Protein kinase A (PKA) is targeted to discrete subcellular locations close to its intended substrates through interaction with A kinase-anchoring proteins (AKAPs). Ion channels represent a diverse and important group of kinase substrates, and it has been shown that membrane targeting of PKA through association with AKAPs facilitates PKA-mediated phosphorylation and regulation of several classes of ion channel. Here, we investigate the effect of AKAP79, a membrane-associated multivalent-anchoring protein, upon the function and modulation of the strong inwardly rectifying potassium channel, Kir2.1. Functionally, the presence of AKAP79 enhanced the response of Kir2.1 to elevated intracellular cAMP, suggesting a requirement for a pool of PKA anchored close to the channel. Antibodies directed against a hemagglutinin epitope tag on Kir2.1 coimmunoprecipitated AKAP79, indicating that the two proteins exist together in a complex within intact cells. In support of this, glutathione S-transferase fusion proteins of both the intracellular N and C domains of Kir2.1 isolated AKAP79 from cell lysates, while glutathione S-transferase alone failed to interact with AKAP79. Together, these findings suggest that AKAP79 associates directly with the Kir2.1 ion channel and may serve to anchor kinase enzymes in close proximity to key channel phosphorylation sites.

The phosphorylation state of ion channels is governed by the activation of protein kinases and phosphatases, which in turn respond to fluctuations in the local concentration of second messengers such as calcium and cyclic AMP. It has been shown recently that much of the specificity seen in intracellular phosphorylation events comes from the targeting of protein kinases and phosphatases to specific subcellular structures and organelles through association with anchoring or adaptor proteins (1, 2). Cyclic AMP-dependent protein kinase (protein kinase A or PKA), for example, is targeted to discrete subcellular locations through interaction with A kinase-anchoring proteins or AKAPs (3, 4). AKAPs form a family of more than thirty different functionally related proteins. Each anchoring protein contains at least two functional motifs: an amphipathic helix that binds the regulatory (RII) subunits of the PKA holoenzyme (5, 6) and a specialized anchoring domain that tethers the AKAP-PKA complex to specific intracellular sites close to their intended substrate (7). The modulation of ion channel activity through protein phosphorylation is an important physiological control mechanism, and ion channels represent a diverse group of kinase substrates. It has been shown that membrane targeting of PKA through association with AKAPs facilitates PKA-mediated phosphorylation and regulation of AMPA-kainate and NMDA glutamate ion channels, skeletal and cardiac calcium channels, and calcium-activated potassium channels (8, 9, 10, 11). Interestingly, AKAP15/18, the anchoring protein that targets PKA to L-type calcium channels does so not only by membrane association through a lipid anchor but also by a possible direct interaction with Ca2+ channel α subunits (12, 13).

Inwardly rectifying potassium (Kir) channels are another group of ion channels whose modulation is influenced by the presence of AKAPs (14). Intrinsic gating and block of the Kir channel pore by intracellular magnesium and polyamines ensures that Kir channels permit K+ entry under hyperpolarization more readily that they permit K+ exit under depolarization (15, 16). This asymmetry in the current-voltage relation of the channel allows modification of the electrical properties of cells without excessive K+ loss. Consequently, Kir channels are involved in setting and maintaining the resting membrane potential, buffering extracellular K+, and the generation of prolonged action potentials in the heart and in fertilized egg cells (17). Kir channels are also regulated by protein phosphorylation and direct G-protein activation and thus play a role in the fine-tuning of cellular excitability (18, 19, 20). Cloning of members of the Kir family and examination of their primary sequences suggest the presence of multiple consensus phosphorylation sites (21, 22, 23), although experimental data for the involvement of kinase enzymes in the regulation of cloned Kir channel activity are contradictory, with evidence in favor as well as against a role for protein kinase A and/or protein kinase C (24, 25, 26).

Here, we present evidence that suggests that AKAP79 (recently renamed AKAP5), a multivalent-anchoring protein that binds PKA, PKC, and the protein phosphatase calcineurin (PP2B) (27, 28), is not only required for the reliable modulation
of the strong inward rectifying K⁺ channel, Kir2.1, but further that AKAP79 is targeted to the intracellular N and C domains of Kir2.1 to anchor kinases close to key channel phosphorylation sites.

**EXPERIMENTAL PROCEDURES**

**Antibodies, Polyacrylamide Gel Electrophoresis, and Immunoblotting**—The following primary antibodies were used: rabbit polyclonal HA-probe (Y-11; Santa Cruz Biotechnologies, Inc.); mouse monoclonal anti-AKAP79 (clone 22; BD Transduction Laboratories), Horseradish peroxidase-, fluorescein (FITC-) and Texas red-conjugated anti-rabbit and anti-mouse secondary antibodies from Jackson ImmunoResearch Laboratories, Inc. Protein extracts were resolved by SDS-polyacrylamide gel electrophoresis on 10% polyacrylamide-Tris gels and transferred electrophoretically onto nitrocellulose membranes (Hybond ECL, Amersham Pharmacia Biotech). Membranes were blocked overnight at 4 °C in blocking solution containing 5% (w/v) skim milk powder and 0.1% Tween 20 in Tris-buffered saline. Primary antibodies were diluted in blocking solution containing 1% skim milk powder and 0.1% Tween 20 in Tris-buffered saline. Primary antibodies were diluted in blocking solution containing 1% skim milk powder and 0.1% Tween 20 in Tris-buffered saline and incubated with the membranes for 2–3 h at room temperature. Membranes were washed in Tris-buffered saline and then incubated with horseradish peroxidase-conjugated secondary antibody for a further hour at room temperature. Labeled bands were visualized using enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech) according to the manufacturer's protocol.

**Cells and Cell Transfection**—Chinese hamster ovary (CHO), COS-7, and HEK-293 cells were grown in minimal essential medium (without nucleosides), Dulbeco's modified Eagle's medium, and minimal essential medium supplemented with 1% non-essential amino acids, respectively. Medium was supplemented with 10% (v/v) fetal bovine serum. All medium and reagents were from Life Technologies, Inc. No antibiotics were used. Cells were transiently transfected using FuGENE 6® transfection reagent (Roche Molecular Biochemicals) according to the manufacturer's protocol. Transfections were performed in six-well culture plates with cells at 50–80% confluence.

**Plasmids and DNA Constructs**—The Kir2.1 expression construct in pcDNA3 has been previously described (29). The AKAP79 expression construct in pcDNA3 was a generous gift from Dr. John Scott, Vollum Institute, Portland, OR. The enhanced green fluorescent protein-Kir2.1 construct in pcDNA3 was a generous gift from Dr. N. W. Davies (University of Leicester, UK). Electrodes were pulled from borosilicate glass (outer diameter 1.5 mm; Clarke Instruments, UK) and fire polished to give a final resistance of around 4 MΩ. Electrodes were back-filled with 2 M KCl or 2 M K2SO4. The pipette solution was saturated with 95% O2 and 5% CO2 and maintained at 37 °C. A 1.5-MΩ resistor was placed in series with the pipette to ground. Pipettes were filled with 0.25 M KCl. Cells were held at −200 mV and stimulated with 2–4 pulses of 50 ms at 1 Hz. The amplitude of the membrane potential change was digitized at 10 kHz using a DigiData 1200 interface (Axon Instruments) and analyzed using software written in AxoBasic (Axon Instruments) by Dr. N. W. Davies (University of Leicester, UK). The following day, the pipettes were replaced with fresh ones. The control experiments were performed at normal room temperature (18–22 °C) and the results are expressed as mean ± S.E. Statistical significance was evaluated using the Student's unpaired t test.

**Immunocytochemistry**—HEK-293 or COS-7 cells were plated onto six-well plates and transiently transfected with cDNA encoding AKAP79 and HA-tagged Kir2.1. Cells were fixed with 4% formaldehyde for 15 min and permeabilized with 0.1% Triton X-100. Following blocking, samples were incubated with rabbit polyclonal HA-probe (Y-11) or 5 μg of rabbit non-immune control serum (Santa Cruz Biotechnologies) for 2 h at room temperature, washed, and incubated for 15 min at 4 °C. 100 μl of prewashed goat anti-rabbit A-garose beads (Santa Cruz Biotechnologies) were added to the antibody/lysozyme mix and incubated with gentle inversion for 90 min at 4 °C. The protein-coupled agarose beads were then pelleted by gentle centrifugation and washed five times in PBS. Immunoprecipitated proteins were recovered in 2× loading buffer, resolved by 10% SDS-polyacrylamide gel electrophoresis, and analyzed by immunoblotting as described above.

**Electrophysiology**—Whole-cell currents were recorded from either CHO or COS-7 cells typically 24–48 h post-transfection using an Axopatch 200a amplifier (Axon Instruments). Currents recorded in response to 250 ms voltage steps at 5 kHz (~33 pulses/second) were digitized at 10 kHz using a DigiData 1200 interface (Axon Instruments), and analyzed using software written in AxoBasic (Axon Instruments) by Dr. N. W. Davies (University of Leicester, UK). The junction potential between pipette and extracellular solutions was sufficiently small (~1.5 mV) to be neglected. As far as possible, analogue means were used to correct capacity transients. Up to 90% compensation was routinely used to correct for series resistance. All experiments were performed at room temperature (18–22 °C), and the results are expressed as mean ± S.E. Statistical significance was evaluated using the Student’s unpaired t test.
AKAP Targeting to Inward Rectifier K⁺ Channels

Results

Coexpression of AKAP79 and Kir2.1 Has No Effect upon Channel Function—Membrane currents were recorded from CHO or COS-7 cells using the conventional whole-cell clamp technique typically 24–48 h after transfection with the cDNAs encoding Kir2.1 and AKAP79, or with Kir2.1 alone. To confirm the presence of AKAP79 within cells during electrophysiological recordings, AKAP79 was subcloned into the bicistronic expression vector pIRES2-EGFP (CLONTECH). This permits AKAP79 and EGFP to be translated simultaneously from the same mRNA transcript and allows visual selection of successfully transfected cells expressing AKAP79. The presence of Kir2.1 was confirmed by recording whole-cell Kir currents. For cells expressing Kir2.1 alone, we cotransfected the cDNA encoding Kir2.1 with the empty (no AKAP79) pIRES2-EGFP expression vector. Fig. 1A shows whole-cell currents recorded from a CHO cell transfected with both Kir2.1 and AKAP79. Currents were recorded in response to voltage steps from a holding potential of −35 mV (the K⁺ equilibrium potential, E_K) to test potentials ranging from +55 mV and −105 mV in 10 mV increments. Voltage steps positive to E_K elicited only small outward currents, whereas steps negative to E_K produced substantial inward currents. No significant whole-cell currents were detected in non-transfected cells. The presence of the kinase-anchoring protein, AKAP79 within the cells had no significant effect upon whole-cell Kir2.1 current amplitudes (Fig. 1B) or voltage-dependent properties of Kir channel currents, such as the relationship between whole-cell conductance and voltage (Fig. 1C), or the time constant of Kir2.1 channel gating (Fig. 1D). This suggests that basal Kir2.1 channel activity is unaffected by coexpression with AKAP79.

AKAP79 Enhances the Response of the Kir2.1 to cAMP but Only in the Presence of Phosphatase Inhibitors—Preliminary results indicated that elevation of intracellular CAMP had little effect upon whole-cell Kir2.1 currents either in the presence or the absence of AKAP79 (Fig. 2, A and B). In these experiments, whole-cell currents recorded at −80 mV from cells expressing AKAP79 and Kir2.1 increased by only 13.4 ± 1.9% (mean ± S.E.; n = 6) during a 5-min exposure to 100 μM intracellular CAMP. Cells expressing the channel alone showed a similar 11.1 ± 2.8% (n = 6) increase in whole-cell current over the same time interval. It has previously been reported that CAMP-dependent phosphorylation of L-type Ca²⁺ channel subunits in HEK-293 can only be detected in the presence of phosphatase inhibitors (10). We therefore tested whether the phosphatase inhibitors okadaic acid (an inhibitor of protein phosphatases 1 and 2A) and cypermethrin (a potent inhibitor of calcineurin, phosphatase 2B) augmented the effect of CAMP on Kir2.1 currents. Following a 30–40-min pretreatment of the cells in 200 nM okadaic acid and 200 nM cypermethrin, CHO cells expressing both AKAP79 and Kir2.1 showed a 25.6 ± 3.6% (n = 5) increase in whole-cell Kir2.1 current over a 5-min period when exposed to 100 μM intracellular CAMP (Fig. 2, C and D). In contrast, cells transfected with Kir2.1 alone showed an 8.1 ± 2.5% (n = 5) increase in Kir2.1 current when exposed to 100 μM CAMP over the same period.

In the presence of phosphatase inhibitors and AKAP79, the increase in whole-cell Kir current in response to elevated intracellular CAMP was reflected in a 10 mV rightward shift in the relationship between chord conductance and voltage (Fig. 3). The voltage at which the chord conductance was half-
maximal ($V_{0.5}$) shifted from $V_{0.5} = -35.1 \pm 1.0$ mV in control to $V_{0.5} = -25.0 \pm 0.8$ mV in cAMP-exposed cells expressing AKAP79 and Kir2.1 (Fig. 3D). The conductance-voltage relation of cells expressing Kir2.1 alone shifted from $V_{0.5} = -34.3 \pm 1.0$ mV in control to $V_{0.5} = -29.0 \pm 1.2$ mV in cAMP-exposed cells.

AKAP79 coimmunoprecipitates with HA-tagged Kir2.1—The results of the functional studies outlined above indicate that AKAP79 facilitates cAMP-induced modulation of Kir2.1, presumably by anchoring PKA in close proximity to the ion channel. To establish whether any physical interaction between Kir2.1 and AKAP79 occurs within intact cells, coimmunoprecipitation experiments were performed. COS-7 cells were transfected with cDNAs encoding AKAP79 and HA-tagged Kir2.1. HA-tagged Kir2.1 channels were found to be functionally indistinguishable from untagged wild-type Kir2.1 channels (data not shown). Antibodies directed against the HA epitope tag on Kir2.1 were able to immunoprecipitate AKAP79 (Fig. 4), suggesting that Kir2.1 and AKAP79 exist in a complex within cells.

AKAP79 interacts with the intracellular N and C domains of Kir2.1—Kir channels have a relatively simple structure compared with Na$^+$ and Ca$^{2+}$ channels and members of the voltage-gated K$^+$ (Kv) channel family. Each subunit in the Kir tetramer consists of an intracellular N and C terminus and two membrane-spanning domains (M1 and M2) separated by a short stretch of amino acids (the H5 or P-region) that dips back into the membrane from the extracellular side to form the selectivity filter and channel pore (21, 22). The only intracellular portions of the channel subunit are found at the N terminus (residues 1–71) before the channel protein disappears into the membrane to form M1, and at the C terminus when the protein emerges from M2 (182–428). It is therefore relatively straightforward to create GST fusion proteins of the intracellular portions of the Kir channel and test for direct interaction with AKAP79 in pull-down assays.

To identify possible interactions, GST fusion proteins of the intracellular N (residues 1–71) and C (302–428) terminal domains of Kir2.1 were screened for their ability to bind AKAP79 from lysates of COS-7 cells transfected with cDNA encoding AKAP79. GST fusion proteins of both the N- and C-domains of Kir2.1 were able to isolate AKAP79 from COS-7 lysates, whereas GST alone failed to interact with AKAP79 (Fig. 5A).

It has recently been reported that AKAP79 binds to the Src homology 3 (SH3) and guanylate kinase-like (GK) domains of the PDZ protein, PSD-95/SAP90 (31). The C terminus of Kir2.1 contains the conserved PDZ-domain recognition motif (XS/TiXIVf) that mediates binding to members of the PSD-95/SAP90 protein family. Whereas PSD-95 is not present in either COS-7 cells or Escherichia coli, where the GST fusion proteins were originally expressed (see "Experimental Procedures"), we felt it important to test whether AKAP79 binds to Kir2.1 via interaction with an unidentified, endogenous PDZ protein from these cells. For this purpose we constructed a shortened C-terminal GST fusion protein encoding just the last eight C-terminal amino acids of Kir2.1 (421–428). This shortened protein emerges from M2 (182–428).
GST-C fusion protein, GST-C-(421–428), which includes the Kir2.1 PDZ binding motif, failed to isolate AKAP79, suggesting that AKAP79 binds directly to the intracellular C-domain of Kir2.1 at some point(s) between residues 302 and 421 and not via a C-terminal PDZ protein (Fig. 5B).

AKAP79 and Kir2.1 Are Both Localized to the Plasma Membrane of HEK-293 Cells—Fig. 6 (A and B) shows confocal images of HEK-293 cells that have been transfected with HA-Kir2.1 and AKAP79, stained with antibodies against the HA epitope tag and AKAP79, and visualized with secondary antibodies conjugated with the non-overlapping fluoroprobes, FITC (Fig. 6A) and Texas Red (Fig. 6B), respectively. The images show both proteins to be localized to the plasma membrane, demonstrating proximity between Kir2.1 and AKAP79 within intact cells. Interestingly, we were unable to use EGFP-tagged Kir2.1 channels to monitor Kir2.1 distribution, as the GST fusion proteins bound to glutathione-Sepharose beads were incubated with cell lysates. Bound proteins were resolved by SDS-polyacrylamide gel electrophoresis, transferred onto nitrocellulose membrane, and immunoblotted with anti-AKAP79. 10% of cell lysate was run in the extract lane. Membranes were stained for protein to ensure that equivalent amounts of each GST fusion protein were used in each pull-down assay.

Fig. 5. AKAP79 binds to the N and C regions of Kir2.1. A, GST fusion proteins of the intracellular N (residues 1–71) and C (residues 302–428) domains of Kir2.1 were screened for their ability to isolate AKAP79 from lysates of COS-7 cells transfected with cDNA encoding AKAP79. GST fusion proteins bound to glutathione-Sepharose beads were incubated with cell lysates. Bound proteins were resolved by SDS-polyacrylamide gel electrophoresis, transferred onto nitrocellulose membrane, and immunoblotted with anti-AKAP79. AKAP79 was specifically retained by GST-C-(421–428) but not control GST. 10% of the cell lysate was run in the input lane. Membranes were stained for protein to ensure that equivalent amounts of each GST fusion protein were used in each pull-down assay. B, GST fusion proteins of the final eight C-terminal amino acids of Kir2.1 (GST-C(302–428)) failed to retain AKAP79.
presence of AKAP79 seemed to quench the EGFP-Kir2.1 fluorescence. Fig. 6 (C and D) show typical confocal images of a HEK-293 cell transfected with EGFP-tagged Kir2.1 (EGFP attached to the N terminus of Kir2.1) and AKAP79. The cell has been permeabilized and stained with anti-AKAP79. EGFP fluorescence is almost undetectable (Fig. 6C). AKAP79 distribution within the same cell is shown in Fig. 6D. The reduction in EGFP fluorescence is not a result of cell permeabilization because permeabilized HEK-293 cells expressing EGFP-Kir2.1 alone retain their brightness (Fig. 6E). Nor is the fluorescence reduction simply because of the coexpression of AKAP79 and EGFP, because cells transfected with EGFP and AKAP79 were indistinguishable from cells transfected with EGFP alone (data not shown). Rather, the effect of reduced fluorescence seems to be a side effect of the association of Kir2.1 and AKAP79. EGFP fluorescence is known to be sensitive to a number of factors, particularly pH, and it seems possible that the anchoring of AKAP79 and its associated proteins may change some factor in the very local environment of the ion channel that affects the fluorescence of the attached EGFP molecule.

**DISCUSSION**

Our findings suggest that the protein kinase A-anchoring protein, AKAP79, associates directly with intracellular regions of the strong inwardly rectifying K⁺ channel, Kir2.1 to anchor PKA close to channel phosphorylation sites. The primary sequence of Kir2.1 suggests the presence of one putative PKA phosphorylation site at position Ser-426 on the C terminus and four putative PKC phosphorylation sites, two on the N terminus (Ser-3, Thr-6) and two on the C terminus (Ser-357, Thr-383). AKAP79, in common with many adaptor proteins, binds several different enzymes, in this case PKA, PKC and the Ca²⁺ calmodulin-dependent protein phosphatase-2B, calcineurin (27, 28). Whereas activators of PKC had no discernable effect upon Kir2.1 currents either in the presence or in the absence of AKAP79, elevation of intracellular cAMP caused a marked increase in whole-cell Kir2.1 current in the presence but not in the absence of AKAP79. Interestingly, the effects of elevated cAMP were only observed in cells pretreated with the phosphatase inhibitors okadaic acid and cypermethrin. Phosphatase anchoring via association with AKAP79 could potentially increase phosphatase activity close to the channel, although cypermethrin, which specifically inhibits calcineurin with an IC₅₀ in the subnanomolar range, was found to be largely ineffective on its own (data not shown). Alternatively, the reliance on phosphatase inhibitors may simply reflect a relatively high endogenous phosphatase activity within these cell systems and suggests that channel phosphorylation and dephosphorylation may be a fast, dynamic process. There has been a report of similar findings for cloned L-type Ca²⁺ channels expressed in HEK-293 cells where phosphorylation of the α₁c channel subunit was only observed in the presence of the phosphatase inhibitors, okadaic acid and FK506, both being ineffective on their own (10). This and other studies highlight one of the features of heterologous expression systems, which while offering the advantage of a controlled experimental environment, often do not mimic the native conditions needed for ion channel modulation. For example, the weak inward rectifying K⁺ channel Kir1.1 (ROMK1) is predominantly expressed in the kidney where it plays an important role in K⁺ homeostasis (22). The anti-diuretic hormone, vasopressin is known to enhance the activity of native Kir1.1 channels in the kidney via a cAMP-dependent pathway (32, 33). When cloned Kir1.1 channels are expressed in intact Xenopus oocytes, however, the channels appear insensitive to elevation of intracellular cAMP, unless coexpressed with the kinase-anchoring protein, AKAP79 (14). Overlay assays using the AKAP-binding RII subunit of PKA as a probe show the presence of a 100–120 kDa RII-binding protein in kidney membranes, which is absent in oocytes. This suggests that cAMP-induced modulation of Kir1.1 relies upon an anchored pool of PKA, which is not naturally present within oocyte membranes.

The functional effects on channel activity of coexpressing AKAP79 and Kir2.1 suggest that AKAP79 also facilitates the phosphorylation of Kir2.1 by anchoring PKA close to the ion channel. The low molecular weight anchoring protein AKAP15/18 has been shown to target PKA to L-type Ca²⁺ channels by membrane association through myristoylation and palmitoylation of residues at its N terminus and, possibly, by direct interaction with Ca²⁺ channel subunits (12, 13). AKAPs contain at least two functional domains: an amphipathic helix that binds the regulatory (RII) subunits of the PKA holoenzyme (5, 6) and a specialized targeting region that tethers the AKAP-PKA complex to specific intracellular sites (7). Early studies suggested that AKAP79 targeting was mediated through interactions with cytoskeletal proteins (34, 35), although more recent studies suggest that AKAP79 is anchored to the plasma membrane through direct binding of basic domains on the protein to acidic phospholipids, including the
phosphoinositide PtdIns(4,5)P$_2$ (36). While the primary target of AKAP79 is the plasma membrane, there may be a subsidiary interaction with intracellular regions of channel proteins to create a channel/AKAP complex that could enhance the speed and specificity of PKA-induced phosphorylation. Coimmunoprecipitation of AKAP79 with HA-tagged Kir2.1 certainly suggests that these two proteins exist in a complex within intact cells. Kir channels have a relatively simple structure when compared with Na$^+$ and Ca$^{2+}$ channels, and K$_v$ channels, and this makes it possible to create GST fusion proteins of the entire intracellular portion of the Kir channel and test for direct interaction with AKAP79. GST pull-down assays demonstrate that AKAP79 binds to both the N and C intracellular domains of Kir2.1. Interaction between AKAPs and their target proteins at multiple sites is not unprecedented because AKAP79 has recently been shown to bind to the $\beta$$_2$-adrenergic receptor through sites on both the third intracellular loop and at the C-terminal tail (37).

AKAP79 also binds to the Src homology 3 (SH3) and guanylate kinase-like (GK) domains of the membrane-associated guanylate kinase (MAGUK) proteins, PSD-95 and SAP97. This recruits PKA to ionotropic glutamate receptors because PSD-95 interacts with NR2B subunits of the NMDA receptor, and SAP97 binds to GluR1 subunits of AMPA receptors (31). The C-terminus of Kir2.1 contains the PDZ-domain recognition motif (X(S/T)(V/I)) that mediates binding to the PSD-95/SAP90 protein family, and Kir2.1 has been shown to interact with PSD-95 in yeast two-hybrid screens and heterologous expression systems (38). The C-terminal AKAP binding we detect for Kir2.1 is unlikely to be via interaction with a PDZ protein because GST-C(421–428), a shortened C-terminal fusion protein harboring the PDZ motif, failed to isolate AKAP79 in pull-down assays. Nevertheless, the ability of AKAP79 to bind to PSD-95 and directly to intracellular regions of Kir2.1 may offer additional stability to protein partner complexes containing the ion channel, PDZ protein and AKAP. It is also notable that NMDA receptors retain the option of recruiting PKA through the simultaneous association of two separate anchoring proteins: AKAP79, which binds to the receptor via PSD-95 (31) and yotiao, which binds directly to the C-terminal C1 exon cassette of the NMDA receptor (9). Because the native AKAP partner of Kir2.1 has yet to be identified, it is tempting to speculate that a similar situation may exist for K$^+$ channels.

In conclusion, we present evidence for the direct association of a protein kinase Aanchoring protein with a K$^+$ channel. This is potentially important given the unique role of K$^+$ channels in shaping the electrical properties and firing patterns of cells, and the profound effects that K$^+$ channel modulation has upon cellular excitability. It seems increasingly likely that in vivo K$^+$ channels, like Ca$^{2+}$ channels and ionotropic glutamate receptors, exist in highly organized complexes made up of AKAPs and associated signaling proteins that ensure rapid and efficient phosphorylation of the channel protein in response to localized cell signals.

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