Dopaminergic Modulation of Voltage-Gated Na^+ Current in Rat Hippocampal Neurons Requires Anchoring of cAMP-Dependent Protein Kinase

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Activation of D1-like dopamine (DA) receptors reduces peak Na^+ current in acutely isolated hippocampal neurons via a modulatory mechanism involving phosphorylation of the Na^+ channel α subunit by cAMP-dependent protein kinase (PKA). Peak Na^+ current is reduced 20–50% in the presence of the D1 agonist SKF 81297 or the PKA activator Sp-5,6-dichloro-I-β-D-ribofuranosyl benzimidazole-3′,5′-cyclic monophosphorothionate (cBIMPS). Co-immunoprecipitation experiments show that Na^+ channels are associated with PKA and A-kinase-anchoring protein 15 (AKAP-15), and immunocytochemical labeling reveals their co-localization in the cell bodies and proximal dendrites of hippocampal pyramidal neurons. Anchoring of PKA near the channel by an AKAP, which binds the RII α regulatory subunit, is necessary for Na^+ channel modulation in acutely dissociated hippocampal pyramidal neurons. Intracellular dialysis with the anchoring inhibitor peptides Ht31 from a human thyroid AKAP and AP2 from AKAP-15 eliminated the modulation of the Na^+ channel by the D1-agonist SKF 81297 and the PKA activator cBIMPS. In contrast, dialysis with the inactive proline-substituted control peptides Ht31-P and AP2-P had little effect on the D1 and PKA modulation. Therefore, we conclude that modulation of the Na^+ channel by activation of D1-like DA receptors requires targeted localization of PKA near the channel to achieve phosphorylation of the α subunit and to modify the functional properties of the channel.

Key words: Na^+ current; neuromodulation; cAMP-dependent protein kinase; A-kinase-anchoring protein; hippocampus; dopamine receptors; phosphorylation

Voltage-gated Na^+ current is the primary inward current underlying excitability in the hippocampus and throughout the CNS. The α subunit of the brain voltage-gated Na^+ channel is a target for phosphorylation by cAMP-dependent protein kinase (PKA) at multiple consensus sites on the intracellular loop between domains I and II (Costa et al., 1982; Costa and Catterall, 1984; Rossie and Catterall, 1987, 1989; Rossie et al., 1987). PKA activation reduces peak Na^+ current amplitude in cultured rat brain neurons and in mammalian cells (Li et al., 1992) or *Xenopus* oocytes (Gershon et al., 1992; Smith and Goldin, 1996, 1997) expressing rat brain Na^+ channels. Similarly, activation of D1-like dopamine (DA) receptors, which couple to the stimulation of adenylyl cyclase, decreases endogenous Na^+ current in acutely isolated striatonigral and hippocampal neurons (Surmeier et al., 1992; Schiffmann et al., 1995; Cantrell et al., 1997). This modulatory effect requires direct phosphorylation of the Na^+ channel α subunit by PKA at Ser-573 on the intracellular loop connecting domains I and II (Cantrell et al., 1997; Smith and Goldin, 1997). This modulation occurs very rapidly, suggesting that PKA may be concentrated near the channel via a targeting mechanism.

The type II PKA holoenzyme is a heterotetramer consisting of a dimer of regulatory subunits, each of which binds to and inactivates one catalytic subunit. After binding of cAMP, the regulatory subunit dimer reversibly releases activated catalytic subunits, which phosphorylate target proteins (Krebs and Beavo, 1979; Taylor, 1989). An important factor in determining the specificity of PKA signaling is localization and anchoring of the kinase in the vicinity of the protein to be phosphorylated. Numerous A-kinase anchoring proteins (AKAPs) have been described, which anchor PKA near target proteins by binding to the RII regulatory subunits (Rubin, 1994; Dell'Acqua and Scott, 1997; Murphy and Scott, 1998). AKAPs possess a conserved amphipathic helix that binds to the RII dimer and an additional unique targeting domain that mediates localization of the kinase to specific subcellular targets within cells (Carr et al., 1991, 1992). Recent experiments have identified a novel AKAP, AKAP-15, which is associated with skeletal muscle Ca^{2+} channels (Gray et al., 1997). AKAP-15 has an N-terminal lipid anchor, which targets it to the plasma membrane and an amphipathic alpha helix, which binds PKA (Gray et al., 1998a). It associates with Ca^{2+} channels in skeletal muscle fibers and in transfected cells (Gray et al., 1998a), and it is implicated in cAMP-dependent modulation of calcium channels in skeletal muscle cells (Gray et al., 1998a) and

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in regulation of calcium channels and insulin release from pancreatic β cells (Lester et al., 1997; Fraser et al., 1998).

Because AKAPs are abundantly expressed in the CNS, we hypothesized that anchoring of PKA near the Na^+ channel protein might be required for D1-like DA receptor modulation of the Na^+ current. In agreement with this idea, AKAP-15 was recently isolated from partially purified preparations of brain Na^+ channels (Tibbs et al., 1998). PKA activity co-purifies and co-immunoprecipitates with the brain Na^+ channel, suggesting that PKA is physically associated with the channel (Tibbs et al., 1998). AKAP-15 is the prominent AKAP in these preparations and is likely to be involved in targeting PKA to the brain Na^+ channel (Tibbs et al., 1998). In the experiments described here, we provide further evidence for association and co-localization of the RIIα regulatory subunit of PKA and AKAP-15 with brain sodium channels, and we demonstrate that localization of the kinase near the Na^+ channel is required for D1- and PKA-dependent modulation of the voltage-gated Na^+ current in rat hippocampal pyramidal neurons.

MATERIALS AND METHODS

Co-immunoprecipitation and phosphorylation of the Na^+ channel. Rat brain sodium channels were purified as described by Hartshorne and Catterall (1984). Approximately 10 pmol of these partially purified sodium channels were incubated at 0°C with 10 μg of affinity-purified anti-RIIα antibody (kindly provided by Dr. Stanley McKnight, Department of Pharmacology, University of Washington) or control antibody for 2 hr at 4°C in a final volume of 0.5 ml in 50 mM Tris-HCl, pH 7.4, 75 mM NaCl, 2.5 mM EDTA, 0.1% Triton X-100 (bRIA buffer). In some cases, purified sodium channel was first preincubated with 0.2 mM Ht31 or 0.2 mM Ht31-P peptide (Carr et al., 1991, 1992) for 30 min at room temperature in 0.5 ml bRIA buffer containing aprotinin (10 μg/ml), leupeptin (10 μg/ml), pepstatin A (1 μM), benzamidine (15.7 μg/ml), and 4-(aminoethyl)benzenesulfonyl fluoride (1 mM) before incubation with 10 μg of affinity-purified anti-RIIα antibody. Preswollen protein A-Sepharose (2–5 mg) beads were added to each 0.5 ml reaction and incubated 0.5 hr at 4°C. Protein A-Sepharose beads containing precipitated complexes were sedimented by centrifugation for 5 sec at 2000 $\times$ *g* and washed three times with 1 ml bRIA buffer and twice with 50 mM Tris-HCl, pH 7.5, 10 mM magnesium chloride, 1 mM EGTA, and 0.1% Triton X-100 (phosphorylation buffer). Immunoprecipitated samples were incubated for 15 min at 37°C in 0.05 ml of phosphorylation buffer containing 0.25 μM PKA and 0.1 mM [γ-^{32}P]ATP (0.005 mCi/mmol). The precipitated complexes were then washed three times with 1 ml of phosphorylation buffer, and proteins were eluted by incubation in SDS-sample buffer at 65°C for 10 min and separated on 6% Tris-glycine polyacrylamide gels. γ-^{32}P-labeled phosphoproteins were detected by autoradiography.

Immunocytochemical detection of AKAP-15 in the hippocampus. Single-label immunocytochemical studies in rat brain were performed using the methods described previously (Westenbroek et al., 1998). Anti-AP1 antibodies (Gray et al., 1997) were affinity-purified, diluted 1:20 in a solution containing 0.1% Triton X-100 and 1.0% normal goat serum in 0.1 M Tris-buffered saline, and used to detect AKAP-15. Bound anti-AP1 antibodies were visualized with biotinylated goat anti-rabbit IgG and fluorescein-labeled avidin, and the sections were examined in a Bio-Rad (Hercules, CA) MRC-600 confocal microscope. Control sections incubated in normal rabbit serum or with no primary antibody showed no specific staining.

Acute dissociation of hippocampal neurons. Hippocampal neurons from adult (>25 d postnatal) male rats were acutely isolated using procedures previously described (Bargas et al., 1994; Howe and Surmeier, 1995; Cantrell et al., 1996). In brief, rats were decapitated under metofane anesthesia. Brains were then quickly removed, iced, and blocked before slicing. Four hundred to 500 μm slices were cut and transferred to a low-calcium (100 μM), HEPES-buffered saline solution containing (in mM): 140 Na isethionate, 2 KCl, 4 $MgCl_2$, 0.1 $CaCl_2$, 23 glucose, 15 HEPES, pH 7.4 (300–305 mOsm/l). All solutions were bubbled with 100% O_2 before slicing. Slices were then incubated for 1–6 hr in $NaHCO_3$-buffered Earle's balanced salt solution (Sigma, St. Louis, MO) bubbled with 95% O_2 and 5% CO_2, pH 7.4 (300–305 mOsm/l). Single slices were removed into the low-calcium buffer, and with the aid of a dissecting microscope, regions of hippocampus were removed and placed in a treatment chamber containing protease type XIV (Sigma; 0.7 mg/ml) in HEPES-buffered HBSS (Sigma) at 35° C, pH 7.4 (300–305 mOsm/l). After 5–10 min of enzyme treatment, the tissue was rinsed several times in the low-calcium buffer and mechanically dissociated using a series of fire-polished Pasteur pipettes. The cell suspension was then plated into a 35 mm tissue culture dish (Corning, Corning, NY) mounted on the stage of an inverted microscope containing 1 ml of HEPES-buffered phosphate-free HBSS saline. After allowing the cells to settle (~5 min), the solution bathing the cells was changed to normal external recording solution.

Whole-cell recording. Whole-cell currents were recorded from pyramidally shaped hippocampal neurons that had at most one or two short processes (Hamill et al., 1981; Bargas et al., 1994; Howe and Surmeier, 1995). Electrodes were pulled from 75 μl micropipette glass (VWR Scientific, West Chester, PA) and fire-polished before use. The external recording solution consisted of (in mM): 20 NaCl, 10 HEPES, 1 $MgCl_2$, 0.4 $CdCl_2$, 55 CsCl, 5 $BaCl_2$, and 80 glucose, pH 7.3, with NaOH (300–305 mOsm/l). The internal recording solution consisted of (in mM): 189 *N*-methyl D-glucamine, 40 HEPES, 4 $MgCl_2$, 1 NaCl, 0.1 BAPTA, 25 phosphocreatine, 2–4 Na_2ATP, 0.2 Na_3GTP, and 0.1 leupeptin, pH 7.2, with H_2SO_4 (270–275 mOsm/l). SKF 81297 (Research Biochemicals, Natick, MA) was prepared as a fresh concentrated stock in water and frozen in aliquots before use. Sp-5,6-DClcBIMPS (cBIMPS; BioLog, La Jolla, CA) was prepared as a concentrated stock in DMSO and diluted before use. Appropriate vehicle controls were performed where necessary.

Electrode resistances were typically 3–6 MΩ in the bath. Final series resistance values averaged 6–8 MΩ, of which >80% was compensated electronically. Series resistance compensation did not change significantly during the course of the experiments. Recordings were obtained using an Axon Instruments (Foster City, CA) 1C patch clamp. Voltage pulses were delivered and currents recorded using a personal computer running Basic-FASTLAB software to control an analog-to-digital/digital-to-analog interface (IDA; Indec Systems, Capitola, CA).

Drugs were applied using a gravity-fed "sewer pipe" system. The array of application capillaries (~150 mm inside diameter) was positioned a few hundred micrometers away from the cell under study. Solution changes were made by altering the position of the array with a DC drive system controlled by a microprocessor-based controller (Newport-Klinger, Inc., Irvine, CA). Complete solution changes were achieved within <1 sec as judged by TTX block and changes in reversal potential.

Data analysis. Data were collected using standard voltage step protocols. Least-squares curve fitting and statistical analysis were done using Sigma Plot (Jandel Scientific, Corte Madera, CA). Statistics are presented as means ± SEM.

RESULTS

Activation of D1-like DA receptors, which are coupled to the stimulation of adenylyl cyclase, decreases the Na^+ current in acutely isolated hippocampal neurons via phosphorylation of the α subunit by PKA (Cantrell et al., 1997). A low molecular weight AKAP, AKAP-15, has been identified recently and demonstrated to bind to the brain Na^+ channel, suggesting that anchoring of PKA may be an important element of this signaling pathway (Tibbs et al., 1998). To further demonstrate a physical association between PKA and the Na^+ channel α subunit, the complex of RIIα and Na^+ channels was co-immunoprecipitated using anti-RIIα antibody, and the immunoprecipitated Na^+ channels were then radiolabeled by phosphorylation in the presence of PKA and [γ-^{32}P]ATP and analyzed by SDS-PAGE. Co-immunoprecipitation of the Na^+ channel was observed with anti-RIIα antibody but not with preimmune IgG (Fig. 1*A*). Co-immunoprecipitation was blocked by preincubating channel preparations with the Ht31 anchoring inhibitor peptide, whereas preincubation with a control peptide (Ht31-P) had no effect (Fig. 1*A*). The phosphoprotein of ~190 kDa present in the Ht31 and Ht31-P experiments is a proteolytically cleaved fragment of the α subunit generated during incubation with the peptides at room

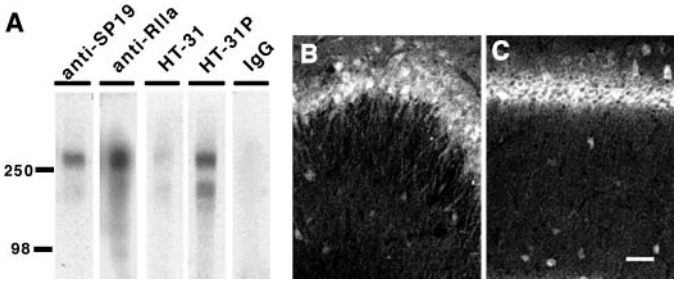

Figure 1. Brain Na^+ channel co-immunoprecipitates with RIIα, and AKAP-15 is localized in hippocampal pyramidal neurons. *A,* RIIα was immunoprecipitated from purified sodium channel preparations with anti-RIIα antibody or nonimmune IgG, phosphorylated by PKA, and analyzed by SDS-PAGE and immunoblotting as described in Materials and Methods. Where indicated, purified sodium channel was first preincubated with 0.2 mM Ht31 or 0.2 mM Ht31-P peptide for 30 min at room temperature before immunoprecipitation with 10 μg of affinity-purified anti-RIIα antibody. A control reaction using 10 μg of an α subunit-specific antibody, anti-SP19, was also performed. Molecular mass markers are represented as $M_r \times 10^{-3}$. *B,* Localization of AKAP-15 in the cell bodies and proximal dendrites of pyramidal neurons in the CA3/CA2 regions of the hippocampus. *C,* AKAP-15 localization in the CA1 region of the hippocampus. Scale bar, 100 μm.

temperature despite the presence of protease inhibitors. These co-immunoprecipitation experiments provide further evidence for physical association between the Na^+ channel and the RIIα subunit of PKA.

Sodium channels have been shown previously to be localized in low density in the cell body of hippocampal pyramidal neurons and in higher density in the mossy fibers and axons of the fimbria, fornix and perforant path (Westenbroek et al., 1989, 1992). Immunocytochemical studies were performed to determine whether AKAP-15 is also present in the cell bodies of these neurons, from which we have made electrophysiological recordings. Using AP1, an antibody that recognizes AKAP-15 (Gray et al., 1997), we observed labeling of the cell bodies of pyramidal neurons located in the CA3/CA2 (Fig. 1*B*) and the CA1 (Fig. 1C) regions of the hippocampus. Low levels of labeling were observed in the proximal dendrites of neurons located in the CA3/CA2 regions (Fig. 1*B*), but axon tracts were not labeled. These experiments provide evidence that Na^+ channels and AKAP-15 are localized together in the cell bodies and proximal dendrites of hippocampal pyramidal neurons but not in the major axon tracts in the hippocampus.

To determine whether anchoring of PKA near the Na^+ channel is necessary for modulation of Na^+ currents in hippocampal neurons via the DA/cAMP pathway, we used the peptides Ht31 (Carr et al., 1991, 1992) and AP2 (Gray et al., 1998a). These peptides span the RII binding domain of human thyroid AKAP Ht-31 and brain AKAP-15, respectively. Dialysis with these peptides disrupts the interaction between the kinase and the AKAP by blocking the binding domain of the AKAP, resulting in disruption of kinase targeting and localization (Carr et al., 1991). Proline-substituted versions of these peptides (Ht31-P and AP2-P), which do not bind RII and have no effect on the anchoring ability of the kinase, are used as negative controls. If D1-like DA receptor modulation of the Na^+ current requires anchoring of the kinase near the Na^+ channel α subunit, the modulation should be eliminated in neurons dialyzed with Ht31 or AP2 peptides but not in cells dialyzed with control solution or with the proline-substituted control peptides.

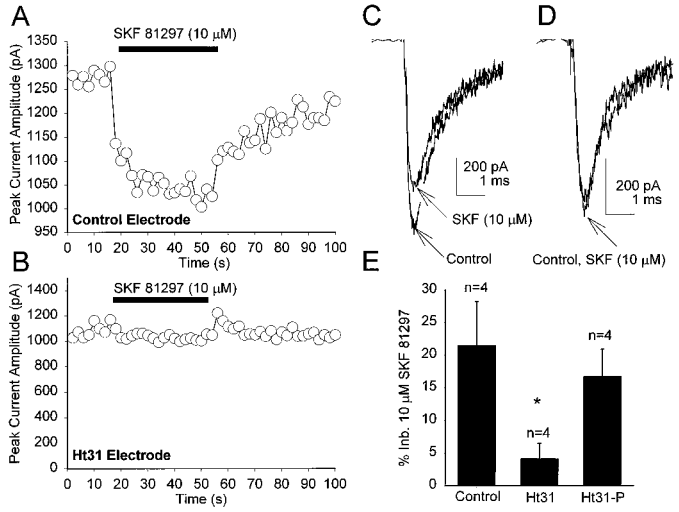

Figure 2. D1-like DA receptor modulation of the Na^+ channel in the rat hippocampus is prevented by dialysis with Ht31 peptide. *A, B,* Plot of peak, Na^+ current elicited by a test pulse to 0 mV from a holding potential of −70 mV versus time in control and in the presence of the D1 agonist SKF 81297 (10 μM), as indicated by the *black bar* for a neuron dialyzed with control solution (*A*) or 500 μM Ht31 peptide (*B*). *C, D,* Representative whole-cell current traces in control extracellular solution or SKF 81297 (smaller trace) from either a cell dialyzed with control solution (*C*) or 500 μM Ht31 (*D*). *E,* Statistical summary of the percentage reduction in peak current for cells dialyzed with control solution, 500 μM Ht31, or 500 μM Ht31-P. *Statistical significance; Student's *t* test, $p < 0.05$.

As illustrated in Figure 2, the ability of the D1-like DA receptor agonist SKF 81297 to modulate the whole-cell Na^+ current was eliminated by dialysis with 500 μM Ht31. Whole-cell Na^+ current was elicited by a test pulse to 0 mV from a holding potential of −70 mV. After stabilization of the current amplitude, SKF 81297 was applied via rapid perfusion, and its effect on the whole-cell current was recorded. As previously reported (Cantrell et al., 1997), application of SKF 81297 (10 μM) resulted in a rapid reduction in the magnitude of the peak current in cells dialyzed with control internal solution (Fig. 2*A,C,E*; 21.5 ± 6.7%; $n = 4$). This modulation occurred without significant changes in the kinetics or voltage dependence of the current. In cells dialyzed with 500 μM Ht31, the reduction in peak current in response to SKF 81297 was greatly attenuated (Fig. 2*B,D,E*; 4.2 ± 2.3%; $n = 4$; $p < 0.05$). In contrast, cells dialyzed with the proline-substituted Ht31-P peptide (500 μM) responded nearly normally to the application of SKF 81297 (Fig. 2*E*; 16.8 ± 4.0%;$n = 4$; $p > 0.05$). Because 10 μM SKF 81297 is sufficient to maximally activate D1-like DA receptors (Cantrell et al., 1997), this result indicates that anchoring by AKAPs is required for modulation of voltage-gated Na^+ channels by the D1-like DA receptor pathway, even when the DA receptor is fully activated.

To directly activate anchored PKA, modulation by the membrane-permeant activator cBIMPS (50 μM) was also studied (Fig. 3). The reduction in peak Na^+ current caused by direct activation of PKA was attenuated similarly to modulation by SKF 81297 in the presence of the Ht31 peptide (Fig. 3). These data indicate that anchoring of the kinase near the Na^+ channel plays an important role in mediating cAMP-dependent modulation of the Na^+ current in rat hippocampal neurons after activation of D1-like DA receptors.

The novel brain AKAP, AKAP-15, targets PKA to voltage-gated brain Na^+ channels as well as voltage-gated skeletal muscle

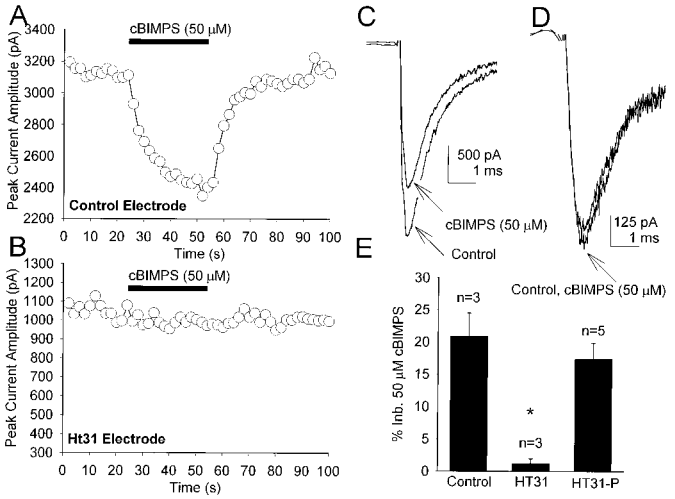

Figure 3. Modulation of the Na^+ channel by the PKA activator cBIMPS in the rat hippocampus is prevented by dialysis with Ht31 peptide. *A, B,* Plot of peak current elicited by a test pulse to 0 mV from a holding potential of −70 mV versus time in control and in the presence of the PKA activator cBIMPS (50 μM), as indicated by the *black bar* for a neuron dialyzed with control solution (*A*) or 500 μM Ht31 peptide (*B*). *C, D,* Representative whole-cell current traces in control extracellular solution or cBIMPS (*smaller trace*) from a cell dialyzed with either control solution (*C*) or 500 μM Ht31 (*D*). *E,* Statistical summary of the percentage reduction in peak current for cell dialyzed with control solution, 500 μM Ht31, or 500 μM Ht31-P. *Statistical significance; Student's *t* test, $p < 0.05$.

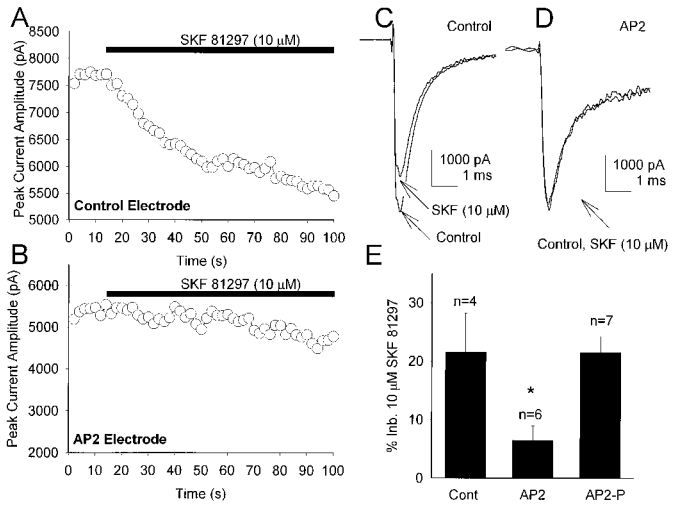

Figure 4. D1-like DA receptor modulation of the Na^+ channel in the rat hippocampus is prevented by dialysis with AP2 peptide. *A, B,* Plot of peak current elicited by a test pulse to 0 mV from a holding potential of −70 mV versus time in control and in the presence of the D1 agonist SKF 81297 (10 μM), as indicated by the *black bar* for a neuron dialyzed with control solution (*A*) or 500 μM AP2 peptide (*B*). *C, D,* Representative whole-cell current traces in control extracellular solution or SKF 81297 (*smaller trace*) from a cell dialyzed with either control solution (*C*) or 500 μM AP2 (*D*). *E,* Statistical summary of the percentage reduction in peak current for cells dialyzed with control solution, 500 μM AP2, or 500 μM AP2-P. *Statistical significance; Student's *t* test, $p < 0.05$.

calcium channels (Gray et al., 1997, 1998a; Tibbs et al., 1998). A PKA-binding peptide containing the amphipathic helix derived from AKAP-15 (AP2) blocks voltage- and PKA-dependent potentiation of Ca^{2+} channel activity in skeletal muscle (Gray et al., 1998a). Because AKAP-15 is abundantly expressed in the CNS and is associated with Na^+ channels, we repeated our experiments using the AP2 and proline-substituted AP2-P peptides. As shown in Figure 4, dialysis with the AP2 peptide also attenuated the ability of the D1 agonist to reduce the Na^+ current (6.6 ± 2.4%; $n = 6$; $p < 0.01$), whereas dialysis with the AP2-P peptide had no effect (21.5 ± 2.8%; $n = 7$). These results further substantiate our conclusion that PKA anchoring near the Na^+ channel by an AKAP is necessary for D1-like DA receptor modulation. Because AKAP-15 is co-immunoprecipitated with brain Na^+ channels and is co-localized with them in hippocampal pyramidal neurons, it is likely that it is the AKAP responsible for anchoring PKA near the Na^+ channel to mediate neuromodulation by DA and other activators of adenylyl cyclase.

DISCUSSION

Our experiments demonstrate the importance of PKA anchoring in neurotransmitter modulation of voltage-gated brain Na^+ channels by showing that this form of ion channel modulation is blocked specifically by anchoring inhibitor peptides. We previously found that D1-like DA receptor activation resulted in the modulation of the functional properties of the voltage-gated Na^+ current in these neurons by reducing the peak current amplitude (Cantrell et al., 1997). The modulation occurred without significant alterations in the voltage dependence or kinetics of channel activation or inactivation (Li et al., 1992; Cantrell et al., 1997). We further demonstrated that the modulation involved the activation of PKA and resulted from PKA-dependent phosphorylation of the α subunit at Ser-573 in the intracellular loop between domains I and II of the α subunit (Cantrell et al., 1997; Smith and Goldin, 1997). The present work defines an additional required element in the signaling pathway–anchoring of PKA to the Na^+ channel via an AKAP.

Signal transduction mechanisms that involve PKA-dependent phosphorylation events often require that the kinase activity be precisely localized within the cell to confer signaling specificity. This is achieved in part by subcellular targeting of the kinase through association with A-kinase-anchoring proteins or AKAPs (Rubin, 1994; Dell'Acqua and Scott, 1997; Gray et al., 1998a,b). AKAPs act as additional regulatory mechanisms allowing for the activation of compartmentalized pools of PKA. This is an extremely important mechanism, because PKA has been implicated in the control of a number of physiological processes in neurons, including the modulation of multiple classes of ion channels (Brandon et al., 1997). Specific targeting and anchoring of PKA ensures that the proper substrates become phosphorylated in response to incoming signals.

Numerous AKAPs have been purified from a variety of tissues in the past several years (Rubin, 1994; Dell'Acqua and Scott, 1997). Each AKAP possesses two binding sites, a conserved RII binding domain, which mediates binding to the kinase, and an additional, unique targeting domain, which mediates localization of the kinase to particular subcellular compartments or substrates. The recently discovered plasma membrane AKAP, AKAP-15 (Gray et al., 1997, 1998a; Tibbs et al., 1998), is a likely candidate for mediating targeting of PKA to the Na^+ channel. Purified preparations of rat brain Na^+ channels are phosphorylated by co-purifying PKA (Costa et al., 1982; Tibbs et al., 1998). Analysis of these preparations using an RII overlay technique to detect proteins with high affinity for the RII subunit of PKA identified AKAP-15 in these preparations, suggesting that this

AKAP is likely to target PKA to neuronal Na^+ channels (Tibbs et al., 1998). Co-immunoprecipitation experiments support the conclusion that PKA is associated with Na^+ channels, and immunocytochemical labeling shows that AKAP-15 and Na^+ channels are co-localized in the cell bodies of hippocampal pyramidal neurons (Fig. 1). Block of PKA modulation by the anchoring inhibitor peptide Ht31 shows that anchored PKA is required for Na^+ channel modulation. Our electrophysiological results using the AKAP-15-derived peptide AP2 further support the conclusion that this novel AKAP is required for Na^+ channel modulation. However, our results are not conclusive on this point, because the AP2 peptide is expected to bind RII and thereby prevent its association with all endogenous AKAPs. Na^+ channels in the major axon tracts in the hippocampus are not associated with AKAP-15. Either they are not regulated by PKA or their regulation does not require anchoring by AKAP-15. Experiments are currently in progress to define the mechanism of interaction of Na^+ channels and AKAP-15 and to determine their subcellular localization in different classes of neurons. These experiments should further clarify the role of AKAP-15 in regulating the activity of the Na^+ channel in the CNS.

AKAPs have recently been demonstrated to play a role in mediating PKA-dependent regulation of other voltage-gated ion channels in skeletal, cardiac, and smooth muscle tissue. For example, in skeletal muscle transverse tubules and in cardiac muscle regulation of L-type calcium currents requires anchoring of PKA (Johnson et al., 1994, 1997; Gao et al., 1997), as does regulation of calcium-activated potassium channels in tracheal myocytes (Wang and Kotlikoff, 1996). AKAP-15 is implicated in regulation of L-type calcium channels in skeletal and cardiac muscle and in pancreatic β cells (Fraser et al., 1998; Gray et al., 1998a). Thus, the anchoring of PKA near ion channels through AKAP targeting is emerging as an important theme in ion channel regulation, and AKAP-15 may be involved in this regulation in a wide range of cell types. Our current work broadens the scope of this regulatory mechanism to voltage-gated Na^+ channels in hippocampal neurons.

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