

ABSTRACT (EN)

Purinergic P2X receptors are ATP-gated cation channels with multiple physiological roles and are emerging as important therapeutic targets in a range of diseases. P2X subunit consists of two transmembrane helices (TM1 and TM2), an extracellular ATP-binding domain, and intracellular N- and C- termini. Seven different P2X subunits (P2X1-7) can assemble to form homotrimeric or heterotrimeric ion channels permeable for monovalent cations and calcium. P2X are ubiquitously expressed. Among them, P2X2, P2X4, and P2X7 are the most abundant within the brain. The activity of P2X depends not only on the presence of ATP but also on allosteric modulators that may inhibit or potentiate the activity of these channels. Our aim was to identify new molecules that could interact with allosteric binding sites on P2X receptors, design and synthesize new analogues of neurosteroids, and define crucial receptor domains and amino acids important for neurosteroid binding. By using a patch-clamp electrophysiology technique we recorded ATP-induced currents in HEK293T cells transfected with rat P2X2, P2X4, and P2X7, as well as in the rat anterior pituitary cells and hypothalamic neurons endogenously expressing these receptors. We found that 17 β -ester derivatives of testosterone, namely testosterone butyrate and testosterone valerate, selectively potentiate P2X2 and P2X4, but not P2X7. These compounds allosterically modulate the gating of P2X possibly by binding within the transmembrane domain. We also showed that secondary bile acids, lithocholic acid and 4-dafachronic acid are strong inhibitors of the P2X2 but positive allosteric modulators of the P2X4, and to a small extent also positive modulators of the P2X7. Neurosteroid modulation of ATP-evoked responses mediated by endogenously expressed P2X2 and P2X4 receptors was confirmed in native cells. Moreover, we described explicit structural requirements of these compounds for modulatory effect. Both testosterone derivatives and lithocholic acid were able to inhibit partially the effect of ivermectin, P2X4-specific positive allosteric modulator. We showed that the potentiating effect of lithocholic acid is inhibited in Y42A and significantly reduced in F48A and V43A mutants that belong to the determinants of the ivermectin binding site in P2X4. This indicates the binding site for neurosteroids identified here overlaps with the binding site for ivermectin that interacts with the transmembrane domain.