Identification of a Domain Involved in ATP-gated Ionotropic Receptor Subunit Assembly*

(Received for publication, March 2, 1999, and in revised form, May 14, 1999)

Gonzalo E. Torres, Terrance M. Egan, and Mark M. Voigt†‡

From the Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, St. Louis, Missouri 63104

P2X receptors are ATP-gated ion channels found in a variety of tissues and cell types. Seven different subunits (P2X1-P2X7) have been molecularly cloned and are known to form homomeric, and in some cases heteromeric, channel complexes. However, the molecular determinants leading to the assembly of subunits into P2X receptors are unknown. To address this question we utilized a co-immunoprecipitation assay in which epitope-tagged deletion mutants and chimeric constructs were examined for their ability to co-associate with full-length P2X subunits. Deletion mutants of the P2X2 receptor subunit were expressed individually and together with P2X3 or P2X4 receptor subunits in HEK 293 cells. Deletion of the amino terminus up to the first transmembrane domain (amino acid 28) and beyond (to amino acid 51) did not prevent subunit assembly. Analysis of the carboxyl terminus demonstrated that mutants missing the portion of the protein downstream of the second transmembrane domain could also still co-assemble. However, a mutant terminating 25 amino acids before the second transmembrane domain could not assemble with other subunits or itself, implicating the missing region of the protein in assembly. This finding was supported and extended by data utilizing a chimera strategy that indicated TMD2 is a critical determinant of P2X subunit assembly.

Ligand-gated ion channels (ionotropic receptors) are oligomeric protein complexes formed by the specific association of homologous subunits (1). All of the known ionotropic receptors are members of families composed of multiple subunit genes, each encoding proteins with unique biophysical and pharmacological properties (2). Co-assembly among the subunit proteins of any particular family results in the formation of homo- and/or hetero-oligomeric receptors. For some families, this subunit-subunit interaction occurs in a promiscuous fashion, whereas in other families, there appears to be specific restrictions placed on which productive interactions are allowed (3–5). The biochemical and cellular mechanisms underlying assembly of subunits into functional receptor complexes are complex and poorly understood (for review, see Refs. 6 and 7).

Receptor families formed by subunits containing four transmembrane domains (4TMD)1 have been extensively used as models to investigate the rules governing ionotropic receptor assembly and stoichiometry. The best studied is the muscle nicotinic acetylcholine receptor (nAChR), which contains four different subunits that combine to form pentameric hetero-oigomericomers (8). The nAChR appears to be assembled using a stepwise pathway, and critical motifs involved in initial subunit assembly events reside in the large extracellular amino-terminal domain of each subunit (9–11). However, this receptor type has rigid constraints placed on the composition and stoichiometry of its constituent subunits such that only particular subunit-subunit interactions are allowed as intermediates and as final complexes (4, 7). Such constraints are not observed in other 4TMD receptor families, and this divergence in assembly rules raises a concern of whether the process found for the nAChR is representative of a template used by all ionotropic receptors.

Unlike the 4TMD receptors mentioned above, the ionotropic receptors for extracellular ATP (P2X receptors) have been found to have a much different topological arrangement. P2X subunits have only two TMDs, yielding a topology that places their amino- and carboxyl termini in the intracellular compartment and the loop connecting the two TMD extracellular (12, 13). Recent evidence indicates that, like the 4TMD receptors, P2X subunits can participate in the formation of homo- and hetero-oligomeric channel assemblies (14–17). Thus, the P2X receptors represent a new and perhaps simpler model system for the investigation of mechanisms involved in the subunit assembly of other members of the ligand-gated receptor family.

In an effort to identify the domain(s) of the P2X subunit protein that are critically important for productive subunit assembly, we systematically examined the involvement of the amino terminus, carboxyl terminus, and TMDs in subunit-subunit interaction between subunits transiently expressed in HEK 293 cells. Using a co-immunoprecipitation assay to analyze homomeric and heteromeric assembly of a combination of truncated and chimeric constructs from different P2X subunits, we report here that in contrast to the nAChR, it is the second TMD of P2X subunits that carries a critical determinant of specific subunit-subunit interactions.

EXPERIMENTAL PROCEDURES

DNA Constructs—The full-length cDNA for the rat P2X2 receptor subunit was obtained from Dr. D. Julius, whereas the cDNAs for P2X3, P2X4, and P2X2 receptor subunits were cloned as described previously (17). PCR-based mutagenesis (36 cycles at 94 °C for 15 s, 55 °C for 1 min, 72 °C for 1 min) was used to incorporate the FLAG (DYKDDDDK) epitope into the amino terminus, following the first methionine (P2X2-NFLAG) or into the carboxyl-terminal end, followed by a stop codon (P2X2-CFLAG), of the P2X2 subunit. After PCR mutagenesis, restriction fragments containing epitope-tagged sequences were digested with

---

† To whom correspondence and reprint requests should be addressed: Dept. of Pharmacological and Physiological Science, Saint Louis University School of Medicine, 1402 S. Grand Blvd., St. Louis, MO 63104. Tel.: 314-577-8545; Fax: 314-577-8233; E-mail: voigtm@slu.edu.

‡ To whom correspondence and reprint requests should be addressed: 314-577-8545; Fax: 314-577-8233; E-mail: voigtm@slu.edu.

1 The abbreviations used are: TMD, transmembrane domain; nAChR, nicotinic acetylcholine receptor; PCR, polymerase chain reaction; aa, amino acid(s); MEM, minimal essential medium; HA, hemagglutinin, HEK 293 cells, human embryonic kidney 293 cells; TBS-T, Tris-buffered saline-Tween.

© 1999 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.
appropriate restriction enzymes and subcloned into the mammalian expression vector pRK5. Amino-terminal truncations ΔN7, ΔN14, ΔN22, ΔN28, ΔN35, and ΔN51 (numbers indicate the quantity of residues deleted starting from the wild type-initiating Met) were generated using P2X2-CFLAG as a PCR template, a flanking 3′ primer, and 5′ oligonucleotide primers containing methionine codons at the initial methionine. These were subcloned into pRK5. For 1.3.1, post-TMD1 overlap oligos were used having the following sequences: for 1.3.1, post-TMD1 overlap oligos for 1 h with streptavidin coupled to horseradish peroxidase. Filters were incubated with 10 μg/ml of the anti-FLAG antibody. After several washes with TBS-T, filters were incubated with peroxidase-conjugated sheep anti-mouse antibody for 1 h. Filters were washed extensively in TBS-T, and immunoreactivity was detected with the ECL detection kit.

**RESULTS**

**Role of the Amino Terminus in P2X2 Homo- or Hetero-oligomerization**—Similar to all other known ligand-gated ion channels, P2X subunits can form homo- and/or hetero-oligomeric receptor channels when expressed in heterologous systems such as HEK 293 cells (17). As the amino-terminal region of 4TMD ionotropic receptors has been implicated in subunit assembly (10), we wanted to determine whether this domain was also involved in P2X2 subunit assembly. We therefore created deletion mutants of P2X2 lacking the first 7, 14, 22, and 28 aa (designated ΔN7 through ΔN28, Fig. 1A). In each case an initial methionine was engineered into the truncated sequence to ensure correct translation. These nested deletions cover the portion of the protein up to the first TMD (predicted to span aa 32–52, approximately), and the truncated proteins were tagged with the FLAG epitope at the carboxyl terminus to allow for immunodetection after expression in HEK 293 cells. As seen in Fig. 1B, all deletion mutants were efficiently synthesized and were of the predicted size. When the four deletion mutants were tested for functionality, only the ΔN7 mutant gave whole cell currents gated by ATP (Fig. 1C). Currents from cells expressing ΔN7 were similar to those observed in cells transfected with the full-length P2X2 receptor and, superficially, the activation and deactivation kinetics for the responses did not differ from wild type P2X2. To determine whether the lack of function of the other three deletion mutants was a result of disruption in the mutants' protein processing and/or sorting, leading to the absence of receptor molecules on the plasma membrane, cells transfected with these constructs were incubated with Sulfo-NHS-LC-biotin. This compound binds to free amino groups of proteins and, because it is membrane impermeant in intact cells, can be used to distinguish between cell surface and intracellular proteins. Membranes labeled with Sulfo-NHS-LC-biotin were solubilized with 1% Nonidet P-40 and subjected to immunoprecipitation with the anti-FLAG antibody. Precipitates were then electrophoresed on SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose, and biotinylated protein was detected with streptavidin. As shown in Fig. 1D, all four amino-terminal deletion mutants yielded labeled proteins when transfected, indicating that they were present on the cell surface. This suggested that the absence of functional expression was not because of lack of their delivery to the cell membrane.

Next, we examined the ability of the amino-terminal deletion mutants to co-associate with full-length P2X2 receptor subunits. Because assembly would be predicted to depend upon the interaction of specific recognition sequences between subunits, we hypothesized that if the amino-terminal domain did contain a motif critical for P2X2 subunit-subunit interaction, then deletion mutants missing such a motif would fail to interact with full-length P2X2 subunits. To test this hypothesis, we utilized a co-immunoprecipitation assay in which each of the FLAG-tagged P2X2 deletion constructs were individually co-expressed with the full-length P2X2 subunit tagged with the HA epitope. Deletion mutants were then immunoprecipitated with anti-FLAG antibody, and the presence of P2X2-HA was detected on Western blots using the anti-HA antibody. This strategy pro...
Domain Involved in P2X Receptor Assembly

Fig. 1. Functional and cell surface expression of amino-terminal deletions of P2X2. A, schematic representation of wild type (WT) and amino-terminal deletions Δ7.FLAG, Δ14.FLAG, Δ22.FLAG, and Δ28.FLAG (numbers indicate the residues deleted starting from the wild type-initiating Met) of P2X2. Black boxes represent transmembrane domains, whereas the insertion of the FLAG epitope at the carboxyl-terminal domain of all subunits is indicated by flags. B, immunoblot analysis using the anti-FLAG antibody showing the levels of expression of wild type and amino-terminal deletion P2X2 deletion mutants expressed in HEK 293 cells. C, whole-cell currents recorded from HEK 293 cells expressing wild type and amino-terminal deletion P2X2 mutants. Each nonfunctional mutant was tested at least 20 times (scale bars, 1 nA, 3 s). D, cell surface expression of wild type and amino-terminal deletion mutants. HEK 293 cells were transfected with the indicated constructs and labeled with Sulfo-NHS-LC-biotin. Solubilized receptors were immunoprecipitated with the anti-FLAG antibody and detected using horseradish peroxidase-conjugated streptavidin.

Fig. 2. P2X2 amino-terminal deletion mutants co-immunoprecipitate with full-length P2X2 or P2X3 HA-tagged subunits. A, HEK 293 cells were transfected with the following combination of constructs: P2X2.FLAG/P2X2.HA (lane 2), Δ7.FLAG/P2X2.HA (lane 4), Δ14.FLAG/P2X2.HA (lane 6), Δ22.FLAG/P2X2.HA (lane 8), or Δ28.FLAG/P2X2.HA (lane 10). The cells were then lysed with a buffer containing 1% Nonidet P-40. FLAG-tagged subunits were immunoprecipitated with the anti-FLAG antibody, and HA-tagged subunits were detected by Western blot with the anti-HA antibody. Mixing experiments were performed from cells expressing individual subunits before immunoprecipitation (lanes 1, 3, 5, 7, and 9). B, HEK 293 cells expressing P2X2.FLAG/P2X3.HA (lane 1), Δ7.FLAG/P2X3.HA (lane 2), Δ14.FLAG/P2X3.HA (lane 3), Δ22.FLAG/P2X3.HA (lane 4), Δ28.FLAG/P2X3.HA (lane 5) were lysed with 1% Nonidet P-40. FLAG-tagged subunits were immunoprecipitated with the anti-FLAG antibody, and HA-tagged subunits were detected by Western blot with the anti-HA antibody.

The data provided the means to specifically isolate and analyze stable complexes consisting of mutant and wild type subunits.

Membranes from transfected cells were solubilized with detergent (1% Nonidet P-40), and P2X receptor complexes were immunoprecipitated with the anti-FLAG monoclonal antibody. As a positive control, we also tested the co-expression of P2X2.FLAG with P2X2.HA. As expected, P2X2.FLAG co-precipitated with P2X2.HA, and this interaction was observed only when both constructs were co-expressed in cells, as simply mixing lysates from cells expressing individual subunits did not result in co-precipitation (Fig. 2A). Similarly, immunoprecipitation of each of the deletion mutants with the anti-FLAG antibody also resulted in the co-precipitation of full-length P2X2.HA (also Fig. 2A). Thus, deletion of the amino terminus of P2X2 up to the first TMD did not prevent homomeric association of P2X2 receptor subunits.

Because P2X2 can also co-associate with P2X3 to form hetero-oligomeric receptors, we tested whether the amino terminus of P2X2 might be involved in hetero-oligomeric assembly by cotransfecting each of the deletion mutants with P2X3.HA. In each case we detected specific and stable interactions (Fig. 2B). These results indicate that in the absence of the amino terminus, the mutant P2X2 subunits still contained recognition sequences directing both homo- and hetero-oligomeric channel assembly.

Role of the Intracellular Carboxyl-terminal Domain of P2X2 in Subunit Assembly—Because the previous experiments ruled out the amino-terminal domain as being critical for assembly, our attention next turned to the carboxyl terminus. To investigate the role of this region in P2X receptor assembly, we made a series of deletions of the P2X2 receptor subunit at amino acid positions 362, 370, 380, and 387 (designated ΔC362 through ΔC387, Fig. 3A) by introducing stop codons immediately following those residues. These constructs were tagged with the FLAG epitope at the amino terminus. All carboxyl-terminal-truncated subunits expressed well in HEK 293 cells and gave proteins of the appropriate molecular size (Fig. 3B). Whole cell recordings from HEK 293 cells expressing each of these mutants revealed that progressive deletions of the carboxyl terminus of P2X2 to amino acid 362 (which is just on the presumed intracellular side of the TMD2 region, thought to span from aa 328 to 356) did not prevent the formation of functional ATP-gated channels (Fig. 3C). Although the kinetics of activation...
and desensitization for the ΔC387, ΔC380, and ΔC370 mutants compared with wild type channels were not altered, there was an appreciable difference in both the size and the rate of desensitization of currents given by ΔC362.

The fact that all of these carboxyl-terminal mutants were functional implied that they retained the ability to form homo-oligomers, and indeed we found that these carboxyl-terminal deletion mutants immunoprecipitated co-transfected P2X2.HA (Fig. 3D). These interactions occurred only after co-expression of the full-length and mutant subunits and were not detectable when lysates from cells expressing individual subunits were mixed before immunoprecipitation. These findings were also extended to hetero-oligomeric assembly properties, as specific and stable interactions were observed between each of the carboxyl-terminal deletion mutants and P2X3.HA (Fig. 3E). These results therefore provide direct evidence that the bulk of ATP-gated channels.

As shown in Fig. 4B, ΔN51 was expressed at high levels in transfected cells. Its apparent molecular weight was decreased after tunicamycin treatment (Fig. 4B), indicating that the protein was glycosylated and had achieved the appropriate transmembrane orientation. This mutant was able to co-assemble with either P2X2.HA or P2X3.HA (Fig. 4C), thus effectively ruling out TMD1 as being critical for assembly. In contrast, ΔC304 did not assemble with either P2X2.HA or P2X3.HA (Fig. 4D). Additionally, no self-assembly of ΔC304 was observed in cells co-transfected with two different ΔC304 constructs, differentially tagged with the FLAG and HA epitopes (Fig. 4D), ruling out the possibility that the negative co-assembly results were because of the mutant protein preferentially associating with itself rather than with the full-length wild type proteins. Another possible explanation for the lack of assembly could be that the truncated protein is not inserted into the membrane correctly. However, tunicamycin treatment of cells transfected

---

2 G. E. Torres, T. M. Egan, and M. M. Voigt, unpublished observation.
with ΔC304 produced a downward shift in the apparent molecular mass of the protein (Fig. 4E), indicating that glycosylation had occurred. This in turn suggests that the truncated protein was inserted into the endoplasmic reticulum membrane in the appropriate orientation, as the only N-glycosylation consensus sites present in the protein exist in the extracellular loop. Taken together, the results of studies using both of these truncated proteins support the hypothesis that the region of the P2X2 subunit lying between aa 304 and aa 362 is critical for co-assembly, with an obvious candidate for such an interaction being TMD2.

Role of TMDs in Subunit Assembly—To verify the role of TMD2 in assembly, we used intact proteins rather than truncated ones so that obvious concerns regarding appropriate subunit folding would be reduced. Therefore, a chimeric approach was chosen to investigate the question of TMD2 involvement in assembly. We have previously reported that the hetero-oligomeric association of different P2X subunits is selective (17). One result pertinent to our experimental design was that P2X6 meric association of different P2X subunits is selective (17). We reasoned that exploiting these intrinsic subunit properties through the use of chimeric receptor subunits would enable us to establish that it is a TMD and not the extracellular domain that is critical for productive P2X subunit assembly. In designing the chimeric constructs, we were cognizant that two subunits with such widely differing biophysical and pharmacological properties as P2X2 and P2X3 could yield problematic chimeric proteins. So instead of P2X2, we chose to use the P2X1 subunit, which functionally is nearly identical to P2X3. Two chimeric constructs were then engineered: 1.3.1, in which only the extracellular domain was from P2X3 and the rest of the flanking protein from P2X1, and 3.1.3, in which the extracellular domain of P2X1 was flanked by the amino and carboxyl termini of P2X3 (shown schematically in Fig. 5A). We inserted the FLAG epitope into the carboxyl termini of both constructs and then tested whether either of these chimeric constructs could co-precipitate with P2X6.HA when co-expressed in HEK 293 cells. As Fig. 5B shows, the chimera 1.3.1.FLAG was able to co-precipitate with P2X6.HA when co-transfected, whereas 3.1.3.FLAG could not. In contrast, both chimeras were able to individually co-precipitate P2X3.HA (Fig. 5C). This supports the contention that a TMD contains a critical motif for subunit-specific assembly.
amino acids of the amino terminus of P2X2 were found not to subunits in HEK 293 cells. Nested deletions of the initial 28 functional channels and to specifically associate with wild type units were constructed and assayed for the ability to form series of amino- and carboxyl-terminal deletions mutant sub-units in the homo- and hetero-oligomerization of P2X receptors, a initial step to examining the molecular determinants involved

FIG. 5. The chimeric construct 1.3.1.FLAG but not 3.1.3.FLAG associates with P2X6.HA. A, schematic representation of the chimeric construct 1.3.1.FLAG and 3.1.3.FLAG. Boxes represent transmembrane domains. The FLAG epitope was inserted at the carboxyl termini of both constructs. B, HEK 293 cells expressing P2X1.FLAG/P2X6.HA, 1.3.1.FLAG/P2X6.HA, 3.1.3.FLAG/P2X6.HA, or P2X3.FLAG/P2X6.HA as indicated. Transfected cells were lysed with 1% Nonidet P-40, and FLAG-tagged subunits were immunoprecipitated with the anti-FLAG antibody. HA-tagged subunits were then detected by Western blot with the anti-HA antibody. Mi represents the mixing of lysates from cells transfected with the individual subunits before immunoprecipitation with the anti-FLAG antibody, whereas Co indicates the co-expression of subunits.

DISCUSSION

Most previous studies investigating the processes underlying the assembly and formation of ionotropic receptors have used the 4TMD muscle-nAChR as the model system. Such studies have demonstrated that assembly occurs in a stepwise fashion, with the four monomeric subunits assembling into specific hetero-oligomeric intermediates through interactions of motifs present on their amino-terminal domains before the mature pentameric assembly is formed (6, 7). However, not all ionotropic receptors have a similar stoichiometry or topology to the nAChRs (e.g. ionotropic glutamate or P2X receptors). Therefore, we sought to determine whether the knowledge garnered from the nAChR studies had general applicability to other ionotropic receptors, especially the P2X receptor.

We and others have previously established that P2X2 sub-units can assemble into homo- and/or hetero-oligomeric channels when co-expressed in HEK 293 cells (e.g. 17, 19). As the initial step to examining the molecular determinants involved in the homo- and hetero-oligomerization of P2X receptors, a series of amino- and carboxyl-terminal deletions mutant sub-units were constructed and assayed for the ability to form functional channels and to specifically associate with wild type subunits in HEK 293 cells. Nested deletions of the initial 28 amino acids of the amino terminus of P2X2 were found not to prevent homo- or hetero-oligomeric channel assembly. Identical results were obtained using the carboxyl-terminal-truncated subunits, in which the construct with the most minimal intracellular tail (of some 10 aa) was still able to co-assemble with either P2X2 or P2X3.

Interestingly, our data indicate that although the amino terminus does not play a critical role in the membrane insertion and plasma membrane targeting of the protein, it does influence the functional attributes of the receptor shows. With the exception of ΔN7, all other amino-terminal deletions resulted in nonfunctional channels despite the fact that these proteins were expressed on the cell surface. One possible mechanism underlying these results is that the agonist binding and/or gating properties of the mutant channels are altered through effects mediated by the first TMD. In contrast to the results with the amino-terminal deletions, we found that removal of almost the entire intracellular carboxyl-terminal domain did not result in loss of channel function. However, the results from the carboxyl-terminal nested deletions did show that the desensitization properties of the P2X subunit can be modulated by a stretch of eight amino acids just downstream of TMD2. This stretch of primary sequence contains a number of charged and aromatic amino acids, and future site-directed mutagenesis experiments should be useful in delineating the roles that these residues play in receptor desensitization.

As neither the amino- nor carboxyl-terminal domains appeared to be important for assembly, the next step was to investigate the role of either of the TMDs. We were unable to observe any interaction between a deletion mutant subunit lacking the second transmembrane domain (ΔC304) with either of the wild type subunits. This lack of interaction was not the result of improper targeting and insertion of this protein into the endoplasmic reticulum membrane as evidenced by its glycosylation (as the only N-linked consensus sites are present in the extracellular domain of the protein). In fact, the inability of this mutant to even co-assemble with itself suggests that it does not contain recognition sequences required for subunit assembly. We could not, however, rule out the possibility that the lack of co-assembly by ΔC304 was the result of an inadequate secondary or tertiary structure. An additional consideration was a report by Kim et al. (20), in which they described the self-assembly into tetramers of a bacterial fusion protein containing the extracellular domain of P2X2. Therefore we also wanted to rule in, or out, the importance of the extracellular domain of full-length P2X subunits in their assembly.

For those reasons, our next step was to use an alternative approach for verifying the deletion mutant analysis. The truncation studies had pointed to the area of the P2X2 protein next
to and including the second TMD as carrying a pivotal determinant for subunit co-assembly. We therefore constructed chimeras derived from P2X1 and P2X3 so that only the extracellular domain, which lies between the two TMDs, was exchanged and then tested for their co-assembly with P2X6 (which will co-assemble with P2X1, but not P2X3). These experiments demonstrated that an extracellular loop comprised of P2X1 sequence (construct 3.1.3) was not sufficient to direct co-assembly with P2X6 and that an extracellular domain derived from P2X3 (construct 1.3.1) did not prevent co-assembly with P2X6. In both cases, the individual chimeras did co-assemble with P2X1, thus demonstrating that the failure of the 3.1.3 chimera to co-assemble with P2X6 is not because of impairment of assembly but rather to the lack of a domain promoting or stabilizing specific subunit-subunit interactions. These findings lead us to two conclusions. First, they demonstrate that the extracellular region on its own is not sufficient for assembly of full-sized subunits to occur, and second, they support the idea that a TMD is involved in allowing productive subunit-subunit interaction to occur. When these results are combined with those from the deletional studies, the most parsimonious explanation is that the TMD2 of P2X subunits contains a critical determinant for productive subunit co-assembly. This postulate does not rule out other areas of the protein as being involved in assembly, but it assumes that such domains play a subservient role in allowing and/or maintaining subunit assembly than does TMD2.

In summary, we report here that the assembly of the ATP-gated P2X receptors is dependent upon a motif(s) present in the second TMD of the protein. This is in marked contrast to what has been shown for the 4TMD muscle-nAChR, in which the amino terminus has been shown to be important for assembly. Thus, the findings presented in this report demonstrate that not all ligand-gated ion channels undergo assembly using a generic process.

REFERENCES

1. Unwin (1989) Neuron 3, 665–676
2. Barnard, E. A. (1992) Trends Biochem. Sci. 17, 368–374
3. Seeberg, P. H. (1993) Trends Neurosci. 16, 359–365
4. Kreienkamp, H. J., Maeda, R. K., Sine, S. M., and Taylor, P. (1995) Neuron 14, 635–644
5. Barnard, E. A., Skolnick, P., Olsen, R. W., Mohler, H., Sieghart, W., Biggio, G., Braestrup, C., Bateson, A. N., and Langer, S. Z. (1998) Pharmacol. Rev. 50, 291–313
6. Green, W. N. (1999) J. Gen. Physiol. 113, 163–169
7. Keller, S. H., and Taylor, P. (1999) J. Gen. Physiol. 113, 171–176
8. Devillers-Thiery, A., Galzi, J. L., Eisele, J. L., Bertrand, S., Bertrand, D., and Changeux, J. P. (1993) J. Membr. Biol. 136, 97–112
9. Yu, X.-M., and Hall, Z. W. (1991) Nature 352, 64–67
10. Verrall, S., and Hall, Z. W. (1992) Cell 68, 23–31
11. Sumikawa, K. (1992) Mol. Brain Res. 13, 349–353
12. Torres, G. E., Egan, T. M., and Voigt, M. M. (1998) FEBS Lett. 425, 19–23
13. Newbolt, A., Stoop, R., Virgolini, C., Surprenant, A., North, R. A., Buell, G., and Rassendren, F. (1998) J. Biol. Chem. 273, 15177–15182
14. Radford, K. M., Virgolini, C., Surprenant, A., North, R. A., and Kawashima, E. (1997) J. Neurosci. 17, 6529–6533
15. Torres, G. T., Haines, W. R., Egan, T. E., and Voigt, M. M. (1998) Mol. Pharmacol. 54, 989–993
16. Le, K. T., Babinski, K., and Seguela, P. (1998) J. Neurosci. 15, 7152–7159
17. Torres, G. E., Egan, T. M., and Voigt, M. M. (1999) J. Biol. Chem. 274, 6653–6659
18. Couturier, S., Bertrand, D., Matter, J. M., Hernandez, M. C., Bertrand, S., Millar, N., Valera, S., Barkas, T., and Ballivet, M. (1999) Neuro 5, 847–856
19. Lewis, C., Neidhart, S., Holy, C., North, R. A., Buell, G., and Surprenant, A. (1999) Nature 377, 432–435
20. Kim, M., Yoo, O. J., and Choe, S. (1997) Biochem. Biophys. Res. Commun. 240, 618–622