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Sodium functions as a negative allosteric modulator of the oxytocin receptor

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Abstract
The oxytocin receptor, a class A G protein coupled receptor (GPCR), is essentially involved in the physiology of reproduction. Two parameters are crucially important to support high-affinity agonist binding of the receptor: Mg^{2+} and cholesterol, both acting as positive modulators. Using displacement assays with a high-affinity fluorescent antagonist (OTAN-A647), we now show that sodium functions as a negative allosteric modulator of the oxytocin receptor. In membranes from HEK293 cells stably expressing the oxytocin receptor, oxytocin binding occurred with about 15-fold lower affinity when sodium chloride was increased from 0 to 300 mM, whereas antagonist binding remained largely unchanged. The effect was concentration-dependent, sodium-specific, and it was also observed for oxytocin receptors endogenously expressed in Hs578T breast cancer cells. A conserved Asp (Asp 85) is known to stabilize the sodium binding site in other GCPRs. Mutations of this residue into Ala or Asn are known to yield non-functional oxytocin receptors. When Asp 85 was exchanged for Glu, most of the oxytocin receptors were localized in intracellular structures, but a faint plasma membrane labeling with OTAN-A647 and the appearance of oxytocin-induced calcium responses indicated that these receptors were functional. However, a sodium effect was not detectable for the mutant D85E oxytocin receptors. Thus, the oxytocin receptor is allosterically controlled by sodium similar to other GPCRs, but it behaves differently concerning the involvement of the conserved Asp 85. In case of the oxytocin receptor, Asp 85 is obviously essential for proper localization in the plasma membrane.

Keywords: Oxytocin receptor; sodium; cholesterol; calcium response; affinity state;

Abbreviations: CCM, cholesterol consensus motif; CRAC, cholesterol recognition amino acids consensus domain; GPCR, G protein coupled receptors; HBS, Hepes buffered saline; MβCD, methyl-β-cyclodextrin; OT, oxytocin; OTR, oxytocin receptor

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