine, Coustillier and Khazipov.

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Keywords

Cell-attach, GABA, Inhibitory postsynaptic potentials, Patch-clamp, Rubi-GABA, Uncaged GABA