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Neighboring glycine residues are essential for $P2X_2$ receptor/channel function

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Abstract

The roles of a glycine-rich region in the cloned $P2X_2$ receptor/channel were evaluated by site-directed mutagenesis. Responsiveness to ATP was lost when Gly^{247} was replaced by alanine. The sensitivity to ATP was reduced when Gly^{248} was replaced by alanine, and the responsiveness to ATP was lost when Gly^{248} was replaced by valine. The results suggest that the neighboring glycine residues are essential for $P2X_2$ receptor/channel function. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: P2X receptor/channel; Site-directed mutagenesis; *Xenopus* oocyte

ATP-gated channels have been identified as P2X receptors by molecular cloning. It has been reported that glycine-rich motifs are essential for the binding of mononucleotides, including ATP, to receptive proteins (e.g., Walker et al., 1982; Xu et al., 1998). In the present study, we have introduced site-directed mutagenesis in a glycine-rich region in the $P2X_2$ receptor/channel, and the sensitivity to ATP was examined.

Mutant channels were constructed by polymerase chain reaction (PCR)-based site-directed mutagenesis (Stratagene, La Jolla, CA, USA) from the rat $P2X_2$ receptor clone (Brake et al., 1994). Expression of channels in *Xenopus* oocytes and membrane current measurements were conducted as in our previous report (Nakazawa et al., 1998). Statistical analysis was done with Duncan's multiple comparison.

Glycine residues possess the smallest side-chain group (–H) among amino acid residues. Four highly conserved glycine residues in a glycine-rich region of the $P2X_2$ receptor/channel (Fig. 1A) were replaced by alanine residues, which possess the second smallest side-chain group ($–CH_3$). The sensitivity to ATP in the G237A or G251A channel was not different from that in the wild-type (WT) channel (Fig. 1B). On the other hand, sensitivity was reduced in the G248A channel (Fig. 1B, C), and the responsiveness to ATP was lost in the G247A channel (Fig. 1B). The loss of responsiveness in G247A channel may not be attributable to non-expression of channel proteins because the sensitivity to ATP was significantly reduced, presumably due to the formation of heteromeric channels when the WT channel was co-expressed with the G247A channel [pD_2 values: WT, 4.23 ± 0.05 ($n = 7$); WT + G247A, 3.99 ± 0.05 ($n = 6$); $P < 0.01$]. Responsiveness was also lost when Gly^{248} was replaced by valine, an amino acid having a larger side-chain group [$–CH_2(CH_3)_2$] than does alanine (Fig. 1C). The G247A or G248V channel responded to none of the P2X receptor agonists tested [ADP (1 mM), ATPγS (100 μM), 2-methylthio ATP (100 μM), or CTP (300 μM)].

The above results suggest that Gly^{247} and Gly^{248} are essential for $P2X_2$ receptor/channel function. The glycine-rich region, especially these two neighboring

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A.

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P2X2  235 KAGENFTELAHKGGVIGVI 253
P2X1  238 ESGQDFRSLAEKGGVVGIT 256
P2X3  224 FAGQDFAKLARTGGVLGIK 242
P2X4  238 DAGHSFQEMAVEGGIMGIQ 256
P2X5  240 WAGADFQDIALKGGVIGIY 258
P2X6  242 MTGGDFEDLALLGGAVGIN 260
P2X7  237 EIGENFTEVAVQGGIMGIE 255
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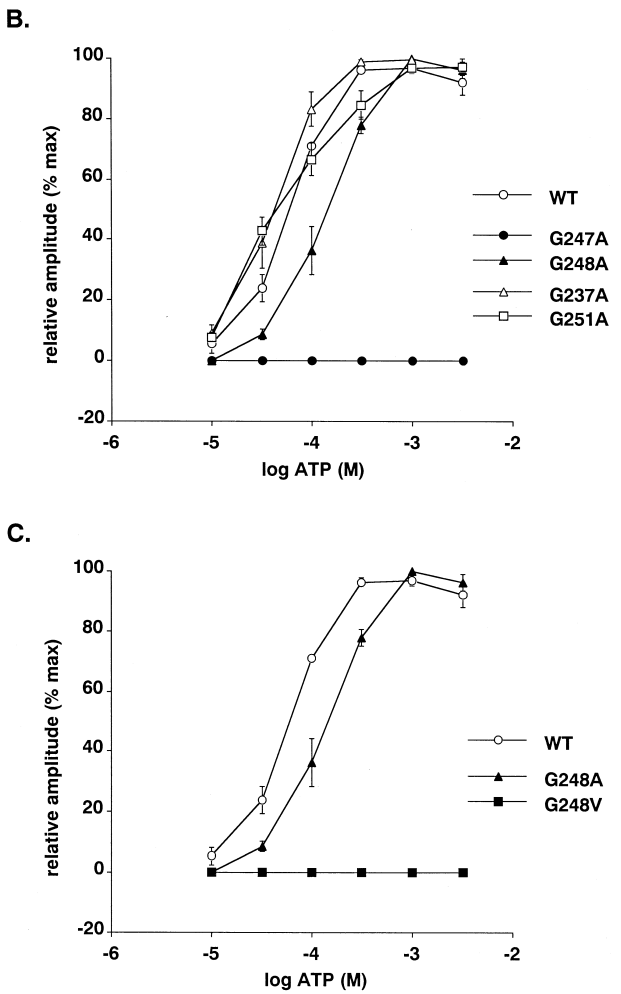

Fig. 1. (A) Glycine-rich regions containing four highly conserved glycine residues in rat P2X receptor subclasses. (B) Concentration–response relationship for ATP-activated current through WT and four glycine-to-alanine substituted mutants. Each symbol and bar represents mean and S.E. (C) Changes in sensitivity to ATP with gradual enlargement of the amino acid side-chain group at position 248.

glycine residues may contribute to a binding motif for ATP molecules. This view may be supported by recent findings that P2X receptor antagonists require a lysine or arginine residue in this glycine-rich region for antagonism (Buell et al., 1996; Collo et al., 1996), and that a sequence stretch in P2X receptors similar to an ATP-binding motif proposed for class II aminoacyl-tRNA synthetases (Freist et al., 1998) involves the glycine-rich region.

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