Determinants of Apamin and d-Tubocurarine Block in SK Potassium Channels*

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Small conductance calcium-activated potassium channels show a distinct pharmacology. Some, but not all, are blocked by the peptide toxin apamin, and apamin-sensitive channels are also blocked by d-tubocurarine. Cloned SK channels (small conductance calcium-activated potassium channel) recapitulate these properties. We have investigated the structural basis for these differences and found that two amino acid residues on either side of the deep pore are the primary determinants of sensitivity to apamin and differential block by d-tubocurarine. Therefore, the pharmacology of SK channels compared with other potassium channels correlates with structural differences in the outer pore region. However, introduction of a tyrosine residue in the position analogous to that which determines sensitivity to external tetraethylammonium for voltage-gated potassium channels endows SK channels with an equivalent tetraethylammonium sensitivity, indicating that the outer vestibules of the pores are similar. The pharmacology of channels formed in oocytes coinjected with SK1 and SK2 mRNAs, or with SK1-SK2 dimer mRNA, show that SK subunits may form heteromeric channels.

Small conductance calcium-activated potassium channels (SK channels)1 are responsible for the slow afterhyperpolarization (sAHP) following an action potential. With sustained stimulus, spike frequency adaptation occurs where the repetitive firing of action potentials is self-limiting, because the depth and time course of the sAHP are extended, preventing the membrane potential from reaching threshold (1–4). The sAHP demonstrates a distinct pharmacology. In hippocampal interneurons, the sAHP is blocked by the peptide toxin, apamin (5, 6), while in pyramidal cells, it is not affected (7). Apamin-sensitive sAHPs (8, 9) and SK channels (10) are also sensitive to the plant alkyloid, d-tubocurarine (dTC). Therefore, the structural relationship between the channels underlying apamin-sensitive and apamin-insensitive sAHPs was unclear.

Recently, the amino acid sequences of several SK channels have been described (11). Compared with other cloned K+ channels, and consistent with their distinct pharmacology and biophysical properties, SK channels form a separate branch of the potassium channel family tree. They show no clear homology to other K+ channels except in part of the pore region. Heterologously expressed SK2 channels are blocked by apamin with an IC50 of 60 pM, while highly homologous SK1 channels are not blocked by 100 nM apamin. Also, SK2 channels are more sensitive to dTC than SK1 channels (11). In situ hybridization studies in rat brain showed a good correlation between the pattern of apamin-sensitive SK channel mRNAs, SK2 and SK3, and radiolabeled apamin binding sites, while SK1 mRNA was detected in cell types with apamin-insensitive AHPs (11–14).

Apamin-sensitive SK channels have been implicated in several important physiological processes. In the central nervous system, the sensory motor portion of the inferior colliculus is capable of seizure generating activity, which may be evoked by application of apamin (15).2 Intracerebroventricular injections of apamin disturbed the circadian cycle and disrupted normal sleep patterns (16). Rats injected with apamin prior to but not following training showed accelerated acquisition rates and retention times of learned tasks (17), and increased levels of c-fos and c-jun mRNAs in the hippocampus (18). In the periphery, apamin application to guinea pig proximal colon blocked neurotensin-induced relaxation and resulted instead in contraction (19). Denervated skeletal muscle (20, 21), or skeletal muscle from patients with myotonic dystrophy (22), contained apamin-sensitive SK channels and radiolabeled apamin binding sites, while normal adult skeletal muscle did not. Moreover, the hyperexcitability associated with both of these conditions may be ameliorated by direct application of apamin (21, 23, 24). These studies suggest that apamin-sensitive SK channels may be effective therapeutic targets.

We investigated the molecular basis for the different pharmacology among cloned SK channels. The results show that two amino acids residing on opposite sides of the deep pore determine the relative sensitivities to both apamin and dTC. Despite the structural and pharmacological differences between SK channels and other potassium channels, introduction of a tyrosine residue in SK1 at the position analogous to that which determines TEA (tetraethylammonium) sensitivity in voltage-gated K+ channels (25, 26) endows equivalent TEA sensitivity, suggesting that the architecture of the outer vestibules of these two classes is similar. In addition, expression of SK1-SK2 dimers results in channels which have intermediate apamin and dTC sensitivities, indicating that more than one subunit mediates toxin binding and that SK subunits may coassemble into heteromeric channels.

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1 The abbreviations used are: SK, small conductance calcium-activated potassium channel; sAHP, slow afterhyperpolarization; dTC, d-tubocurarine; TEA, tetraethylammonium; PCR, polymerase chain reaction.

2 T. McCown, personal communication.
Channels formed following expression of chimeric subunits were tested. Hatched regions represent SK1 and the use of common restriction endonuclease sites or by a method described after agarose gel electrophoresis. Chimeras were constructed either by polymerase (Life Technologies, Inc.); following synthesis, mRNAs were verified before use (Sequenase, U. S. Biochemical Corp.). Oligonucleotides of all mutations, chimeric junctions, and PCR products were generated by overlap extension of PCR primers that encoded the desired sequence (chimera 1: 1–338 of SK1 and 350–581 of SK2; chimera 2: 1–396 of SK1 and 407–581 of SK2; chimera 3: 1–407 of SK2 and 595–581 of SK2). Site-directed mutations were created either by overlap PCR (see above) or by the altered sites method (Promega) as described previously (28). Dimer construction also used the overlap PCR method, introducing a stretch of 10 glutamine residues between the C terminus of SK1 and the N terminus of SK2, as described previously (29). All PCRs were performed using Vent polymerase (New England Biolabs), and the nucleotide sequences of all mutations, chimeric junctions, and PCR products were verified before use (Sequenase, U. S. Biochemical Corp.). Oligonucleotides were from Genosys, and sequence comparisons were performed with the GAP program from the Genetics Computer Group suite.

**EXPERIMENTAL PROCEDURES**

**Molecular Biology**—All channel subunits were subcloned into the oocyte expression vector pBF, which provides 5′- and 3′-untranslated regions from the Xenopus β-globin gene flanking a polylinker containing multiple restriction sites. In vitro mRNAs were generated using SP6 polymerase (Life Technologies, Inc.); following synthesis, mRNAs were evaluated spectrophotometrically and by ethidium bromide staining after agarose gel electrophoresis. Chimeras were constructed either by the use of common restriction endonuclease sites or by a method described by Horton et al. (27) in which the chimeric junctions were generated by overlap extension of PCR primers that encoded the desired sequence (chimera 1: 1–338 of SK1 and 350–581 of SK2; chimera 2: 1–396 of SK1 and 407–581 of SK2; chimera 3: 1–407 of SK2 and 595–581 of SK2). Site-directed mutations were created either by overlap PCR (see above) or by the altered sites method (Promega) as described previously (28). Dimer construction also used the overlap PCR method, introducing a stretch of 10 glutamine residues between the C terminus of SK1 and the N terminus of SK2, as described previously (29). All PCRs were performed using Vent polymerase (New England Biolabs), and the nucleotide sequences of all mutations, chimeric junctions, and PCR products were verified before use (Sequenase, U. S. Biochemical Corp.). Oligonucleotides were from Genosys, and sequence comparisons were performed with the GAP program from the Genetics Computer Group suite.

**Electrophysiology—Xenopus** care and handling were in accordance with the highest standards of institutional guidelines. Frogs underwent no more than two surgeries, separated by at least 3 weeks, and surgeries were performed using well established techniques. Frogs were anesthetized with an aerated solution of 3-aminobenzoic acid ethyl ester. Oocytes were studied 2–14 days after injection with 0.2–2 ng of mRNA.

Inside-out macropatches were excised into an intracellular solution containing 116 mM potassium gluconate, 4 mM KCl, 10 mM HEPES (pH 7.2, adjusted with KOH) supplemented with CaCl₂ to give free calcium concentration of 5 μM. The proportion of calcium binding to gluconate was determined by a computer program (CaBuf) assuming a stability factor for Ca³⁺ gluconate of 15.9 μM⁻¹ (30). Electrodes were pulled from thin-walled, filamented borosilicate glass (World Precision Instruments) and filled with 116 mM potassium gluconate, 4 mM KCl, 10 mM HEPES (pH 7.2). For experiments with apamin, the toxin was added to the patch pipette, because bath application did not yield satisfactorily reproducible results. Patch formation and excision were performed in ~20 s, and the initial ramp reflected control currents before block. For outside-out patches, the solutions were reversed, with calcium containing solution in the bath and calcium-free solution in the patch pipette. dTC and TEA were added to the bath solution, as described in the text. Electrode resistance was typically 2–5 MΩ. Membrane patches were voltage-clamped using an Axopatch 1B (Axon Instruments) or an EPC-9 (HEKA Electronics) amplifier. The data were low-pass Bessel-filtered at 1 kHz and acquired using Pulse software.
Determinants of Apamin and d-Tubocurarine Sensitivity in SK Channels

RESULTS

Determinant of Apamin and d-Tubocurarine Sensitivity—To initially localize the sites of interactions between apamin or d-tubocurarine and SK channels, chimeric subunits between sensitive and insensitive SK subunits were constructed. The results presented in Fig. 1A show that channels containing the pore region of SK1 were not sensitive to 100 nM apamin, while those containing the pore region from SK2 were blocked.

The pore sequences of cloned potassium channels contain a consensus motif centered around GYG, which endows the characteristic selectivity for potassium ions (31). Fig. 1B shows an alignment of three SK subunit pore sequences with representative sequences from other K⁺ channel subfamilies. The sequences show progressively less homology with distance from the GYG motif, particularly in the N-terminal domain, suggesting that these differences might endow the outer vestibules with the different pharmacologies. The pore regions of the apamin-sensitive channel, SK2, and the apamin-insensitive channel, SK1, contain three different residues, all on the periphery of the deep pore region. To examine the residues mediating the differential pharmacology among SK channel types, site-directed mutants were constructed and expressed.

Determining the blocking potency of apamin was complicated by the slow blocking rate at concentrations near the $K_{0.5}^*$ and by SK current rundown. Also, apamin application to outside-out patches did not yield reproducible results; block was not readily reversed, and significant amounts of apamin were retained in the bath chamber, even after extensive solution exchange. In contrast, apamin added to the patch pipette solution in the inside-out configuration yielded consistent and reproducible results. Therefore, apamin block was examined in inside-out patches (Fig. 2). Representative current traces from an inside-out patch, evoked by voltage ramp commands either without or with 200 pt apamin in the patch pipette, are shown in A and B, respectively. Currents were evoked every minute after excision, and the current amplitudes at $-100$ mV for each time point from four individual patches excised from the same oocyte are plotted in Fig. 2, C (without apamin) and D (with apamin). The first trace was recorded within 20 s of gigahm seal formation and rapid patch excision. The data were normalized by comparing the current at $-100$ mV for a given time point with the initial current (Fig. 2E); the averaged data from those patches without or with apamin are shown in Fig. 2F. Patches recorded in the absence of apamin show that even though the absolute amplitudes of the initial and final currents varied between patches, due at least in part to differences in patch pipette resistance, the currents rundown with a similar time course and were stable by 20 min. In the presence of apamin the current also reached steady state by 20 min after patch excision. For each oocyte, the relative decrease of current amplitudes at $-100$ mV with apamin in the patch pipette was determined in four patches and was corrected for rundown by comparison with four patches from the same oocyte recorded without apamin in the pipette. This analysis yielded a ratio of 0.18 for 200 pt apamin block of SK2 channels, equivalent to an 82% block, consistent with our previously published data (11). None of the mutations described below, or application of either toxin, obviously affected rundown.
The amino acid residues responsible for the differential sensitivity were identified by examining SK1 subunits with one, two, or three of the different residues in the pore converted to those found in SK2 (see Fig. 1B). Substitution of SK1 His<sup>357</sup> by Asn (H357N) endowed the channels with partial apamin sensitivity, being 21 ± 2% blocked by 200 μM and 52 ± 8% by 2 mM. Similar results were obtained by substituting E330D (19 ± 1% block by 200 μM; 25 ± 7% by 2 mM). These mutations were additive, as the double mutant, E330D,H357N, was 49 ± 11% blocked by 200 μM apamin, almost completely converting the insensitive SK1 channel to that of SK2. SK1 channels in which Lys<sup>328</sup> was changed to Gln were not blocked by 100 nM apamin, without or with the indicated concentrations of dTC (μM) in the bath (external) solution.

As for native SK channels (10), apamin-sensitive SK2 channels, being 50% blocked by 2 mM apamin (not shown).

As for native SK channels (10), apamin-sensitive SK2 channels are blocked by dTC (IC<sub>50</sub> = 5.4 μM, n = 4), while apamin-insensitive SK1 channels are less sensitive (IC<sub>50</sub> = 354.3 μM, n = 4). In contrast to apamin, block by dTC developed rapidly, was reproducible, and the toxin readily washed out from outside-out patches. Therefore, structural determinants for dTC sensitivity were examined using outside-out patches with 5 μM Ca<sup>2+</sup> in the internal (patch pipette) solution; patches were allowed to stabilize for 20 min prior to addition of dTC. As for apamin, the effects of the mutations SK1 E330D (IC<sub>50</sub> = 62.6 μM, n = 7) and H357N (IC<sub>50</sub> = 11.1 μM, n = 4) were additive; the double mutant shifted dTC sensitivity to that of SK2 channels (IC<sub>50</sub> = 6.3 μM, n = 4; Fig. 4). Changing Lys<sup>328</sup> to Gln did not significantly alter dTC sensitivity.

Sensitivity to External TEA—TEA, a small quaternary amine with 4-fold symmetry, has been employed as a molecular ruler, probing the pore dimensions of cloned K<sup>+</sup> channels (32–35). Like their native counterparts, cloned potassium channels show different sensitivities to external TEA. Among the voltage-gated K<sup>+</sup> channels, Kv1.1 channels are most sensitive (IC<sub>50</sub> = 0.3 mM (36, 37), while closely related Kv1.2 channels are only weakly blocked (IC<sub>50</sub> = 150 mM). Mutation of Val<sup>323</sup> in Kv1.2 to Tyr, the residue present in Kv1.1 shifts the sensitivity almost to that of Kv1.1 (25, 26). The structural differences between the pores of SK channels and voltage-gated K<sup>+</sup> channels is reflected by their distinct pharmacologies. However, the C-terminal domain of SK channel pores is identical to that of Kv1.2 over seven residues, including the major determinant of sensitivity to TEA (38). To investigate whether the architecture of the outer pore of SK channels is similar to voltage-gated potassium channels, block by external TEA was examined. SK1...
channels were blocked with an IC_{50} = 14.6 nM (n = 4). Changing Val^{355} to Tyr, the amino acid in Kv1.1, which mediates high TEA sensitivity, increased sensitivity to external TEA to that seen for Kv1.1 channels (IC_{50} = 0.3 nM, n = 4; Fig. 5). This result shows that the general architecture of the external vestibule of SK channel pores is similar to that of voltage-gated K^+ channels.

**SK Subunits Form Heteromeric Channels**—Several highly homologous SK subunits have been cloned. In situ hybridization in rat brain and Northern blot analysis of peripheral tissues indicates widespread, but distinctive, expression patterns in rat brain and Northern blot analysis of peripheral tissues indicates widespread, but distinctive, expression patterns. In situ hybridization in rat brain show a correlation between the expression pattern of rSK2 and rSK3 mRNAs and in situ hybridization in rat brain show a correlation between the expression pattern of rSK2 and rSK3 mRNAs and 125I-apamin binding sites (11, 13, 14). Taken together, these results suggest that SK channels may be the sole class of apamin receptors in brain.

The pore regions of all cloned K^+ channels contain clear primary sequence conservation. However, as reflected by their different pharmacologies, the overall structure of the outer vestibules may be quite different. For example, attempts to endow the pore regions of inward rectifier K^+ channels with sensitivity to external TEA resulted in nonfunctional channels (39). However, introducing a tyrosine residue at the position that mediates external TEA sensitivity in voltage-gated K^+ channels endows SK channels with TEA sensitivity equivalent to that for Kv1.1, demonstrating that the overall architecture of the outer vestibule is similar for the two channel types.

Apamin is an 18-amino acid peptide with two internal disulfide bridges that hold the peptide in a tight, pear-shaped tertiary conformation (40, 41) similar to that proposed for several other larger peptide ion channel blockers such as α-dendrotoxin and β-bungarotoxin (42). Structure-activity studies showed that one of the two adjacent Arg (arginine) residues (Arg^{13}, Arg^{14}) and Gln^{17} are crucial for activity, likely through electrostatic as well as hydrophobic interactions (5, 41, 43, 44).

The results presented here are consistent with a model in which Asp^{341} on the SK2 channel interacts with one of the Arg residues on the toxin, and Asn^{368} on the channel interacts with Gln^{17} on the toxin.

For both blockers, the effects of the two channel residues are additive, and channels formed by expression of SK1-SK2 dimers have intermediate sensitivities between SK1E330D and SK1H357N. Channels formed from dimers are expected to contain two SK2D341 and two SK2N368 residues, while homomeric SK2 channels will contain four of each, suggesting that maximal sensitivity requires interactions between the toxin and at least two channel subunits.

Several brain regions and peripheral tissues express more than one SK channel mRNA (11,4), and the results obtained from coexpression of SK1 and SK2 and expression of the SK1-SK2 dimer indicate that SK subunits may form heteromeric channels, giving rise to structural and functional diversity among this class of K^+ channels. The cloned SK subunits form channels with similar calcium sensitivities and conduction properties. However, the intracellular N and C termini demonstrate considerable sequence divergence, and these domains may mediate specific functions in response to intracellular signals, such as regulation by cyclic AMP-dependent protein kinase (12). The structural differences in the outer vestibule may reflect differential regulation by endogenous extracellular ligands. An endogenous peptide with structural and functional

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**FIG. 6. SK subunits may form heteromeric channels.** A, apamin block (open bars, 1 nM; closed bars, 100 nM) of channels formed from SK1-SK2 dimers, SK1, or SK2 channels. Channels formed from expression of SK1-SK2 dimers showed intermediate apamin sensitivity (33% block by 1 nM and 84% blocked by 100 nM apamin, determined from direct comparisons of current amplitudes at –100 mV). Currents were compared 5 min after patch excision as the block developed quickly in the presence of 100 nM apamin. B, concentration-response curve for block by dTC. Channels formed from expression of SK1-SK2 dimers showed intermediate dTC sensitivity. Open triangles, SK1; open circles, SK2; open squares, SK1-SK2; error bars are S.D.

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4 T. M. Ishii, J. Maylie, and J. P. Adelman, unpublished observation.

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**DISCUSSION**

The results presented here demonstrate that two residues residing on opposite sides of the outer vestibule of the SK pore determine sensitivity to the bee venom peptide toxin, apamin, and the plant alkaloid, d-tubocurarine. No other class of K^+ channels is blocked by these drugs, and among cloned K^+ channels the residues that endow sensitivity are present at those positions only in SK2 and SK3. Autoradiographic studies and in situ hybridization in rat brain show a correlation between the expression pattern of rSK2 and rSK3 mRNAs and 125I-apamin binding sites (11, 13, 14). Taken together, these results suggest that SK channels may be the sole class of apamin receptors in brain.
similarities to apamin in brain has been reported (45), and apamin application has profound physiological effects, presumably through its interactions with SK channels. Therefore, understanding the molecular determinants of apamin binding may provide a framework for the design of novel therapeutic agents affecting seizures (15), circadian cycle (16), learning disorders (17), intestinal motility (19), and myotonic dystrophy (23).

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REFERENCES
1. Hotson, J. R., and Prince, D. A. (1980) J. Physiol. 304, 621–634
2. Yarom, Y., Sugimori, M., and Llinás, R. (1985) J. Neurophysiol. 54, 607–620
3. Lang, D. G., and Ritchie, A. K. (1990) J. Physiol. (Lond.) 425, 117–132
4. Sah, P. (1996) Trends Neurosci. 19, 150–154
5. Vincent, J.-P., Schweitz, H., and Lazdunski, M. (1975) Biochemistry 14, 2521–2525
6. Zhang, L., and McBain, C. J. (1995) J. Physiol. 498, 661–672
7. Lancaster, B., and Adams, P. R. (1986) J. Physiol. 373, 499–514
8. Dun, N. J., Jiang, Z. G., and Mo, N. (1986) J. Physiol. 373, 499–514
9. Goh, J. W., and Pennefather, P. S. (1987) J. Physiol. (Lond.) 384, 315–330
10. Park, Y. B. (1994) J. Physiol. (Lond.) 481, 555–570
11. Kohler, M., Hirschberg, B., Bond, C. T., Marrion, N. V., Kinzie, J. M., Maylie, J., and Adelman, J. P. (1996) Science 273, 1709–1714
12. Nicoll, R. A. (1988) Science 241, 545–551
13. Morris, C., Schmid-Antamarchi, H., Hugues, M., and Lazdunski, M. (1984) Eur. J. Pharmacol. 100, 115–116
14. Gein, D. R., and Gackenheimer, S. L. (1993) Neuroscience 52, 191–205
15. McCown, T. J., and Breese, G. R. (1990) J. Physiol. 373, 499–514
16. Gandolfo, G., Schweitz, H., Lazdunski, M., and Gottesmann, C. (1996) Brain Res. 736, 344–347
17. Messier, C., Moreau, C., Bontempi, B., Sif, J., Lazdunski, M., and Destrade, C. (1991) Brain Res. 551, 322–326
18. Heurteaux, C., Messier, C., Destrade, C., and Lazdunski, M. (1993) Mol. Brain Res. 18, 17–22
19. Hugues, M., Schmid-H, Romey, G., Duval, D., Frelin, C., and Lazdunski, M. (1982) EMBO J. 1, 1039–1042
20. Schmid-Antamarchi, H., Renaud, J.-P., Romey, G., Hugues, M., Schmid, A., and Lazdunski, M. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 2188–2191
21. Blatz, A. L., and Mapley, K. L. (1986) Nature 323, 718–720
22. Renaud, J.-P., Desnuelle, C., Schmid-Antamarchi, H., Hugues, M., Serratrice, G., and Lazdunski, M. (1986) Nature 319, 678–680
23. Behrens, M. J., and Vergara, C. (1992) Am. J. Physiol. 263, C784–C802
24. Behrens, M. J., Julié, P., Serrani, A., Vergara, F., and Alvarez, O. (1994) Muscle & Nerve 17, 1264–1270
25. MacKinnon, R., and Yellen, G. (1990) Science 250, 276–279
26. Kavanaugh, M. P., Varnum, M. D., Osborne, P. B., Christie, M. J., Busch, A. E., Adelman, J. P., and North, R. A. (1991) J. Biol. Chem. 266, 7583–7587
27. Horton, R. M., Hunt, H. D., Ha, S. N., Pullen, J. K., and Pease, L. R. (1989) Gene (Amst.) 77, 61–68
28. Adelman, J. P., Bond, C. T., Pessia, M., and Maylie, J. (1995) Neuron 15, 1449–1454
29. Pessia, M., Tucker, S. J., Lee, K., Bond, C. T., and Adelman, J. P. (1996) EMBO J. 15, 2980–2987
30. Dawson, R. M. C., Elliot, D. C., Elliot, W. H., and Jones, U. M. (1969) Data for Biochemical Research, Oxford University Press, New York
31. Heginbotham, L., Lu, Z., Abrahamson, T., and MacKinnon, R. (1994) Biophys. J. 66, 1061–1067
32. Heginbotham, L., and MacKinnon, R. (1992) Neuron 8, 483–491
33. Hurst, R. S., Kavanaugh, M. P., Yalke, J., Adelman, J. P., and North, R. A. (1992) J. Biol. Chem. 267, 23742–23745
34. Kavanaugh, M. P., Hurst, R. S., Yalke, J., Varnum, M. D., Adelman, J. P., and North, R. A. (1992) Neuron 8, 493–497
35. Newland, C. F., Adelman, J. P., Tempel, B. L., and Almers, W. (1992) Neuron 8, 975–982
36. Stühler, W., Stocker, M., Sakmann, B., Seeburg, P., Baumann, A., Grupe, A., and Pongs, O. (1988) FEBS Lett. 242, 199–206
37. Christie, M. J., Adelman, J. P., Douglass, J., and North, R. A. (1989) Science 244, 221–224
38. McKinnon, D. (1989) J. Biol. Chem. 264, 8239–8246
39. Bond, C. T., Pessia, M., Xia, X. M., Lagrutta, A., Kavanaugh, M. P., Adelman, J. P. (1994) Recept. Channels 2, 183–191
40. Pease, J. H. B., and Wemmer, D. E. (1988) Biochemistry 27, 8491–8498
41. L labour-Julius, C., Granier, C., Albericio, F., Defendini, M.-L., Boerd, G. R., and Van Riel, H., and Van Rietzhoven, J. (1991) Eur. J. Biochem. 196, 639–645
42. Harvey, A. L., and Anderson, A. J. (1985) Pharmacol. Ther. 31, 45–55
43. Granier, C., Pedros-Muller, E. P., and Van Rietzhoven, J. (1978) Eur. J. Biochem. 82, 293–299
44. Sanders, B. E. B. (1979) Int. J. Protein Res. 13, 327–333
45. Fosset, M., Schmid-Antamarchi, H., Hugues, M., Romey, G., and Lazdunski, M. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 7228–7232
46. Kubo, Y., Bladwin, T. J., Jan, Y. N., and Jan, L. Y. (1993) Nature 362, 127–133
47. Lesage, F., Guellemare, E., Fink, M., Duprat, F., Lazdunski, M., Romey, G., and Barhanin, J. (1996) EMBO J. 15, 1004–1011
48. Ketchum, K. A., Joiner, W. J., Sellers, A. J., Kaczmarek, L. K., and Goldstein, S. A. N. (1995) Nature 376, 690–695
49. Butler, A., Tsuoda, S., McCobb, D. P., Wei, A., and Salikoff, L. (1993) Science 261, 221–224
50. Sanguinetti, M. C., Jiang, C., Currans, M. E., and Reaing, M. T. (1995) Cell 81, 299–307
51. Trudeau, M. C., Warmke, J. W., Ganetzky, B., and Robertson, G. A. (1995) Science 269, 92–95
52. Ludwig, J., Terlau, H., Wunder, F., Bruggemann, A., Pardo, I. A., Marquardt, A., Stühler, W., and Pongs, O. (1994) EMBO J. 13, 4451–4458
53. Stühler, W., Ruppersberg, J. P., Schreter, K. H., Sakmann, B., Stocker, M., Giese, K. P., Porecheke, A., Baumann, A., and Pongs, O. (1989) EMBO J. 8, 3235–3244