Transient receptor potential (TRP) channels form a large family of plasma membrane cation channels. Mammalian members of the "short" TRP family (TRP channel (TRPC) 1–7) are Ca²⁺-permeant, non-selective cation channels that are widely expressed in various cell types, including neurons. TRPC activity is linked through unknown mechanisms to G-protein-coupled receptors or receptor tyrosine kinases that activate phospholipase C. To investigate the properties and function of TRPC4 in neuronally derived cells, we transiently expressed mouse TRPC4 and histamine H₁ receptor in mouse adrenal chromaffin cells and PC12 cells. Histamine, but not thapsigargin, stimulated Mn²⁺ influx in transfected cells. In the whole-cell patch clamp mode, histamine triggered a transient current in TRPC4-expressing cells. No current was evoked by perfusion with inositol 1,4,5-trisphosphate. When exocytosis was monitored with the capacitance detection technique, the magnitude of the membrane capacitance increase (∆Cᵢ) on application of histamine in H₁ receptor/TRPC4-expressing chromaffin cells was comparable with that triggered by a train of depolarizing pulses. Our results indicate that TRPC4 channels behave as receptor, but not store-operated, channels in neuronally derived cells. TRPC4 channels can provide sufficient Ca²⁺ influx to trigger a robust secretory response in voltage-clamped neurosecretory cells. Similar mechanisms may modulate exocytosis in other neuronal systems.

The transient receptor potential (trp) Drosophila mutants were discovered in 1969 on the basis of their defective visual response to prolonged illumination (1). Hardie and Minke (2) demonstrated that the product of the trp gene functions as a Ca²⁺-permeable channel required for inositol-mediated Ca²⁺ entry and that flies deficient in trp product lack sustained Ca²⁺ entry. Since the cloning of the original trp gene (3), the "TRP" family of proteins has expanded rapidly. Recently, TRPs were subdivided into three groups, short, long, and Osm (4). Mammalian "short" TRP channels (TRPCs) form a seven-member group of second messenger-operated, non-selective Ca²⁺-permeable cation channels (TRPC1–7) that can be activated by G-protein-coupled receptors or tyrosine receptor kinases. Phospholipase C plays a key role in stimulation of TRPC activity, although the specific mechanism of channel activation is unclear (5). Several activation mechanisms have been proposed, including stimulation by store depletion, direct coupling with IP₃ receptor/ryanodine receptors, and activation by second messengers such as diacetylgluceral (6–10).

TRPCs are widely expressed in mammalian tissues, and the mRNAs of some isoforms such as TRPC4 and TRPC5 are expressed predominantly in brain (11–13). To date, almost all studies of TRPC isoforms have been performed in non-excitable expression systems such as HEK293 cells, Chinese hamster ovary cells, and oocytes (5). There is no universal agreement on the ion selectivity and kinetic properties of individual TRPCs. At least part of the controversy arises because TRPC isoforms can exhibit different characteristics in different cellular environments (14).

Neurotransmitter release in neurons and neuroendocrine cells is triggered by a rise in [Ca²⁺]. Voltage-gated Ca²⁺ channels (VGCC) provide highly localized, rapid [Ca²⁺] elevations. Additional Ca²⁺ entry pathways, however, may trigger or modulate exocytosis. For example, numerous metabotropic presynaptic receptors can modulate synaptic release via second messenger systems. G-protein-coupled receptors that activate phospholipase Cβ cause release of Ca²⁺ from intracellular stores and may activate additional cationic conductances such as store-operated or other cation channels. To determine whether TRPCs can respond to G-protein-coupled receptor signaling and mediate exocytosis in neuronal cells, we transiently expressed TRPC4 in mouse adrenal chromaffin cells, which are developmentally related to sympathetic neurons.

We found that stimulation of TRPC4 via the Gₛᵥ₃₁-linked histamine H₁ receptor induced exocytosis in chromaffin cells co-transfected with TRPC4 and H₁-R. Thapsigargin and IP₃ did not stimulate TRPC4 in the cells, suggesting that activation is not store-operated. Because TRPC4 is abundant in hippocampal pyramidal neurons and cortical neurons (11), we propose that activation of by agonists of metabotropic receptors may regulate secretory responses in neurons.

Experimental Procedures

Molecular Biology—TRIzol Reagent (Invitrogen) was used to isolate total RNA from the mouse brain. The primers used for the polymerase chain reaction were 5'-GTCGACCGCCACATGCTAGTCGTTGCTAT-TACAAGG (sense) and 3'-GGATCGCGTCTCAATCTTGTGTCGTCATAATC (antisense). The β (accession number AAD10168) isoform of mouse TRPC4 was amplified from total RNA by employing SuperScript one-step reverse transcription-PCR with Platinum Taq System. The PCR product was subcloned into the pCR2.1 cloning vector using the Original TA cloning kit (Invitrogen) and then subcloned into the SalI BamHI sites of the pEYFP-N1 vector or the pEYFP-C1 vector.
(CLONTECH, Palo Alto, CA). The mouse H1 histamine receptor was amplified using primers 5'-TCTCTATGGATTATGTGG (sense) and 5'-CGACCAGAGAATGATCG (antisense). The product was subcloned into the BamHI/Xhol sites of the pEYFP-N1 vector. The identity of the inserts was confirmed by sequencing.

Mouse Chromaffin Cells; Isolation, Cell Culture, and Transient Transfection—Chromaffin cells were isolated using a modified Tischler method (15). Briefly, adrenal glands were dissected from 4–10-week-old outbred Swiss Webster mice and placed into Ca2+- and Mg2+-free Locke's buffer that contained 154 mM NaCl, 2.6 mM KCl, 2.2 mM KH2PO4, 0.95 mM KH2PO4, 10 mM glucose, 10 mM HEPES (pH 7.2), supplemented with penicillin (200 μg/ml) and streptomycin (50 μg/ml) at room temperature. Glands were cleaned free of surrounding fatty and cortical tissue. Isolated medullae were digested in Locke's buffer containing first 1.5 mg/ml collagenase P (Roche Molecular Biochemicals) followed by 1.25 mg/ml trypsin (1:250) (Invitrogen) and 0.075 mg/ml DNase I (Sigma) at 37 °C. Chromaffin cells were isolated by trituration through a flame-polished Pasteur pipette in F12-K medium supplemented with 15% heat-inactivated horse serum and 2.5% fetal bovine serum and were plated onto Matrigel-coated 25-mm circular glass coverslips (Fisher).

PC12 cells (a kind gift of Dr. Lisa Elferink, Wayne State University) were maintained in F12-K medium supplemented with 15% heat-inactivated horse serum and 2.5% fetal bovine serum and were seeded onto poly-t-lysine-coated coverslips. Only undifferentiated PC12 cells were used in experiments.

Chromaffin cells as well as PC12 cells were transfected using LipoFECTAMINE 2000 (Invitrogen). Mouse histamine H1 receptor fused to EYFP (H1-R) was expressed alone or co-expressed with TRPC4 (ratio 1:4). Only about 25% of cells were found expressing EYFP. Cells that were co-transfected with both constructs (H1-R/TRPC4) exhibited enhanced cell death, possibly because of increased Ca2+ influx and/or exocytosis. All experiments were performed 1–3 days after transfection.

Solutions and Chemicals—The standard extracellular solution contained 150 mM n-methyl-D-glucamine (NMDG)-MeSO3, 1 mM MgCl2, 1.2 mM CaCl2, 10 mM glucose, 10 mM HEPES (pH 7.2). To study capacitance changes activated by trains of depolarizing pulses in undifferentiated PC12 cells, we increased extracellular [Ca2+] to 10 mM. “High K+” solution contained 90 mM NaCl, 100 mM KCl, 1 mM MgCl2, 1 mM CaCl2, 10 mM glucose, 10 mM HEPES (pH 7.2). The fura-loading buffer contained 130 mM NaCl, 2 mM KCl, 1 mM MgCl2, 1 mM CaCl2, 10 mM glucose, 10 mM HEPES (pH 7.2). During fluorescence measurement the standard extracellular solution was supplemented with 100 μM LaCl3 to block Ca2+ entry through voltage-gated Ca2+ channels and 1 mM MnCl2 to perform fura-2 quench experiments. The pipette solution contained 115 mM CsMeSO3, 20 mM triethylammonium chloride, 1 mM MgCl2, 0.5 mM ATP, 0.5 mM EGTA, 20 mM HEPES (pH 7.2). All chemicals were from Sigma except for thapsigargin and IP3, which were purchased from Calbiochem.

Fluorescence Imaging—A monochromator-equipped imaging system (TILL-Photonics, Martinsried, Germany) was used to monitor fluorescence activities in Fura-2 Molecular Probes, Inc., Eugene, OR) loaded cells. Cells were loaded with fura-2/AM (2–5 μM) for 30 min in a fura-loading buffer at room temperature. After loading, cells were washed 3 times with loading buffer without fura-2/AM and then incubated for an additional 30 min at room temperature. Fura-2 was excited at 340 and 380 nm for determinations of [Ca2+]i and at the isosbestic wavelength of 380 nm for determining Mn2+ quench. Depending on loading, exposure times were 10–100-ms intervals, indicated in the text. Emitted light was collected with a 510-nm long pass filter. A Hamamatsu Plan Fluar 40× oil immersion objective was used. Data were analyzed using TILLvisION software (version 3.02). Background fluorescence was subtracted. The intracellular concentration of Ca2+ was determined as described by Grynkwicz et al. (16). To study the cellular localization of mTRPC4 fusion proteins, a Nikon PCM2000 confocal microscope with a Plan 63× oil-immersion objective was used. EYFP was excited at 488 nm, and emitted light was collected with a 515-nm long pass filter. The pin-hole diameter was set to ~1 Airy disc.

Electrophysiological Techniques—Whole-cell patch-clamp experiments as well as capacitance measurements were performed using a List EPC-7 patch-clamp amplifier. To monitor current through mTRPC4 channels, cells were voltage-clamped at a potential of −60 mV. Current amplitudes were obtained from voltage ramps. The ramps (1 mV/ms) from −100 to 100 mV were applied at 5-s intervals. The acquisition rate was set to 1 kHz, and currents were filtered at 3 kHz. Data were expressed as means ± S.E.

A computer-based phase-tracking capacitance detection technique in the whole-cell patch clamp mode was used to study exocytosis in the

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FIG. 1. Cellular localization of mouse β-TRPC4 tagged with EYFP in PC12 cells. Confocal images of representative cells are shown. Two different sections in the same cell are shown. In the upper image, a section near the interface between the cells and the glass was taken. The lower image demonstrates a section through the middle of the cells.

RESULTS

To study the role of TRPCs in mediating exocytosis we isolated the cDNAs of mouse brain β-TRPC4 isoform using a reverse transcription-PCR based approach and subcloned the PCR product into the multiple cloning site of the pEYFP-C1 vector upstream of the stop codon of EYFP. Expression of this construct resulted in formation of the EYFP-mTRPC4 fusion protein. We found that in confocal images of PC12 cells the fusion construct of β-TRPC4 was localized to the plasma membrane (Fig. 1, 4 independent experiments). Because fusion of YFP to TRPC4 may affect the channel properties, we subcloned β-TRPC4 into pEYFP-N1. YFP is not expressed in the product of this construct because a stop codon follows the coding sequence for TRPC4. In all subsequent experiments, only the construct without fusion to YFP was used.

Expression of TRPC4 in Chromaffin Cells—In a non-excitatory expression model, TRPC4 can be activated via phospholipase C by Gq/11-protein-coupled receptors such as the histamine H1 receptors (HEK-293 cells; Ref. 18). We tested whether mouse chromaffin cells expressed sufficient endogenous histamine H1 receptors to stimulate phospholipase C. In fura-2-loaded, non-transfected mouse chromaffin cells, histamine (20 μM) evoked only small, infrequent intracellular Ca2+ tran-
sients. The same cells responded to high potassium-induced depolarization with a pronounced Ca$^{2+}$ transient due to Ca$^{2+}$ influx via voltage-gated calcium channels (averages of data from five cells; Fig. 2A). Similar results were observed in three independent experiments. After transient expression of mouse histamine H$_1$ receptors, histamine (10 μM) consistently evoked a large Ca$^{2+}$ transient (Fig. 2B). Transient expression of H$_1$-R did not impair the depolarization-activated Ca$^{2+}$ transient (Fig. 2B, note different time scale). In this and subsequent experiments we used mouse H$_1$ receptors fused to EYFP to serve as a marker of transfected cells.

The histamine-induced intracellular Ca$^{2+}$ transient could be due to Ca$^{2+}$ release from intracellular stores or extracellular Ca$^{2+}$ influx or both. To determine the source of the Ca$^{2+}$, we measured Mn$^{2+}$ quench of fura-2 at the Ca$^{2+}$ isosbestic point (360 nm), which is a reliable indicator of divalent cation influx (19). In H$_1$-R-expressing cells, the slow basal Mn$^{2+}$ leak was increased only slightly but consistently by histamine application (Fig. 3A, upper panel, n = 5 independent experiments (coverslips)). In the same cells, histamine evoked a large increase in the fura-2 340/380 nm fluorescence ratio, indicating that the Ca$^{2+}$ transient evoked by histamine was largely caused by release of Ca$^{2+}$ from intracellular stores (Fig. 3A, lower panel).

Most chromaffin cells co-expressing TRPC4 with H$_1$-R, in contrast, displayed robust Mn$^{2+}$ quench after histamine application (Fig. 3B, upper panel, n = 7 coverslips). In these cells, rapid Ca$^{2+}$ release from intracellular stores was followed by a second rise in [Ca$^{2+}$], that occurred with a delay of 5–60 s (Fig. 3A, lower panel, compare insets), due to Ca$^{2+}$ entry via TRPC4.

To test whether TRPC4 is gated by a store-depletion mechanism, we used thapsigargin (1 μM), a sarco(endo)plasmic reticulum calcium ATPase pump inhibitor, which depletes intracellular Ca$^{2+}$ stores by preventing the re-uptake of passively leaked Ca$^{2+}$. Application of thapsigargin resulted in slow depletion of intracellular Ca$^{2+}$ stores, which in some cells was manifested by a slow rise in [Ca$^{2+}$], before clearance by extrusion or alternative uptake mechanisms (Fig. 3, C and D, lower panels). In H$_1$-R-expressing cells, thapsigargin stimulated a detectable, but small Mn$^{2+}$ influx (n = 10 coverslips, Fig. 3C).

Histamine application after thapsigargin had little effect on Ca$^{2+}$ transients, indicating that stores were depleted. Thapsigargin application also had only a small effect on Mn$^{2+}$ influx in TRPC4/H$_1$-R-expressing cells. Subsequent application of histamine, however, induced a marked Mn$^{2+}$ influx (n = 4 coverslips, Fig. 3D). The fura-2 340/380 ratio showed a robust rise with a delay of 10–60 s, similar to that observed in the absence of thapsigargin (Fig. 3D), indicating a Ca$^{2+}$ rise due to influx through TRPC4.

The Mn$^{2+}$ quench experiments indicate that β-TRPC4 can support divalent cation influx when expressed in mouse chromaffin cells. To examine the kinetics of the TRPC4 current, we performed whole-cell, patch-clamp recordings. TRPC4 currents were identified by applying voltage ramps from –100 mV to +100 mV every 5 s. The extracellular recording solution contained NMDG$^+$ as the sole monovalent cation to avoid contamination by voltage-gated Na$^+$ channels. Before histamine ap-
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Fig. 4. Histamine-induced currents in mouse chromaffin cells co-transfected with TRPC4/H1-R or H1-R alone. The insets show currents during voltage ramps from −100 to +100 mV, recorded at the indicated times. Histamine was applied at the times indicated by the horizontal bars. A, currents recorded at +100 mV (upper trace) and −70 mV (lower trace) in a cell expressing TRPC4/H1-R. B, currents recorded at +100 mV (upper trace) and −60 mV (lower trace) in a cell expressing TRPC4/H1-R. C, currents recorded at +100 mV (upper trace) and −60 mV (lower trace) in a cell expressing H1-R. In B and C the standard intracellular solution was supplemented with IP3 (10 μM).

Application of histamine (10 μM) activated a large outward current via the TRPC4 channel (202.2 ± 123.7 pA at +100 mV, n = 5, Fig. 4A). Under these conditions, TRPC4 currents exhibit a characteristic outward rectification due to Ca2+ influx at positive potentials (Fig. 4A, inset, trace 1). The dip of the current-voltage relation between 0 and 60 mV results from voltage-dependent, Mg2+-induced inhibition of TRPC4 (18). The TRPC4 current inactivated spontaneously despite the continued presence of histamine (tdecay = 37.9 ± 10.1 s, n = 5). The rapid decay of TRPC4 currents was not due to internalization of H1-R because the intensity of EYFP fluorescence at the plasma membrane did not change after stimulation with histamine (data not shown). VGCC currents were effectively inhibited after activation of the histamine H1-receptor (Fig. 4A, trace 3), in agreement with a previous report (20).

Some TRPC channels may be activated by a direct interaction with IP3 receptors (9). To test whether IP3 receptors are involved in the activation of TRPC4 in adrenal chromaffin cells expressing H1-R/TRPC4, we dialyzed the cells with IP3 (10 μM). In these experiments, the VGCC currents were markedly reduced or absent, probably due to Ca2+-dependent inactivation of VGCC after Ca2+ release from intracellular stores by IP3. IP3 did not stimulate TRPC4 outward current (Fig. 4B, trace 1). Subsequent application of histamine induced a current nearly identical to that evoked with no IP3 in the pipette (164 ± 86.5 pA at +100 mV, n = 6, Fig. 4B, trace 2). Currents were induced with a short delay after histamine application and reached a maximum within 10–25 s. TRPC4 currents inactivated relatively quickly (tdecay = 32.8 ± 9.3 s, n = 6). No currents were recorded in control chromaffin cells expressing H1-R alone, with (n = 5) or without IP3 (n = 6, Fig. 4C) in the pipette.

Chromaffin cells are a widely used model system for studying Ca2+-evoked exocytosis. Typically, a train of depolarizing pulses stimulated a robust rise in the cell capacitance, as illustrated in Fig. 5A (n = 11), and the capacitance response was a function of the integral of the Ca2+ current (Fig. 5B). We tested whether TRPC4 could supply sufficient Ca2+ to support exocytosis in these cells. Capacitance changes were measured in chromaffin cells expressing either H1-R alone or a mixture of H1-R and TRPC4. Because inward currents through TRPC4 interfered with capacitance measurements (21), we substituted monovalent cations with NMDG−. TRPC4 currents were monitored at positive voltages by applying depolarizing pulses at regular intervals of −11 s. After application of histamine (10 μM), the ramp evoked an inward current representing the activity of VGCC (Fig. 4A, inset, trace 1), the cell was clamped at −70 mV to reduce contribution of VGCC activation to the currents at a holding potential. Application of histamine (10 μM) activated a large outward current via the TRPC4 channel (202.2 ± 123.7 pA at +100 mV, n = 5, Fig. 4A). Under these conditions, TRPC4 currents exhibit a characteristic outward rectification due to Ca2+ influx at positive potentials (Fig. 4A, inset, trace 1). The dip of the current-voltage relation between 0 and 60 mV results from voltage-dependent, Mg2+-induced inhibition of TRPC4 (18). The TRPC4 current inactivated spontaneously despite the continued presence of histamine (tdecay = 37.9 ± 10.1 s, n = 5). The rapid decay of TRPC4 currents was not due to internalization of H1-R because the intensity of

Fig. 5. Depolarization-stimulated exocytosis in mouse chromaffin cells. A, capacitance trace in a chromaffin cell expressing H1-R. Exocytosis was elicited by a train of 10 depolarizing pulses, 40-ms duration, to +10 mV. The arrows indicate depolarizing pulses. The holding potential was set to −90 mV. The inset shows the first (1) and last (2) current trace of the depolarizing pulse train. B, plot of cumulative ΔCm versus cumulative Ca2+ influx. The Ca2+ ion influx per pulse was determined from the time integral and is expressed as total charge.
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The increase of cell capacitance paralleled the development of the TRPC4 currents (Fig. 7, B and C; Ref. 23). In addition, Ca\(^{2+}\) entry was less effective in triggering exocytosis because more Ca\(^{2+}\) ions were required to stimulate a capacitance response in PC12 cells comparable with that in a chromaffin cell (compare Fig. 5B and 7B; note the different scale). Histamine-stimulated capacitance responses were significantly larger in PC12 cells expressing TRPC4 and H1-R than in control H1-R-expressing cells (130 ± 18.1 versus 35.1 ± 7.8, n = 9 and 13, respectively; Fig. 7, C–E). In summary, even in cells that have less robust secretory responses, TRPC4 can provide sufficient Ca\(^{2+}\) to support exocytosis.

**DISCUSSION**

Here we demonstrate that the receptor-operated cation channel, TRPC4, can provide sufficient Ca\(^{2+}\) influx to support agonist-evoked secretion in both mouse primary chromaffin cells and rat-derived PC12 cells, two widely used neurally related models for studying Ca\(^{2+}\)-stimulated exocytosis. We used chromaffin cells and PC12 cells as a functional expression system in which both the G-protein coupled receptor, H1, and TRPC4 are transiently expressed. Our results demonstrate that all tested aspects of the G-protein-coupled receptor-signaling pathway and the secretory pathway are intact and functional in our neuroendocrine expression system. Both the H1-YFP receptor construct and β-TRPC4-YFP were appropriately targeted to the plasma membrane. Sustained histamine application inhibited VGCC for the duration of the recording in chromaffin cells expressing H1-R or H1-R/β-TRPC4. Similar effects were previously observed by Currie and Fox (20) in non-transfected chromaffin cells. Histamine activation of TRPC4, on the other hand, was transient. A second application of histamine did not re-activate any TRPC4-like currents in the same cell.

Mammalian TRPCs were cloned by homology to the *Drosophila* trp gene, with the goal of identifying store-operated channels, such as I\(_{\text{CRAC}}\), that are critical for the function of certain cells of the blood lineage (24–25). Currently, despite numerous reports postulating store-dependent mechanisms for TRPC stimulation, there is little experimental evidence for this hypothesis (5). The exceptions are reports on TRPC1 (26) and TRPC4 (27), although the last report has been disputed (5). Several other mechanisms of activation have been also proposed for each individual TRPCs, TRPC3, -6, and -7 have been shown to be activated by direct coupling with IP\(_3\) receptor/ryanodine receptors or direct stimulation by diacylglycerol (7–10). β-TRPC4 and TRPC5, two closely related isoforms, are not stimulated by diacylglycerol; however, involvement of some other unidentified messenger downstream of phospholipase C was suggested (18). We corroborate that histamine acting on

![](https://example.com/image-url)
the histamine H₁-receptor, a Gₛ/₁₁-protein-coupled receptor, can stimulate β-TRPC4 in mouse chromaffin cells and rat PC12 cells. Neither thapsigargin nor dialysis with IP₃ stimulated β-TRPC4 in our experiments. In addition, β-TRPC4 was not sensitive to La³⁺, a classical inhibitor of store-operated channels. Therefore, our results do not support the hypothesis that the β-TRPC4 can form a store-operated channel in neuroendocrine cells, at least under our experimental conditions.

The few physiological functions attributed to TRPCs so far are diverse, reflecting the broad tissue distribution of the channels. Most studies have been carried out in non-excitable cells. TRPC1 is thought to be an important avenue for the agonist-stimulated and store-operated Ca²⁺ influx that regulates saliva flow in salivary glands (26). TRPC2 is prominent in the vomeronasal organ, where it may participate in pheromone transduction (27). In addition, TRPC2 appears to be activated during the mouse sperm acrosome reaction, a form of exocytosis (28). Mice deficient in TRPC4 have markedly reduced vasorelaxation and agonist-induced Ca²⁺ entry in endothelial cells (29). The α₁-adrenoreceptor-activated cation channel that is involved in regulating the vascular tone in portal vein resembles TRPC6 (30). In the only report thus far on excitable cells, Li et al. (13) find that TRPC5 may contribute to neurotransphin-stimulated Ca²⁺ and Na⁺ influx in pontine neurons. Thus, the phenomenon is transient, occurring only during a specific stage of development. Therefore, there is little information on which to predict the possible function of the numerous neuronal TRPCs.

Adrenal chromaffin cells are developmentally related to sympathetic neurons and widely used as a neuron-like model system for studying Ca²⁺-stimulated exocytosis. Capacitance changes in the cells directly correlate with the number of secreted vesicles. In chromaffin cells, as in neurons, rapid exocytosis is mediated by VGCC, which can provide high [Ca²⁺]ₙₐₜ beneath the plasma membrane at the sites of exocytosis. At neuronal synapses, it is postulated that certain VGCC directly bind to synaptic vesicle proteins so as to provide Ca²⁺ influx at precisely the locations that require it (31).

There are numerous reports that Ca²⁺ pathways other than VGCC can regulate exocytosis in chromaffin cells. Cheek et al. (32) report that agonists of Gₛ/₁₁-coupled receptors, such as histamine and angiotensin II, stimulated exocytosis in bovine adrenal chromaffin cells by a combination of Ca²⁺ release from internal stores and additional subsequent Ca²⁺ entry, which they postulated was "store-operated." Teschemacher and Seward (33) demonstrate that an angiotensin II-induced voltage-independent capacitance increase in bovine chromaffin cells was associated with a small leak current; however, the authors did not discuss the properties of the current. Fomina and Nowycky (34) demonstrate that exocytosis can be triggered by Ca²⁺ entry via a small current activated on depletion of stores with thapsigargin in bovine chromaffin cells. Zerbes et al. (35, 36) report that histamine can trigger exocytosis in bovine chromaffin cells via both store-operated and store-independent mechanisms.

It is not known whether any of these effects may be mediated by any NTRPC family members. Philipp et al. (37) report that TRPC4 is present in the adrenal gland but only in the cortex and not the medulla. A recent report, however, states that PC12 cells express mRNA for TRPC1–6 (38). In our experimental conditions, histamine evoked a small, rapid, and brief but significant increase in Mn²⁺ influx in cells transfected with H₁-R alone, reflecting the presence of some type of agonist-and/or store-operated pathway (Fig. 2A). In chromaffin cells transfected with TRPC4, however, there is a much larger his-
tamine-evoked divalent cation influx. Further studies will be needed to correlate the various inward currents activated by different experimental protocols.

In 1.2 mM external Ca\(^{2+}\), the inward current at negative potentials had a small amplitude compared with that of VGCC. However, β-TRPC4 was as effective in mediating the secretory responses in chromaffin cells as VGCC stimulated by a train of 10 depolarizing pulses, although the secretory rate was very slow. There are a number of possible reasons for the similar efficacy. TRPC4 channels are open for many seconds, and Ca\(^{2+}\) may accumulate in the cytoplasm or bind to Ca\(^{2+}\) sensors for exocytosis. In addition, activation of phospholipase Cβ generates diacylglycerol, an activator of protein kinase C that potentially facilitates Ca\(^{2+}\)-evoked exocytosis in chromaffin cells (39–41). Additional second messenger pathways may also be activated by sustained elevation of [Ca\(^{2+}\)]. Because we do not have data to compare the absolute permeability of VGCC and TRPC4, a quantitative comparison between the efficacy of Ca\(^{2+}\) influx through the two pathways to evoke exocytosis is not possible.

There is no evidence that TRPCs are closely associated with and can bind to synaptic vesicle proteins, as has been demonstrated for N- and P/Q-type VGCCs (42). In a recent study, TRPC4 and -5 were found to be associated with phospholipase Cβ and NHERF, a regulator of the Na\(^+\)/H\(^+\) exchanger (43). NHERF contains two PDZ domains and, through the second PDZ domain, associates with the actin cytoskeleton via interactions with members of the ezrin/radixin/moesin family. The authors termed the TRPC4/5 complex as a “signalplex” by analogy with the INAD scaffold localizing phospholipase C and members of the ezrin/radixin/moesin family. The PDZ domain, associates with the actin cytoskeleton via interactions with members of the ezrin/radixin/moesin family. The authors termed the TRPC4/5 complex as a “signalplex” by analogy with the INAD scaffold localizing phospholipase C and members of the ezrin/radixin/moesin family. The PDZ domain, associates with the actin cytoskeleton via interactions with members of the ezrin/radixin/moesin family. The authors termed the TRPC4/5 complex as a “signalplex” by analogy with the INAD scaffold localizing phospholipase C and members of the ezrin/radixin/moesin family. The PDZ domain, associates with the actin cytoskeleton via interactions with members of the ezrin/radixin/moesin family. The authors termed the TRPC4/5 complex as a “signalplex” by analogy with the INAD scaffold localizing phospholipase C and members of the ezrin/radixin/moesin family. The PDZ domain, associates with the actin cytoskeleton via interactions with members of the ezrin/radixin/moesin family. The authors termed the TRPC4/5 complex as a “signalplex” by analogy with the INAD scaffold localizing phospholipase C and members of the ezrin/radixin/moesin family. The PDZ domain, associates with the actin cytoskeleton via interactions with members of the ezrin/radixin/moesin family. The authors termed the TRPC4/5 complex as a “signalplex” by analogy with the INAD scaffold localizing phospholipase C and members of the ezrin/radixin/moesin family. The PDZ domain, associates with the actin cytoskeleton via interactions with members of the ezrin/radixin/moesin family. The authors termed the TRPC4/5 complex as a “signalplex” by analogy with the INAD scaffold localizing phospholipase C and members of the ezrin/radixin/moesin family. The PDZ domain, associates with the actin cytoskeleton via interactions with members of the ezrin/radixin/moesin family. The authors termed the TRPC4/5 complex as a “signalplex” by analogy with the INAD scaffold localizing phospholipase C and members of the ezrin/radixin/moesin family. The PDZ domain, associates with the actin cytoskeleton via interactions with members of the ezrin/radixin/moesin family. The authors termed the TRPC4/5 complex as a “signalplex” by analogy with the INAD scaffold localizing phospholipase C and members of the ezrin/radixin/moesin family. The PDZ domain, associates with the actin cytoskeleton via interactions with members of the ezrin/radixin/moesin family. The authors termed the TRPC4/5 complex as a “signalplex” by analogy with the INAD scaffold localizing phospholipase C and members of the ezrin/radixin/moesin family. The PDZ domain, associates with the actin cytoskeleton via interactions with members of the ezrin/radixin/moesin family. The authors termed the TRPC4/5 complex as a “signalplex” by analogy with the INAD scaffold localizing phospholipase C and members of the ezrin/radixin/moesin family. The PDZ domain, associates with the actin cytoskeleton via interactions with members of the ze...
TRPC4 Can Be Activated by G-protein-coupled Receptors and Provides Sufficient Ca\(^{2+}\) to Trigger Exocytosis in Neuroendocrine Cells

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