A Calcium-activated Cation Current by an Alternatively Spliced Form of Trp3 in the Heart*

Received for publication, April 27, 2000, and in revised form, September 1, 2000
Published, JBC Papers in Press, September 12, 2000, DOI 10.1074/jbc.M003606200

Gaku Ohki, Taku Miyoshi, Mitsunobu Murata, Kenichi Ishibashi, Masashi Imai,
and Makoto Suzuki‡
From the Department of Pharmacology, Jichi Medical School, 3311-1, Yakushiji, Minamikawachi,
Tochigi, 329-0498, Japan

To investigate a cDNA encoding cation current, we isolated an alternatively spliced form of a rat Trp3, designated Trp3sv. Trp3sv encodes 736 amino acids with a unique N terminus and six transmembrane segments. Expression of the cRNA in Xenopus oocytes was successfully performed. The cation selective current appeared after the addition of ionomycin or induced by prolonged depolarization but not by hyperpolarization. This induction was not observed by a treatment with thapsigargin, phorbol ester, or ATP. Na⁺, K⁺, tetraethylammonium, and divalent cations were permeable, while N-methylglucamine and chloride were nominally impermeable ions. The currents were not inhibited by flufenamate ruthenium red but nonspecifically by 2 mM Gd³⁺. Northern as well as Western blot suggested lower levels of the expression observed in some organs, while reverse transcriptase-polymerase chain reaction suggested that it widely spread among various organs. Therefore, we may conclude that N-terminal spliced variant of Trp3, Trp3sv, encodes a calcium-activated cation channel in various organs.

A class of Ca²⁺-activated nonselctive cation (CAN) current has been found in primary culture of rat heart cells (1) and Purkinje cells (2). Then CANs are demonstrated in various nonexcitable cells including pancreatic duct (3) and renal tubules (4). In general, the channels show ohmic conductance, a poor selectivity for various monovalent cations, exclude anions, and are activated by intracellular Ca²⁺. The channel is considered to play a role in maintaining depolarization after calcium signal transduction or various such as an arrhythmogenic transient inward current (I₉₋₁) (2, 5, 8) in the heart. A few-second depolarization induced the I₉₋₁ current observed as an oscillatory current after repolarization (2). On the other hand, CAN channels have been also found in intracellular sarcoplasmic reticulum by reconstitution methods (6). Therefore, many kinds of CAN channels would be detected, although the gene family of this class has not been detected.

Whereas some of the Trp (transient receptor potential) channels (Trp1, Trp3, and Trp6) showed a nonselective cation channel (9). Trp1, Trp4, Trp5, and dTrp are mainly permeable to Ca²⁺ and have sensitivities to Ca²⁺ store depletion. These channels are known to contribute the pathway of store-operated Ca²⁺ influx. However, Trp3 and Trp6 are activated under the condition of co-expression of G-protein (10), and controversy remains in functional expression induced by Ca²⁺ store depletion of thapsigargin (11–13). The implication of the mechanism under the activation of these channels by store depletion is not well understood. A dTrp channel included in this family is activated by Ca²⁺ or calmodulin when expressed in Xenopus oocytes (14). Trp3 channel expressed in mammalian Chinese hamster ovary cells is activated by an addition of ionomycin and the whole cell current is activated when Ca²⁺ is added in the pipette (15). Trp6 induces oscillatory calcium waves when expressed with a G-protein-coupled receptor (10). Thus, Trp3 or Trp6 may encode the channel-like Ca²⁺-activated cation current found in various organs (16). Further, Trp3 required the G-protein for the expression and thereby does not induce the current in Xenopus oocyte until co-expression of the G-protein (17).

To investigate molecules inducing CAN in nonneuronal organs, we amplified fragments of the Trp3 and -6 by polymerase chain reaction (PCR). We at first isolated a rat Trp3 fragment as a PCR product of the heart cDNAs, suggesting that Trp or its spliced forms may be present in the heart. Trp3 is mainly expressed in cerebellum, but low level RNA is detected in lung and the heart (18). Recently, mRNA sequence files (expressed sequence tags) also show that the Trp3 fragment was involved in the neonatal rat heart (accession number AI088264) and ovary (accession number AI236441) in addition to the brain. Therefore, Trp3 or its variant molecules have been contained in cardiac tissue, and we cloned a full cDNA to elucidate the function in the present experiment.

MATERIALS AND METHODS
Cloning of cDNA—RNA was isolated by using the guanidine thioxyanate method with organic extraction (Life Technologies, Inc.) from the rat heart. mRNA was prepared by using a poly(A) column (Amersham Pharmacia Biotech). The cDNA library was constructed using a kit (Marathon cDNA construction; CLONTECH) with a minor modification. Rapid amplification of cDNA ends was performed with an appropriate primer set at 94 °C for 30 s and 68 °C for 4 min for a total of 25 cycles using proofreading Taq polymerase (ExTaq; Takara, Japan). Nested primers were constructed for the 5′-rapid amplification of cDNA ends protocol. A total of two fragments consisted of Trp3sv cDNA. The primers for the protein-coding region were then constructed, and a Trp3sv cDNA of about 2.4 kb was recloned from the cDNA library. The cloned cDNA was ligated to pCMV-SPORT (Life Technologies). Both

* This work was supported by grants from the Ministry of Education and Culture of Japan and The Research Award to Jichi Medical School Graduate Student. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EBI Data Bank with accession number(s) AB022931.
‡ To whom correspondence should be addressed: Dept. of Pharmacology, Jichi Medical School, 3311-1, Yakushiji, Minamikawachi, Tochigi, 329-0498, Japan. Tel.: 81-285-58-7326; Fax: 81-285-44-5541; E-mail: masuz@jichi.ac.jp.
† The abbreviations used are: CAN, Ca²⁺-activated nonselctive cation; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase-PCR; kb, kilobase pairs; BAPTA-AM, 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid tetrasodium (acetoxymethyl ester).
Expression of a Calcium-activated Cation Current in Heart

The cDNA fragment (residues 1–405) was then used as a probe in Northern blot hybridization under hybridizing conditions at 65 °C (19.20) using a ready made membrane (Multiple Tissue Northern blots; CLONTECH) under the protocol provided. An extensive wash was performed with 0.1× SSC for 40 min at 42 °C.

RT-PCR was performed using a kit (Takara RNA-PCR, Tokyo, Japan). To detect tissue distribution of the Trp3sv fragments, total RNA of 2 μg was isolated (RNeasy; Qiagen) and used as a template. The RNA-PCR kit was used to amplify the fragment with the primers (5′-GCGGCGAGTCCAACATGATGCTTTAA-3′ and 5′-TCCTCGCTGGACAGCAGCACA-3′) under the manufacturer’s protocol. The PCR products encoding Trp3sv-specific N terminus were partly evidenced by sequence and transferred to nylon membrane followed by Southern blot detection. The Trp3sv cDNA were used as a probe in Southern blot hybridization.

Preparation of Oocyte and cRNA Injection—Mature females of Xenopus laevis were purchased from Hamamatshu animal (Shizuoka, Japan). Xenopus oocytes (stage V) collected from the ovary of frog were exposed to collagenase (1 mg/ml; Sigma Type I) in the modified Barth solution (10 mM KCl, 3 mM MgCl2, 5 mM HEPES, 80 mM NaCl, pH 7.4) for 100 min and then defolliculated manually.

The Trp3sv-inserted pCMVSPORT was transcribed in vitro by SP6 RNA polymerase after digestion with NotI (mMessage mMachine; Ambion, Austin, TX). This transcript (cRNA) was dissolved in sterile water, and 20 ng of 5′-capped cRNA was injected automatically into the oocyte (IM200J; Narishige). After injection, the oocytes were incubated in a modified Barth solution at 19 °C, and an electrophysiological study was undertaken 2–3 days later.

Two-electrode Voltage Clamp—Two-electrode voltage-clamp experiments were carried out with an amplifier (CA-1; Dagan) with microelectrodes, which, when filled with 3M KCl, had a resistance of 1–2 megaohms. The oocyte was voltage-clamped at a given voltage protocol. The stimulation and data storage were recorded at room temperature (20–24 °C), and data were stored and analyzed on a Fujitsu computer with Axon software.

Bath solution contained 90 mM KCl (where K represents monovalent cation) or sodium gluconate, 3 mM MgCl2, and 5 mM HEPES (pH 7.4). For divalent cations, 50 mM XCl, (where X represents divalent cation) solution was used with 5 mM HEPES (pH 7.4). N-Methyl-d-glucamine chloride of 50 mM was used for the experiment for this ion permeation. Flufenamic acid at 300 μM was added to the bath solution to block the endogenous Ca2+-dependent chloride current. The experimental chamber (1-mL volume) was perfused continuously with gravity flow at a rate of 1 mL/min. Various bath solutions were connected to the chamber through a multichannel unit. In each batch of oocytes injected with cRNA, control oocytes injected with water were subsequently voltage-clamped to ensure that there were no endogenous ionic currents. If any significant endogenous current was seen, then all of the oocytes in the batch were discarded. A cell-permeable Ca2+ chelator, BAPTA-AM, was purchased from Molecular Probes (Leiden, The Netherlands).

Preparation of the Antibody—Antibody was raised against N-terminus Trp3sv-specific peptides (LVLTLKSTRA) with polyethylene glycol as a conjugated substrate. Immunogen of 1 mg in Freund’s complete adjuvant was injected intramuscularly into two New Zealand White rabbits followed by biweekly booster injections of the same dose of immunogen in Freund’s incomplete adjuvant. The serum for 4–8 weeks after the injection was a sample for the measurement of the titer by enzyme-linked immunosorbent assay. The serum, which titered over 10,000 times higher than control serum, was obtained from the rabbit, designated anti-N terminus. They were purified by a protein A column (Hitrap™, Amersham Pharmacia Biotech) and affinity-purified by a kit (ProtOn™ kit; MPS, San Diego) under the manufacturer’s protocol.

Western Blot—The total protein samples were collected from the rat heart by using the homogenizer. The sample was centrifuged at 45,000 rpm for 60 min, and the membrane fraction was isolated from the cytosolic fraction. Total protein of 10 μg, obtained from tissues, was fractionated by 10% SDS-polyacrylamide gel electrophoresis, and the protein was transferred to a polyvinylidene difluoride membrane (Immobilon-P membrane; Millipore Corp., Bedford, MA). The membrane
was incubated with 3% skim milk in Tris-buffered saline overnight to block nonspecific absorption. The protein of Trp3sv was subsequently detected by incubating the membrane with a 1:300 dilution of polyclonal anti-rat Trp3sv antibody for 1 h at room temperature followed by incubation with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:3000) for 1 h. The immunocomplexes were visualized by using the ECL detection systems (PerkinElmer Life Sciences). The oocyte injected with Trp3sv cRNA was used for positive control of the Trp3sv signal.

RESULTS

Isolation of Trp3sv—Fig. 1 shows a Northern blot obtained using the Trp3sv fragment as a probe. This involved untranslated Trp3sv-specific nucleotide alignment and Trp3/Trp3sv common alignment. A band with a size of approximately 3.4 kb corresponding to Trp3 was stained positive in the brain. Positive bands with a size of about 2.4 kb were recognized ubiquitously. Using the rapid amplification of cDNA ends procedure, we successfully isolated the spliced variants of the human Trp3 homologue from the constructed heart cDNA library. An alignment of the Trp3sv amino sequence, compared with human Trp3 (21), is shown in Fig. 2. Trp3sv starts from different amino acids with Trp3, but it ends at the same C terminus with Trp3. The alignment of the splicing junction “AGGT” is observed at nucleotide 93, where amino acid alignment is also changed. Recently, genomic sequence corresponding to human Trp3 has been reported in chromosome 5 (locus AC008661), suggesting that Trp3sv is formed by a spliced 5’ alternative of the exon occurring at nucleotide 67. Thus, Trp3sv may be an alternatively spliced variant of the Trp3 gene. The Trp3sv cDNA encodes a 736-amino acid protein in which the six transmembrane segments and pore are common to Trp3. Trp3sv and Trp3 have the same transmembrane, while Trp3sv lacks two ankyrin repeats in its N terminus.

Electrophysiologic Characterization of Trp3sv Current—The current was observed with Xenopus oocytes at 2 days after the injection of Trp3sv cRNA. The essential feature of Trp3sv is its ability to pass a large cation current 3–5 min after the addition of 10–6 M ionomycin with 1 mM CaCl2 in bath solution (Fig. 3). To avoid an activation of endogenous Ca2+-dependent chloride current by depolarization, we tested the effects of ionomycin on the currents at the voltage step from −100 to 0 mV. Slope conductance was significantly higher in Trp3sv-injected oocytes compared with the control. While the addition of ionomycin without bath Ca2+ did not evoke the current, suggesting that Trp3sv encoded Ca2+-activated current influx. The cation current was also observed after maintaining a depolarizing pulse. The oocyte injected by Trp3sv revealed a small current in a steady state (Fig. 4a). A large current was evoked after depolarizing stimulus for a 20-s duration (Fig. 4b). The magnitude of the current was gradually increased, reached a plateau in 1 min, stabilized for a few minutes, and then decreased during the depolarization. In a single pulse, the current showed a time-dependent activation at +60 mV during 6-s maintenance followed by an inward current at −40 mV. Although the feature of this current is similar to arrhythmogenic I\(_{\text{f}}\), no oscillatory current such as I\(_{\text{CaL}}\) was followed in 20 experiments. The slope conductance (from −60 to 0 mV) was increased along with the duration of the depolarization but not evoked by hyperpolarization. When this maintenance stimulus voltage of 20 s was altered from 0 to +80 mV, the evoked conductance was exponentially related to stimulus voltage. The current was activated by a more than +60 mV depolarizing pulse (Fig. 5). Thus the Trp3sv current is evoked in a time- and magnitude-dependent manner by the depolarization pulse. The effect of prepulse from −100 to +90 mV on this evoked current at −60 mV was examined. The magnitude of the current at −60 mV was not related to the prepulse voltage. Thus, the opening was not voltage-dependent. Depolarization increased intracellular Ca2+ via an endogenous mechanism. Chelating intracellular Ca2+ by 50 \(\mu\)M BAPTA-AM for 30 min, the current was not evoked by the prolonged depolarization any more (Fig. 6c). The effect of this incubation on the intracellular Ca2+ was measured using fura-2, an indication that the dose and time of BAPTA-AM was sufficient to deplete the intracellular Ca2+. Therefore, the Trp3sv current was induced by depolarization via a rise in intracellular Ca2+. This induction was useful to test the current, since possibly toxic ionomycin was not required. Following experiments were done with several reagents compared with this induction.

The Trp3sv current was neither evoked by the addition of 2 \(\mu\)M thapsigargin, 30 \(\mu\)M ATP, nor 10−8 M phorbol ester (Fig. 6), as it was suggested to be activated in case of Trp3. To certify the effect of thapsigargin, the water-injected control oocytes and Trp3sv-injected ones were incubated for 3 h with 2 \(\mu\)M thapsigargin in calcium-free modified Barth solution at 19°C (11). Currents were measured in 90 mM NaCl bath solution before and after the addition of 2 mM CaCl2. This

![Figure 3](image-url)
procedure failed to activate the current in control and Trp3sv-injected oocytes. Specific blockers were tested to the depolarization-induced Trp3sv current, involving ruthenium red (0.1 mM), amiloride, and 4-aminopyridine but had no effect. GdCl₃ and LaCl₃ at 2 mM concentration only blocked the current. Although blocking by these trivalent ions was considered nonspecific, we could use these ions for estimation of the leak current.

The I-V relationship under the mean currents of the series of exchanges of the bath solution was obtained (Fig. 7). Em was not remarkably altered between 25 and 110 mV during the substitution of cations. The Trp3sv current is permeable to monovalent cations; the sequence of the conductance was K⁺, Cs⁺, Na⁺, Li⁺, tetraethylammonium, choline. Substitution of Cl⁻ with glutamate in an NaCl bath solution indicated low anion permeability of Trp3sv, because Em was not significantly altered, giving PCl/PNa as less than 0.1. Relative permeability for selected divalent cations was examined such as for monovalent cations. The currents were small but still larger than that in the presence of N-methyl-D-glucamine or in the bath added to 2 mM GdCl₃. Thus, divalent cations might be permeable, resulting in the sequence of relative conductance of Ba²⁺ > Mg²⁺ > Sr²⁺ > Ca²⁺. N-Methyl-D-glucamine was impermeable because Em was shifted toward −50 mV.

Distribution of Trp3sv—Distribution of Trp3sv was examined with a specific antibody against a unique N terminus. Western blot suggested that Trp3sv was present in membrane rather than cytoplasmic fraction of the heart, the aorta, and the brain (Fig. 8a). However, we failed to detect the histologic localization of the TRP3sv protein in the heart. Immunohistochemical findings with heart, aorta, and lung usually revealed a weak signal so that we did not discriminate them from a background noise. A RT-PCR for the Trp3sv-specific N-terminal region followed by Southern blot is shown in Fig. 8b. It indicated that the amplifications were obtained ubiquitously in the organs we tested, although magnification of the amplitude was different.

**DISCUSSION**

We have isolated an alternatively spliced variant of Trp3, Trp3sv, from a rat heart. At first, we loaded a conventional amount of mRNA (20 µg/lane) but could not detect the Trp3sv bands by Northern blot when we used full-length cDNA of Trp3sv as a probe. Although a large amount of RNA (50 µg/lane) was loaded, the Trp3sv was hardly detectable in any other organs including the kidney, skeletal muscle, and the testis. However, when we used a Trp3sv-specific N terminus as a probe and loaded mRNA (2 µg), we could find the positive bands in many organs. We found a positive sequence-matched band by 30 cycle amplification of RT-PCR. We could also find one fragment corresponding to Trp3 N terminus in addition to Trp3sv during the cloning. Thus, both clones may exist at the PCR level in the heart, although the copy number of Trp3 itself is low. The failure of the immunological detection also suggested that Trp3sv might be a minor population in the heart, although it exists in the membrane fraction. The RT-PCR pro-
 Protocol was designed to discriminate DNA from RNA. Following Southern blot, it strongly suggested that this spliced form presents widely in many organs.

Functional expression of Trp3 has not been reported in Xenopus oocytes but has been reported in mammalian cells (13, 15–17, 21–24). A comparison of Trp3sv- with Trp3-induced current might therefore be controversial. Thapsigargin is reported to enhance (13, 21, 24) or not influence (15, 17, 23) the calcium-permeable cation current induced by Trp3 in mammalian cells. Also, rat Trp4 (12) or Drosophila Trp (14) enhanced store-operated Ca$^{2+}$ current in Xenopus oocytes with thapsigargin treatment. This suggests that an endogenous mechanism needed for the capacitative calcium entry current is provided by the Xenopus oocyte. However, in this study, thapsigargin did not influence the Trp3sv-induced current. We also added ATP to activate the endogenous G-protein of the oocyte (22), if present, but did not find any modification of the current ($n = 4$). Thus, the Trp3sv current appeared unrelated to "capacitative Ca entry current." We did not know whether the unresponsiveness is due to a difference in the amino acid alignment or to a difference in the expressing cells. However, both Trp3- and Trp3sv-induced currents were activated by the addition of ionomycin (15). N-Methyl-D-glucamine was reported as impermeable ions for the CAN channel (7). Therefore, Trp3sv possessed some characteristics of a CAN channel widely found in many tissues.

A characteristic feature of CAN current has been the activation by the prolonged depolarization (2, 8). The prolonged depolarization is suggested to enhance an influx of calcium and then activate a CAN channel. When an endogenous calcium oscillatory mechanism follows, the current is repeatedly appeared and disappeared. The $I_{TT}$ in the heart Purkinje (2) is induced by this procedure and thereby considered to be arrhythmogenic. The $I_{TT}$ is induced by CAN or by the calcium-sodium transporter. Although it is not known whether Trp3sv-induced current after the depolarization is a cause of $I_{TT}$, the current in the present study may be sufficient to explain a role of CAN after the depolarization. This role of CAN related to depolarization is suggested by the data with a single channel analysis. On the other hand, CANs in sarcolemmal vesicles are isolated from adult canine ventricle (6), one of which can be blocked by ruthenium red (26). However, ruthenium-resistant CAN is also reported. Trp3sv might be a subunit of this intracellular channel. Prolonged depolarization may open another

**FIG. 5.** Depolarization-dependent induction of the Trp3sv current. Voltage was clamped at various levels from 0 to 80 mV and maintained for 20 s, and step pulses from −100 to 0 mV were followed. Bath solution is 90 mM NaCl, 3 mM MgCl$_2$, 5 mM HEPES, and 300 μM flufenamic acid. The $I$-$V$ relationship of each maintained voltage is shown (a), and the slope conductance from −60 to 0 mV was calculated (b). The mean of three experiments is plotted.

**FIG. 6.** Effect of reagents on the Trp3sv-induced current. The currents were recorded by 0.5-s step pulses from −100 to 0 mV, and slope conductance from −60 to 0 mV was calculated. Bath solution is 90 mM NaCl, 3 mM MgCl$_2$, 5 mM HEPES, and 300 μM flufenamic acid. Influence of the depolarization, 50 nM phorbol ester, or 30 μM ATP is shown (a). *, $p < 0.05$ (analysis of variance). The effect of thapsigargin was independently examined (b). Oocytes with or without Trp3sv were incubated with 2 μM thapsigargin in calcium-free modified Barth solution for 3 h. Then conductance was calculated after the addition of 2 mM CaCl$_2$ (Student’s t test). The inhibitory effect of reagents on the Trp3sv current is shown (c). Before recording the current of BAPTA-AM, oocytes were incubated with 50 μM BAPTA-AM for 30 min (n = 5). Ruthenium red (0.1 mM), LaCl$_3$ (2 mM), or GdCl$_3$ (2 mM) were added to the depolarization-activated current. Mean conductance is expressed with S.E. as a bar. *, $p < 0.05$ (analysis of variance).
Expression of a Calcium-activated Cation Current in Heart

pace-making current termed “sustained inward current” in the sinus node (27). However, the activation of the current occurs at a range of less than −40 mV and is not matched with the Trp3sv activation.

Trp, vanilloid receptor (28), and epithelial calcium channel (29) encode molecules of nonselective cation channels. They all possess six transmembrane segments with long N terminus containing ankyrin repeats. Recently, spliced variants deleting the long N terminus are reported showing different electrophysiologic characteristics (30). The synthetic N terminus of the Trp family inhibits an assembly of the channel formation and thereby alters the functional characteristics (24). Thus, it may be possible that the N-deletion form of the Trp3β is able to be expressed in Xenopus oocyte and play a different functional role in tissues from Trp3. Thus, Trp3 may function as G-protein-coupled and Trp3sv as a CAN activated by depolarization in the organs expressed.

Acknowledgments—We thank Y. Oyama and M. Hashimoto for technical and secretarial assistance.

REFERENCES

1. Colquhoun, D., Neher, E., Reuter, H., and Stevens, C. F. (1981) Nature 294, 752–754
2. Kass, R. S., Lederer, W. J., Tsien, R. W., and Weingart, R. (1978) J. Physiol. (Lond.) 281, 187–208
3. Eguiguren, A. L., Rios, J., Riveros, N., Sepulveda, F. V., and Stutzin, A. (1996) Biochem. Cell Biol. 73, 505–513
4. Teulon, J., Paulais, M., and Bouthier, M. (1987) Biochim. Biophys. Acta 905, 125–132
5. Ehara, T., Noma, A., and Ono, K. (1988) J. Physiol. (Lond.) 403, 117–133
6. Hill, J. A., Jr., Coronado, R., and Strauss, H. C. (1988) Circ. Res. 62, 411–415
7. Champigny, G., Verrier, B., and Lazdunski, M. (1991) Biochem. Cell Biol. 69, 1196–1203
8. Saunders, J. H., Ferrier, G. R., and Moe, G. K. (1973) Circ. Res. 32, 610–617
9. Freichel, M., Schweig, U., Staufs-oberger, S., Freise, D., Schohr, W., and Flockerzi, V. (1999) Cell. Physiol. Biochem. 9, 270–283
10. Boulay, G., Zhu, X., Peyton, M., Jiang, M., Hurst, R., Stefani, E., and Birnbaumer, L. (1997) J. Biol. Chem. 272, 29672–29680
11. Petersen, C. C., Berridge, M. J., Borgese, M. F., and Bennett, D. L. (1995) Biochem. J. 311, 41–44
12. Tomita, Y., Kaneko, S., Funayama, M., Kendo, H., Sato, M., and Akaike, A. (1998) Neurosci. Lett. 248, 185–189
13. Preuss, K. D., Noller, J. K., Krause, E., Gobel, A., and Schulz, I. (1997) Biochem. Cell Biol. 75, 125–132
14. Lan, L., Bawden, M. J., Auld, A. M., and Barritt, G. J. (1996) Biochem. Cell Biol. 74, 8375–8378
15. Ishibashi, K., Kuwahara, M., Gu, Y., Kageyama, Y., Tohsaka, A., Suzuki, F., and Schumacher, M. A. (1997) Nature 383, 411–415
16. Mizuno, N., Kitayama, S., Saishin, Y., Shimada, S., Morita, K., Mitsuhata, C., Kurihara, H., and Dohi, T. (1999) Brain Res. Mol. Brain Res. 64, 41–51
17. Kamouchi, M., Philipp, S., Flockerzi, V., Wissenbach, U., Mamin, A., Raeymaekers, L., Eggermont, J., Droogmans, G., and Nilius, B. (1999) J. Physiol. (Lond.) 518, 345–358
18. Mori, Y., Takada, N., Okada, T., Wakamori, M., Imoto, K., Waniufuchi, H., Oka, H., Oba, A., Ikemoto, K., and Katsumi, T. (1998) Neuronreport 9, 567–571
19. Ishibashi, K., Kuwahara, M., Gu, Y., Kageyama, Y., Tohsaka, A., Suzuki, F., Marumo, F., and Sasaki, S. (1997) J. Biol. Chem. 272, 793–803
20. Zit, C., Obukhov, A. G., Strubing, C., Zobel, A., Kalkbrenner, F., Luckhoff, A., and Schultz, G. (1997) J. Cell Biol. 138, 1333–1341
21. Mirzoe, N., Kiyai, S., Saitosh, Y., Shimada, S., Morita, K., Mitsuhata, C., Kurihara, H., and Dohi, T. (1999) Brain Res. Mol. Brain Res. 64, 41–51
22. Marcus, D. C., Toma, M., Krause, E., Gobel, A., and Schulz, I. (1997) Biochem. Cell Biol. 75, 125–132
23. Zhu, X., Jiang, M., Peyton, M., Boulay, G., Hurst, R., Stefani, E., and Birnbaumer, L. (1996) Cell 85, 661–671
24. Hoffmann, T., Obukhov, A. G., Schaefer, M., Harteneck, C., Gudermann, T., and Schultz, G. (1999) Nature 397, 259–263
25. Zhu, X., Jiang, M., and Birnbaumer, L. (1998) J. Biol. Chem. 273, 133–142
26. Grosseh, K., Hingel, S., Linschinger, B., Balzer, M., Romanin, C., Zhu, X., and Schreibmayer, W. (1998) FEBS Lett. 437, 101–106
27. Marcus, D. C., Toma, M., Krause, E., Gobel, A., and Schulz, I. (1997) Biochem. Cell Biol. 75, 125–132
28. Rousseau, E., Smith, J. S., Henderson, J. S., and Meissner, G. (1986) Neuron 2, 325–332
29. Caterina, M. J., Schumacher, M. A., Tominaga, M., Rosen, T. A., Levine, J. D., and Schumacher, M. A. (1997) J. Biol. Chem. 272, 8375–8378
30. Schumacher, M. A., Moff, I., Jan, S., and Levine, J. D. (2000) J. Biol. Chem. 275, 2756–2762