



Transmembrane potential polarization, calcium influx, and receptor conformational state modulate the sensitivity of the imidacloprid-insensitive neuronal insect nicotinic acetylcholine receptor to neonicotinoid insecticides

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Neonicotinoid insecticides act selectively on insect nicotinic acetylcholine receptors (nAChRs). Recent studies revealed that their efficiency was altered by the phosphorylation/dephosphorylation process and the intracellular signaling pathway involved in the regulation of nAChRs. Using whole-cell patch-clamp electrophysiology adapted for dissociated cockroach dorsal unpaired median (DUM) neurons, we demonstrated that intracellular factors involved in the regulation of nAChR function modulated neonicotinoid sensitivity. DUM neurons were known to express two alpha-bungarotoxin-insensitive nAChR subtypes: nAChR1 and nAChR2. Whereas nAChR1 was sensitive to imidacloprid, nAChR2 was insensitive to this insecticide. Here, we demonstrated that, like nicotine, acetamiprid and clothianidin, other types of neonicotinoid insecticides, acted as agonists on the nAChR2 subtype. Using acetamiprid, we revealed that both steady-state depolarization and hyperpolarization affected nAChR2 sensitivity. The measurement of the input membrane resistance indicated that change in the acetamiprid-induced agonist activity was related to the receptor conformational state. Using cadmium chloride, omega-conotoxin GVIA, and (R,S)-(3,4-dihydro-6,7-dimethoxy-isoquinoline-1-yl)-2-phenyl-N,N-di-acetamide (LOE 908), we found that inhibition of calcium influx through high voltage-activated calcium channels and transient receptor potential gamma (TRPgamma) activated by both depolarization and hyperpolarization increased nAChR2 sensitivity to acetamiprid. Finally, using N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride (W7), forskolin, and cAMP, we demonstrated that adenylyl cyclase sensitive to the calcium/calmodulin complex regulated internal cAMP concentration, which in turn modulated TRPgamma function and nAChR2 sensitivity to acetamiprid. Similar TRPgamma-induced modulatory effects were also obtained when clothianidin was tested. These findings bring insights into the signaling pathway modulating neonicotinoid efficiency and open novel strategies for optimizing insect pest control.

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