Purinergic receptors (P2XRs) activate and desensitize in response to the binding of extracellular nucleotides in a receptor- and ligand-specific manner, but the structural bases of their ligand preferences and channel kinetics have been incompletely characterized. Here we tested the hypothesis that affinity of agonists for binding-domain accounts for a ligand-specific desensitization pattern. We generated chimeras using receptors with variable sensitivity to ATP in order: P2X1R > P2X2aR > P2X3aR > P2X7R. Chimeras having the ectodomain Ile66–Val310 sequence of P2X2R and Glu66–Tyr315 ectodomain sequence of P2X4R in the backbone of P2X7R were expressed but were non-functioning channels. P2X2aR + X,R and P2X2bR + X,R chimeras having the Val66–Tyr315 ectodomain sequence of P2X2R in the backbone of P2X2bR were functional and exhibited increased sensitivity to ligands as compared with both parental receptors. These chimeras also desensitized faster than parental receptors and in an agonist-specific manner. However, like parental P2X2aR and P2X2aR, chimeric P2X2bR + X,R desensitized more rapidly than P2X2aR + X,R, and the rate of desensitization of P2X2bR + X,R increased by substituting its Arg71-Pro76 intracellular C-terminal sequence with the Arg71-Pro76-Gly801 sequence of P2X7R. These results indicate the relevance of interaction between the ectodomain and flanking regions around the transmembrane domains on ligand potency and receptor activation. Furthermore, the ligand potency positively correlates with the rate of receptor desensitization but does not affect the C-terminal-specific pattern of desensitization.

Purinergic receptors (P2XRs)1 are a family of ligand-gated receptor channels that open in response to extracellular ATP. Like other ligand-gated receptor channels, P2XRs also become refractory to the stimulus during the sustained agonist occupancy. This process, called desensitization, was initially characterized in acetylcholine receptors by Katz and Thesleff (1) and occurs because receptors enter stable desensitized states in which ion permeation is blocked or attenuated although ligand remains bound. Significant progress has been made recently in characterizing the ectodomain architecture of other ligand-gated receptor channels and the relationship between ligand-binding domain occupancy and receptor activity (2–4). However, the boundaries of the ectodomain and ATP-binding domain of P2XRs and the molecular mechanisms of transduction of information from ligand-binding domain to the pore of channels are largely unknown (5).

Modification at the triphosphate moiety of ATP served well in identification of native P2XR subtypes and provided useful information about the putative ligand-binding domain. For example, the substitution of bridging oxygen between α- and β-phosphorus with a methylene group resulted in a ligand, called α,β-meATP, which is a high potency agonist for P2X7R and P2X6R, low potency agonist for P2X2R, and partial agonist for P2X5R (6). High sensitivity of P2X7R to α,β-meATP can be transferred to P2X5R and P2X6R subtypes by generating the extracellular chimeras having the ectodomain of the P2X7 subunit in the P2X5- and P2X6-based backbone (7). These chimeras also exhibited enhanced rates of desensitization (7, 8). Within this region, single residue mutation studies have identified positively charged Lys66, Lys70, and Lys369 of P2X7R and the corresponding Lys69 and Lys71 of P2X5R as contributing to the ATP-binding site and control of rate of receptor desensitization (9, 10). In general agreement with these observations, the N-terminal half of the P2X2a ectodomain, from Val60 to Arg306, is necessary for high α,β-meATP sensitivity of the receptor. The attempt to further narrow this region was obstructed, indicating that the ectodomain is sensitive to modification by site-directed mutagenesis (7).

P2XRs activate and desensitize in a receptor- and ligand-specific manner. When stimulated with ATP, P2X1R and P2X2R desensitize very rapidly (in an ms time scale), P2X3R and P2X4R desensitize with a moderate rate (within a few seconds), and P2X5R, P2X6R, and P2X7R show little or no desensitization (11, 12). Two main hypotheses emerged from previous work on desensitization of P2XRs, one based on the structure of channels and the other based on the actions of intracellular messengers. Heteromultimerization results in P2XRs that desensitize with different kinetics from those seen in cells expressing homomeric receptors (13–15). The site-directed mutagenesis experiments indicated the relevance of C-terminal structure on the desensitization of P2XR (15–19), as well as the relevance of a highly conserved N-terminal site for protein kinase C in functional desensitization of receptors (19–21). Phosphorylation of a protein kinase A site in C-terminal of P2X2a may also participate in receptor desensitization (22). In parallel to other ligand-gated receptor channels (23–28), the ligand-binding domain of P2XRs may also contribute to the control of rates of desensitization (7, 8).

Here, we extended investigations on the relevance of the ectodomain structure on ligand selectivity and the pattern of P2XR desensitization. Experiments were done with wild-type

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1 The abbreviations used are: P2XRs, purinergic receptor channels; α,β-meATP, α,β-methylene-ATP; βXATP, 3'-O-(4-benzoyl)benzoyl-ATP; GFP, green fluorescent protein; EGFP, enhanced GFP, AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.

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P2X2R, P2X2bR, and chimeric P2X2a + X2R and P2X2b + X2R containing the ectodomain sequence of P2X2R instead of the corresponding P2X2R sequence. The reverse P2X2 + X2R chimera was also constructed, as well as the P2X2 + X2R chimera containing the ectodomain sequence of P2X2R. To clarify the possible interaction between the ectodomain and C-terminal domain in control of agonist specificity and receptor activity, we also constructed P2X2a-Pro376 to X2R mutant chimera containing the C-terminal 6-residue sequence of P2X2R instead of the corresponding sequence of P2X2a + X2R. Both whole-cell patch clamp current recordings and calcium measurements were used to estimate the activity of receptors in response to ATP, the native agonist for P2XR2s, and two agonist analogs, BzATP and αβ-meATP. The results of these investigations clearly indicate the C-terminal-independent influence of the ectodomain structure on agonist potency and rate of P2XR desensitization.

MATERIALS AND METHODS

DNA Constructs, Cell Culture, and Transfection—The coding sequences of the rat P2X2a, P2X2b, P2X4 subunits were isolated by reverse transcription-PCR (15) and subcloned into the baculovirus expression vector, pBacPac-EFGR (Clontech), at the restriction enzyme sites of Xhol/PstI for P2X2, P2X4 and P2X2b, and Xhol/EcoRI for P2X2R. Chimera P2X2a + X2R, P2X2b + X2R, P2X2 + X2R, and P2X2 + X2R were directly constructed by overlap extension PCR using the corresponding wild-type P2XR2s cDNAs as templates. Tagging primers were pairs of chimeric sense and antisense that were 36-mer long with the joint sites positioned at the center. The constructed P2X2a + X2R, P2X2b + X2R, P2X2 + X2R chimeric subunits replace Ile66–Tyr315 with Val66–Tyr315 extracellular domain of P2XR2, whereas P2X2 + X2R and P2X4 + X2R contain the Ile66–Tyr315 and Val66–Phe313 sequence of P2X2R and P2X4R, respectively, instead of the native Val66–

Y315 of P2XR2. We also constructed a mutant of chimera P2X2a + X2R, termed P2X2a-Pro376 to X2R, by overlap extension PCR, as described previously (15). This mutant contains the Glu376–Gly372 sequence of P2X2R instead of the corresponding Arg77–Pro76 sequence of P2X2a + X2R. These chimeric subunits were verified by gel electrophoresis and by dye termination using the pClamp 8 software packages in conjunction with the Digidata 1322A A/D converter (Axon Instruments). Patch electrodes were filled with a solution containing 140 mM KC1, 0.5 mM CaCl2, 1 mM MgCl2, 5 mM EGTA, and 10 mM HEPES; the pH was adjusted to 7.0 with KOH to 7.2. The osmolarity of the internal solutions was 282–285 mosm. The bath solution contained 142 mM NaCl, 3 mM KC1, 1 mM MgCl2, 2 mM CaCl2, 10 mM glucose, and 10 mM HEPES; the pH was adjusted to 7.3 with 1 mM NaOH. The osmolarity of this solution was 285–289 mosm. A 3 mM KC1 reservoir placed was between the bathing solution and the reference electrode. ATP was applied for 60 s using a fast gravity-driven microperfusion system (BFS-8, ALA Scientific Instruments, Westbury, NY). The application tip was routinely positioned about 500 μm above the recorded cell. Less than 600 ms was required for complete exchange of solutions around the patched cells, as estimated from altered potassium current (10–90% rise time). The time between each ATP application was about 10 min to allow recovery from receptor desensitization.

RESULTS

Characterization of Wild-type and Chimeric P2XR2s—When expressed in GT1 cells under identical experimental conditions, parental receptors P2X2aR, P2X2bR, and P2X4R responded to ATP, BzATP, and αβ-meATP stimulation with a rapid rise in [Ca2+]i, followed by a gradual decline toward steady plateau levels. In accordance with previously published data (8, 30), in all agonist concentrations studied, the peak [Ca2+]i responses were comparable in P2X2aR- and P2X2bR-expressing cells. Fig. 1A illustrates the concentration dependence of three agonists on the peak amplitude of [Ca2+]i, responses for both receptor subtypes combined. The calculated EC50 values (Fig. 1, dotted vertical lines) were vertical in agreement with the data in the literature (6): ATP was the most potent agonist for P2X2R2, followed by BzATP, whereas αβ-meATP acted as a low potency agonist.

On the other hand, the estimated EC50 values for ATP, BzATP, and αβ-meATP in GT1 cells expressing rat P2X2R (Fig. 1B) differed significantly from previously published data (reviewed in Ref. 6). In our experiments, ATP was a highly potent agonist for these receptors, with an EC50 of about 1 μM, and the calculated EC50 for BzATP was 2.5 μM as compared with >500 μM reported in other expression systems. At supramaximal concentrations, the peak amplitudes of ATP- and BzATP-induced [Ca2+]i responses in P2X2R-expressing cells were about 40% of those observed in P2X2R-expressing cells, whereas the peak amplitudes of current responses were comparable (Fig. 1).
Desensitization of P2X Receptors

aβ-meATP-induced peak current/[Ca2+]i responses were 44% as compared with those in ATP- and BzATP-stimulated cells, consistent with the partial agonist action of this ATP analog observed in other expression systems (31), but the calculated EC50 was 4 μM (Fig. 1B).

The P2X2α + X,R and P2X2β + X,R chimeras having the ectodomain of P2X2R in the backbone of P2X2αR and P2X2βR, respectively, were functional and responded to three agonists in a concentration-dependent manner with highly comparable peak amplitudes. Fig. 1C illustrates the combined results for both receptors. Chimeric receptors differed from parental receptors in two respects. First, although the structure of the pore was not altered, the peak amplitudes of [Ca2+]i responses in chimeric receptors were 80% of those observed in P2X2αR- and P2X2βR-expressing cells (p < 0.01 in the 10–1000 μM concentration range of ATP). Second, chimeric receptors exhibited higher sensitivity to ATP as compared with both parental receptors, and aβ-meATP exhibited the full agonistic action as compared with partial agonistic action in P2X2R-expressing cells.

The reverse P2X4R + X,R chimera, having the ectodomain of P2X2R inserted into the backbone of P2X4R, was expressed at the levels comparable with those observed in experiments with P2X2α + X,R and P2X2β + X,R, as estimated by GFP fluorescence intensity. However, the receptor did not respond to ATP in the 1–1000 μM concentration range. The lack of effects of ATP was not due to the endogenous desensitization of receptors because ATP was also ineffective in cells cultured in the presence of apyrase, an ectoATPase. Also, the P2X4R + X,R chimera having the ectodomain of P2X2R instead of P2X4R was expressed but was not functional in the presence or absence of apyrase. All together, experiments with chimeras suggested that although the ectodomain sequences we selected contained several residues critical for ligand binding, they were not sufficient to preserve intact ligand-binding domains. The P2X2R-specific ligand potency was enhanced, whereas the P2X4R- and P2X2R-specific ligand potency was abolished in chimeric channels, indicating the interaction between the ectodomain and nearby residues.

Receptor-specific Desensitization Pattern—To characterize the pattern of receptor desensitization and its impact on calcium signaling, both current and calcium measurements were used. Fig. 2A illustrates typical profiles of ATP (100 μM)-induced current responses in cells expressing wild-type P2X2αR, P2X2βR, and P2X2R. The peak amplitudes of current responses were in high pA (P2X2R) to low nA (P2X2αR and P2X2βR) range. Consistent with previously published data (16), P2X2R desensitized slowly and incompletely, reaching the steady levels with a τ of 22 s, whereas P2X2βR desensitized rapidly with a calculated τ of about 4 s. P2X2R desensitized to the steady levels comparable with P2X2βR but with a τ of about 9 s.

The significance of receptor-specific desensitization pattern on [Ca2+]i response is illustrated in Fig. 2B. Three receptors generated calcium signals, which differed in profiles. The rates of signal desensitization (expressed as τ) were: 96 s in P2X2αR-expressing cells, 18 s in P2X2βR-expressing cells, and 45 s in P2X2R-expressing cells. Differences in the calculated τ values in current and [Ca2+]i measurements illustrate the impact of calcium-handling mechanisms of the cells used in experiments on the rate of receptor desensitization. On the other hand, the relative ratios in the rates of P2X2αR, P2X2βR, and P2X2R desensitization estimated from two measurements were highly comparable. This clearly indicates that [Ca2+]i measurements not only provide information about the physiological relevance of receptor activation and desensitization but also could be used as valuable parameters in characterizing the nature of P2X1R desensitization, at least for slower desensitizing receptors.

A comparison between the patterns of current responses in
cells expressing wild-type P2X$_{2a}$R and P2X$_{2b}$R and chimeric receptors is shown in Fig. 3. The substitution of the native P2X$_{2a}$R ectodomain with P2X$_{4}$ ectodomain dramatically enhanced the rate of receptor desensitization (Fig. 3 B). Significant differences were also observed between P2X$_{2a}$R and chimeric P2X$_{2a}$R + X$_R$ (A) and wild-type P2X$_{2b}$R and chimeric P2X$_{2b}$R + X$_R$ (B). The mean ± S.E. values are shown above traces, and the numbers in parentheses indicate the number of records for each receptor. Asterisks indicate significant differences (p < 0.05) between the pairs.

FIG. 3. Acceleration of P2X$_{2a}$R and P2X$_{2b}$R desensitization by substituting their common ectodomain with the P2X$_{4}$R ectodomain (current recordings). The traces shown are representative for wild-type P2X$_{2a}$R and chimeric P2X$_{2a}$R + X$_R$ (A) and wild-type P2X$_{2b}$R and chimeric P2X$_{2b}$R + X$_R$ (B). The mean ± S.E. values are shown above traces, and the numbers in parentheses indicate the number of records for each receptor. Asterisks indicate significant differences (p < 0.05) between the pairs.

FIG. 4. Influence of ectodomain and C-terminal domain on acceleration of P2X$_{2a}$R + X$_R$ and P2X$_{2b}$R + X$_R$ desensitization (calcium recordings). A, representative traces of calcium responses in cells stimulated with 10 μM ATP (upper traces) and 100 μM ATP (bottom traces). In this and following figures, experimental records are shown by open circles (mean values from at least 15 traces in representative experiments), and fitted curves are shown by full lines. A single exponential function was sufficient to describe the desensitization rates. The fitted function is extrapolated for clarity. B, concentration dependence of ATP on the rate of P2XR desensitization. Asterisks indicate significant differences (p < 0.01) between the pairs. Vertical dotted lines indicate differences in the plateau [Ca$^{2+}$]. C, comparison of 100 μM ATP-induced [Ca$^{2+}$]$_i$ signals in GT1 cells expressing P2X$_{2a}$R + X$_R$, P2X$_{2a}$-6aa + X$_R$, and P2X$_{2b}$R + X$_R$.

FIG. 5. Ligand-specific receptor desensitization pattern of wild-type P2XR. A, representative traces of [Ca$^{2+}$]$_i$ response in cells expressing P2X$_{4}$R (upper traces), P2X$_{2a}$R (central traces), and P2X$_{2b}$R (bottom traces). Traces shown are representative from 3–5 independent experiments. Horizontal dotted lines indicate differences in the plateau [Ca$^{2+}$]. B, mean values of rates of receptor desensitization. Asterisks indicate significant differences (p < 0.01) between the pairs.
Desensitization of P2X Receptors

**Table I**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>P2X2aR</th>
<th>P2X2aR</th>
<th>P2X2a-6aaR</th>
<th>P2X2aR + X4R</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP, 100 μM</td>
<td>59.9 ± 2.2</td>
<td>82.1 ± 1.0</td>
<td>85.1 ± 2.7</td>
<td>100 ± 5.2</td>
</tr>
<tr>
<td>BzATP, 100 μM</td>
<td>44.3 ± 11.4</td>
<td>72.0 ± 1.8</td>
<td>76.7 ± 1.9</td>
<td>100 ± 2.4</td>
</tr>
<tr>
<td>αβ-meATP, 500 μM</td>
<td>45.8 ± 3.2</td>
<td>43.4 ± 2.5</td>
<td>85.5 ± 2.2</td>
<td>97.8 ± 0.5</td>
</tr>
</tbody>
</table>

*p < 0.05 vs. P2X2aR (second and third columns) and P2X2a-6aaR + X4R (fourth and fifth columns). Basal [Ca²⁺], was expressed as 0% and peak [Ca²⁺], response as 100%.

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**Fig. 6.** Comparison of BzATP-induced calcium signals in cells expressing wild-type and chimeric receptors. A, concentration-dependent effects of BzATP on the pattern of calcium response in P2X2aR and P2X2a-6aaR-expressing cells (A) and P2X2aR and P2X2a-6aaR + X4R-expressing cells (B). In A and B, left panels illustrate representative traces, and right panels (bars) illustrate differences in the rates of calcium signal desensitization.

Current and calcium measurements showed that P2X2a-6aaR + X4R desensitized more rapidly than P2X2aR + X4R, consistent with the hypothesis that the C-terminal-specific desensitization pattern was preserved in chimeric channels. To test this hypothesis further, we generated P2X2a-6aaR + X4R mutant chimera, having the Glu³⁷⁶-Gly³⁸¹ C-terminal sequence of P2X2aR instead of the Arg³⁷¹-Pro³⁷⁶ sequence of P2X2bR + X4R. Our earlier studies have shown the relevance of Arg³⁷¹-Pro³⁷⁶ in slowing the rate of receptor desensitization (16). Substitution of this sequence with the Glu³⁷⁶-Gly³⁸¹ sequence of P2X2aR increased the rate of P2X2aR desensitization (15). In accordance with these observations, P2X2a-6aaR + X4R mutant chimera showed an increased rate of desensitization as compared with P2X2aR + X4R chimera (Fig. 4C), and the ratio between the rates of P2X2aR + X4R, P2X2a-6aaR + X4R, and P2X2bR + X4R was comparable with that observed in cells expressing P2X2aR, P2X2a-6aaR, and P2X2bR (15). Thus, the increase in the potency of receptors for ATP increases the rates of receptor desensitization independently of the C-terminal-controlled desensitization.

**Ligand-specific Desensitization Pattern**—In further experiments, we compared the patterns of calcium signals and rates of desensitization in response to ATP, BzATP, and αβ-meATP. Fig. 5 illustrates typical profiles of calcium signals in response to these three agonists in cells expressing parental receptors. In accordance with our previous study (8), the C-terminal-specific desensitization pattern of wild-type P2X2aR observed in response to ATP (Fig. 5A, left traces) was partially mimicked by BzATP (central traces) but was lost in cells stimulated with αβ-meATP (right traces). The ligand specificity of receptor desensitization was also observed in cells expressing wild-type P2X2aR (Fig. 5A, upper traces). The mean values of the rate of receptor desensitization in response to three agonists are shown in Fig. 5B. When stimulated with ATP, three receptors desensitized with significantly different rates (left panel). No differences in the rates of P2X2aR and P2X2bR desensitization were observed in response to BzATP stimulation (central panel), and all three receptors desensitized with highly comparable kinetics when stimulated with αβ-meATP (right panel). The plateau [Ca²⁺] levels in response to three agonists also differed (illustrated by dotted lines in Fig. 5A and quantified in Table I).

In further studies, we examined the ligand-specific desensitization pattern of chimeric receptors. Fig. 6 compares typical calcium signal profiles in cells expressing wild-type P2X2aR and chimeric P2X2a-6aaR + X4R (Fig. 6A) and wild-type P2X2aR and chimeric P2X2a-6aaR + X4R (Fig. 6B) during the prolonged stimulation with increasing BzATP concentrations. The C-terminal-specific desensitization patterns of P2X2aR and P2X2a-6aaR that are present in ATP-stimulated cells (Fig. 5), but are lost in BzATP-stimulated cells (Figs. 5 and 6), were reestablished in BzATP-stimulated cells expressing chimeric receptors. Fig. 6A (left panels) shows a typical pattern of receptor desensitization in wild-type and chimeric P2X2Rs, and bars (right panels) illustrate significant differences in the mean values for the rate of receptor desensitization, whereas Fig. 6B (left and right panels) illustrates more dramatic differences between wild-type and chimeric P2X2aR.

The establishment of C-terminal-specific desensitization pattern was also observed in chimeric receptors stimulated with αβ-meATP. Fig. 7A shows that wild-type P2X2aR do not respond to 10 μM αβ-meATP, whereas chimeric receptors do. Fig. 7B illustrates the lack of receptor-specific desensitization.
pattern in wild-type P2X<sub>2</sub>Rs and a significant difference in the rates of chimeric receptor desensitization in response to 100 μM αβ-meATP. The same conclusions were also derived from cells stimulated with 500 μM αβ-meATP (Fig. 7C). Table I summarizes the level of calcium signal desensitization in wild-type and chimeric P2X<sub>2</sub>Rs. Thus, the introduction of the P2X<sub>4</sub>R ectodomain in P2X<sub>2a</sub>R and P2X<sub>2b</sub>R backbones had three obvious effects: increase of the rate of receptor desensitization for ATP without affecting the C-terminal-dependent desensitization pattern, reestablishment of the C-terminal-dependent receptor desensitization in response to BzATP and αβ-meATP stimulation, and comparable plateau levels in response to three agonists.

**DISCUSSION**

In this study, we used the wild-type P2X<sub>2a</sub>R and P2X<sub>2b</sub>R because of their identical ectodomains and rapid activation properties, but distinct and well defined desensitization patterns in response to sustained stimulation with ATP (30, 32, 33). The P2X<sub>2</sub>R shares about 39% similarity with P2X<sub>3</sub>R and desensitizes with rates comparable with those observed in cells expressing P2X<sub>2b</sub>R, whereas P2X<sub>2</sub>R shares about 26% similarity with P2X<sub>4</sub>R and desensitizes in a manner more comparable with the P2X<sub>2a</sub>R subtype (6, 34). These four receptors also exhibit highly specific ligand potency profiles, including ATP, BzATP, and αβ-meATP. ATP is a highly potent agonist for P2X<sub>2</sub>R, a high to middle potency agonist for P2X<sub>2b</sub>R, and a low potency agonist for P2X<sub>4</sub>R. BzATP is considered as a high potency agonist for P2X<sub>2a</sub>R, a middle potency agonist for P2X<sub>2b</sub>R, and a low potency agonist for P2X<sub>4</sub>R. αβ-meATP acts as a low potency agonist for P2X<sub>2b</sub>R and a partial agonist for P2X<sub>2a</sub>R, whereas P2X<sub>4</sub>R is insensitive to this agonist (6).

The sequences Ile<sup>66</sup>–Tyr<sup>310</sup> of P2X<sub>2a</sub>R, Val<sup>66</sup>–Tyr<sup>315</sup> of P2X<sub>2b</sub>R, and Val<sup>66</sup>–Phe<sup>313</sup> of P2X<sub>4</sub>R used for construction of our chimeric channels contain the majority of residues relevant for ATP binding identified so far. They enclose the 10 conserved cysteine residues among all known P2XRs and three N-linked glycosylated sites (Asn<sup>182</sup>, Asn<sup>239</sup>, and Asn<sup>298</sup> in rat P2X<sub>2</sub>R), which might be responsible for functionality of the channels (35–39). The ectodomain sequences we used also contain several conserved residues, which might contribute to the ATP-binding site, including Lys<sup>69</sup> and Lys<sup>71</sup> in rat P2X<sub>2</sub>R sequence and corresponding to Lys<sup>59</sup> and Lys<sup>70</sup> in human P2X<sub>2</sub>. (9, 10), Trp<sup>256</sup> in rat P2X<sub>2a</sub>R, and Lys<sup>230</sup> in human P2X<sub>2</sub>. (9). Because those are common residues for all seven channels, it is obvious that other residues account for ligand specificity among receptors. By exchanging the ectodomain sequences, we hoped that the ligand selectivity profiles would be preserved and thus enabled us to examine the dependence of channel activity on the ligand-binding-specific domains. In accordance with this, our previously published data with P2X<sub>2b</sub>R+X<sub>R</sub>R chimeras having the Val<sup>66</sup>–Phe<sup>313</sup> ectodomain sequence of P2X<sub>2a</sub>R instead of the native Ile<sup>66</sup>–Tyr<sup>310</sup> sequence showed highly comparable ligand potency with parental P2X<sub>2</sub>R (7, 8).

However, the present data clearly indicate that the transfer of these ectodomains alters the native agonist selectivity and potency. First, the ATP potency for P2X<sub>2a</sub>+X<sub>B</sub>R and P2X<sub>2b</sub>+X<sub>B</sub>R chimeras was higher than that observed in both parental receptors. In parallel to that, two chimeras desensitized more rapidly than parental receptors. Second, αβ-meATP is a partial agonist for P2X<sub>2a</sub>R (31) and a full and highly potent agonist for P2X<sub>2b</sub> and P2X<sub>4</sub>R chimeras. Third, P2X<sub>2a</sub>+X<sub>B</sub>R and P2X<sub>2b</sub>+X<sub>B</sub>R were expressed but were not functioning receptors. These results suggest the effects of transmembrane domain flanking sequences on ligand specificity and agonist potency. We may speculate that these sequences act as “dominant-positive” and “dominant-negative” domains, depending on the structure of the main ectodomain sequence, to change the sensitivity of receptors for agonists. In accordance with this view, it has been reported recently that point mutations in the first transmembrane domain affect the ligand selectivity of rat P2X<sub>2</sub>R (41).

In this study, we also progressed in understanding the mechanism by which the putative ligand-binding domain may influence the rate of receptor desensitization and the relationship between the ectodomain and C-terminal domain in control of desensitization. The main conclusion that emerged from this work is that the P2XR desensitization pattern is receptor- and ligand-specific. The P2X<sub>2a</sub>R-, P2X<sub>2b</sub>R-, and P2X<sub>4</sub>R-specific desensitization patterns were observed in response to ATP, the native agonist for these channels, but were less obvious when stimulated with BzATP and were lost when receptors were stimulated with αβ-meATP. The increase in EC<sub>50</sub> values for all
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three agonists induced by substituting the ectodomains indicated that the potency of agonists reflects the ligand specificity of receptor desensitization, i.e. highly potent agonists trigger the subtype-specific desensitization pattern, whereas agonists with lower potency are less effective or are ineffective. Our effort to further establish this hypothesis by generating receptors with decreasing sensitivity for ATP was unsuccessful because P2X2a + X4R and P2X2b + X4R, whereas the P2X2R and P2X3R ectodomains and the backbone of P2X3R generated nonfunctional channels. This suggests that flanking sequences around the transmembrane domains may act as modulatory regions. A parallelism in the leftward shift of EC50 and DC50 for ATP further suggests that the potency of agonists underlines the ligand-specific patterns of receptor desensitization.

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Dependence of Purinergic P2X Receptor Activity on Ectodomain Structure
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