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Article *in* The Journal of Neuroscience : The Official Journal of the Society for Neuroscience · April 2001

DOI: 10.1523/JNEUROSCI.21-06-01964.2001 · Source: PubMed

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Direct Interaction of a Brain Voltage-Gated K^+ Channel with Syntaxin 1A: Functional Impact on Channel Gating

Oded Fili,[1] Itzhak Michaelevski,[1] Yaniv Bledi,[2] Dodo Chikvashvili,[1] Dafna Singer-Lahat,[1] Hassia Boshwitz,[2] Michal Linial,[2] and Ilana Lotan[1]

[1]*Department of Physiology and Pharmacology, Sackler School of Medicine, Tel-Aviv University, 69978 Ramat-Aviv, Israel, and* [2]*Department of Biological Chemistry, Life Sciences Institute, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel*

Presynaptic voltage-gated K^+ (Kv) channels play a physiological role in the regulation of transmitter release by virtue of their ability to shape presynaptic action potentials. However, the possibility of a direct interaction of these channels with the exocytotic apparatus has never been examined. We report the existence of a physical interaction in brain synaptosomes between Kvα1.1 and Kvβ subunits with syntaxin 1A, occurring, at least partially, within the context of a macromolecular complex containing syntaxin, synaptotagmin, and SNAP-25. The interaction was altered after stimulation of neurotransmitter release. The interaction with syntaxin was further characterized in *Xenopus* oocytes by both overexpression and antisense knockdown of syntaxin. Direct physical interaction of syntaxin with the channel protein resulted in an increase in the extent of fast inactivation of the Kv1.1/Kvβ1.1 channel. Syntaxin also affected the channel amplitude in a biphasic manner, depending on its concentration. At low syntaxin concentrations there was a significant increase in amplitudes, with no detectable change in cell-surface channel expression. At higher concentrations, however, the amplitudes decreased, probably because of a concomitant decrease in cell-surface channel expression, consistent with the role of syntaxin in regulation of vesicle trafficking. The observed physical and functional interactions between syntaxin 1A and a Kv channel may play a role in synaptic efficacy and neuronal excitability.

Key words: Kv channel; potassium channel; SNARE complex; syntaxin 1A; gating; K^+ channel; Kv1.1 subunits; Kvβ subunits; Xenopus *oocytes; rat brain synaptosomes*

It is well established that presynaptic voltage-gated K^+ (Kv) channels play a role in neurotransmitter release, where their function is thought to be exerted through their ability to shape action potentials invading nerve terminals (Roeper and Pongs, 1996; Meir et al., 1999). However, the possibility of direct interaction between K^+ channels and the exocytotic machinery regulating transmitter release has never been investigated.

Syntaxin (Bennett et al., 1992) is a component protein of a molecular complex that controls the docking and fusion of synaptic vesicles with the presynaptic membrane (Bennett, 1995; Hanson et al., 1997; Hay and Scheller, 1997; Linial, 1997). The minimal complex common to all secretory processes consists of the three soluble *N*-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor (SNARE) proteins (Sollner et al., 1993): syntaxin (HPC-1), VAMP, and SNAP-25. In nerve terminals the complex also contains synaptotagmin and voltage-dependent Ca^{2+} channels (Bajjalieh and Scheller, 1995; Bennett, 1995; Sudhof, 1995; Linial and Parnas, 1996; Hanson et al., 1997). Recently, we showed that presynaptic muscarinic ACh receptors interact with the core complex of rat brain synaptosomes (Linial et al., 1997; Ilouz et al., 1999).

The interaction of N- and L-type Ca^{2+} channels with syntaxin was shown to have regulatory effects on the functions of the channels (Bezprozvanny et al., 1995; Wiser et al., 1996; Bergsman and Tsien, 2000; for review, see Catterall, 2000). A number of other ion channels, including CFTR Cl^- (Naren et al., 1998; Peters et al., 1999) and epithelial Na^+ channels (Qi et al., 1999; Saxena et al., 1999), were shown to interact physically and functionally with syntaxin 1A, a neuronal form of syntaxin. However, opinions differ as to the existence of a causative relationship between these interactions. In addition, neuronal voltage-gated Na^+ channels were shown to interact physically with synaptotagmin (Sampo et al., 2000). No physiological relevance of the exocytotic apparatus was demonstrated in any of these cases. Rather, the synaptotagmin–Na^+ channel complex was shown to be distinct from the synaptotagmin–SNARE protein complex.

Pore-forming α subunits of voltage-gated channels (Kvα) have been detected at presynaptic nerve terminals in a number of mammalian brain structures (Meir et al., 1999). Also, colocalization of Kv1.1 (an α subunit of the Kv1 subfamily) with Kvβ1.1 (a peripheral subunit of the Kvβ subfamily that can associate with Kv1.1) (Rettig et al., 1994) was demonstrated in synaptic terminals in specific regions of rodent brain (Rhodes et al., 1995; Veh et al., 1995), implying a role for these subunits in repolarizing the membrane in the synaptic terminals and hence in controlling transmitter release. Indeed, evidence from peripheral nerves indicates that blockade of Kv1.1 channels with specific antibodies can increase transmitter release (Shillito et al., 1995). In addition, neuronal deficiency of either Kv1.1 (Meiri et al., 1997) or Kvβ1.1

Received Nov. 14, 2000; revised Dec. 27, 2000; accepted Dec. 27, 2000.

This work was supported by grants from the Israel Academy of Sciences (I.L.), the State of Israel Ministry of Health (I.L., M.L.), and the United States–Israel Binational Science Foundation (I.L.). We thank Nathan Dascal and Eitan Reuveny for critical reading of this manuscript, Dafna Atlas for advice and for the L-753-893 GST-fusion construct, Julia Pranis for initial characterization of the cross-linking experiments, Leonid Mittelman for help with the confocal analysis, and Rachel Barzilay for generating the GST fusion constructs.

O.F. and I.M. contributed equally to this study.

Correspondence should be addressed to Prof. Ilana Lotan, Department of Physiology and Pharmacology, Sackler School of Medicine, Tel-Aviv University, 69978 Ramat-Aviv, Israel. E-mail: ilotan@post.tau.ac.il.

(Giese et al., 1998) impaired certain types of learning and memory.

We showed previously, using *Xenopus* oocytes, that Kv1.1/Kvβ1.1 channels are modulated by cellular factors including protein kinases A and C, a PSD-95-related protein, G-protein $\beta\gamma$-subunits, and microfilaments (Levin et al., 1995, 1996a,b; Peretz et al., 1996; Jing et al., 1997, 1999; Levy et al., 1998). Here we describe a modulation of this channel that involves its direct interaction with syntaxin 1A. The interaction also occurs in fresh synaptosomes, involves synaptotagmin and SNAP-25, and is altered after the triggering of transmitter release.

MATERIALS AND METHODS

Constructs and antibodies. The primary antibodies used were Kv1.1–C terminus (Alomone Labs, Jerusalem, Israel), Kv1.1–N terminus, and Kvβ–C terminus (Ivanina et al., 1994), polyclonal syntaxin 1A (Alomone), monoclonal anti-HPC-1 (Sigma Israel, Rehovot, Israel) synaptophysin (Boehringer Mannheim, Mannheim, Germany), and monoclonal SNAP-25 (Signal Transduction, Lexington, KY). GIRK1 antibody and GIRK1,2,4 mRNAs were the generous gift of N. Dascal (Tel-Aviv University, Israel). Kv1.1 and Kvβ1.1 [kindly donated by O. Pongs (ZMNH, Hamburg, Germany)] cDNAs and their mRNAs were described in Levin et al. (1996a). DNAs of Kv1.1 fragments and Kvβ1.1 to create GST fusion proteins were described in Jing et al. (1999). Enzymes were purchased from Boehringer Mannheim, Promega (Madison, WI), or MBI Fermentas (Vilnius, Lithuania). The degenerate phosphorothioate antisense oligodeoxynucleotides (AS-ODNs) (including 5′ and 3′ end capping of 2- and 4-phosphorothioates, respectively, and a phosphorothioate at every third internal position to enhance nuclease resistance) were targeted against the following nucleotide sequences: AS-linker: 5′-GA(GA)GA(AG)(TC)T(TCG)GA(AG)GA(N)ATG(CT)T(N)GA3-′ [encoding amino acids EELE(ED)ML(ED)]; AS-HS: 5′-GA(AG)-(CU)U(N)CA(UC)GA(CU)AUGUU(CU)AUGGA(CU)AUG-3′ (encoding amino acids ELHDMFMDM). AS-linker corresponds to amino acids 163–170 in the linker separating helixes H2B and H3, and AS-H3 corresponds to amino acids 210–220 within the H3 helix of human syntaxin 1A. The ODNs are expected to hybridize to syntaxins from human, rodent, bovine, chick, *Aplysia*, leech, and sea urchin homologs, as well as to rat syntaxins 3 and 4.

The sequence ODN 5′-ATCGTTTGTGAGCGCTTCGGCATCGGT-3′ was used as a non-sense oligomer.

Oocytes and electrophysiological recording. Oocytes of *Xenopus laevis* were prepared as described (Dascal and Lotan, 1992). Oocytes were injected (50 nl per oocyte) with 150−300 ng/μl K_v1.1 and 1−3 μg/μl $K_v\beta$1.1 mRNAs for biochemical studies, and with 5−10 ng/μl K_v1.1 and 15−1000 ng/μl $K_v\beta$1.1 mRNAs for electrophysiological experiments. Syntaxin mRNA (3−50 ng/μl) was injected for both biochemical and electrophysiological experiments. Two-electrode voltage-clamp recordings were performed as described (Levin et al., 1995). To avoid possible errors introduced by series resistance, only current amplitudes up to 4 μA were recorded. Currents were elicited by stepping up the membrane potential from a holding potential of −80 mV to +50 mV for 250 msec. Current–voltage relationships were obtained by depolarizing steps from −80 mV to the indicated voltages. Net current was obtained by subtracting the scaled leak current elicited by a voltage step from −80 to −90 mV. Oocytes with a leak current of >3 nA/1 mV were discarded.

Immunoprecipitation in oocytes. Oocytes were subjected to immunoprecipitation (IP) as described (Levin et al., 1995). Briefly, immunoprecipitates from 1% Triton X-100 homogenates of either plasma membranes (PMs) or internal fractions (IFs) [separated mechanically, as described in Ivanina et al. (1994)] were analyzed by SDS−PAGE (usually on gradients of 8 or 5−15% to separate syntaxin from the lower band of Kvβ1.1). Digitized scans were derived by PhosphorImager (Molecular Dynamics, Eugene, OR), and relative intensities were quantitated by ImageQuant.

Immunoprecipitation and immunoblotting in synaptosomes. For all experiments described in Figure 1, *D* and *E*, fresh synaptosomes were prepared from rat brains (P2 fraction) (Pearce et al., 1991) and used within 3 hr of preparation. The physiological state of the synaptosomes was monitored by a glutamate release assay, as described previously (Linial, 1997). For the experiments described in Figure 1*A*—*C*, we used fresh synaptosomes that had been stored in aliquots at −70°C and were thawed once. IP was performed as described (Linial, 1997). Briefly, antibodies were prebound to protein G–Sepharose or protein A–Sepharose beads (Zymed, South San Francisco, CA) in HKA buffer (50 mM HEPES–KOH, pH 7.4, 140 mM K-acetate, 1 mM $MgCl_2$, and 0.1 mM EGTA) supplemented with 0.1% gelatin and 0.1% bovine serum albumin (BSA). Aliquots of synaptosomes (150 μg) were incubated for 30 min at 25°C in Ca^{2+}-free BSS buffer (10 mM HEPES/NaOH, pH 7.4, 128 mM NaCl, 2.4 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM KH_2PO_4, and 10 mM D-glucose). For stimulation of the preparation, 1.6 mM Ca^{2+} was added, and 60 mM NaCl was replaced by KCl. Synaptosomes were washed gently twice and solubilized for 1 hr at 4°C in IP buffer containing HKA buffer with the addition of either 2% freshly prepared y3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid (CHAPS) (Boehringer Mannheim) or 1% Triton X-100. Protease inhibitors (10 μg/ml aprotonin, leupeptin, and pepstatin; Boehringer Mannheim) and 10 mM 4-2-(aminoethyl)benzenesulfonylfluoride, HCl (Calbiochem, Darmstadt, Germany) were added to the IP buffer. After overnight incubation of the prebound beads (4°C) with solubilized synaptosomes, the bound proteins were thoroughly washed (in IP buffer with only 0.2% CHAPS), separated by SDS-PAGE, and subjected to Western blot analysis using the ECL detection system (Amersham, Buckinghamshire, UK). Special precautions were taken to avoid nonspecific interactions with syntaxin adhering to protein A– or protein G–Sepharose beads. Such adhesion was minimized by including gelatin in the experiment and 5% glycerol in the final washing step. The intensity of nonspecific immunoreactive signals for syntaxin on protein G–Sepharose did not exceed 5% of the signal obtained by including the relevant antibody. The amounts of Kv–syntaxin complex were insensitive to varying protein concentrations (ranging from 1.5 to 0.1 mg/ml) during the immunoprecipitation experiments. Immunoprecipitation reactions were performed at a protein concentration of 0.15 mg/ml. Using a competition-quantified ELISA assay using recombinant proteins and a Kv peptide, we estimated the ratio between Kv1.1 channels and syntaxin to be 1:9. This value refers to the molar ratio of the proteins only in the plasma membrane.

Cross-linking of synaptosomal proteins. P2 fractions (2 mg/ml) in either DMSO (10%) or 2.5 mM dithiobis (succinimidyl propionate) (DSP; Pierce, Rockford, IL) in 10% DMSO were incubated for 30 min at 25°C. The reaction was terminated by the addition of 150 mM Tris, and synaptosomes were immediately solubilized in 1% SDS (2 hr, 25°C). The undissolved material was discarded after centrifugation (16,000 × *g*, 15 min), and the soluble fraction was diluted 20-fold (final protein concentration 0.1 mg/ml) in HKA buffer and CHAPS for immunoprecipitation experiments. Reduction of the thiol groups of DSP was performed using 100 mM dithiothreitol (DTT).

"Pull-down" of synaptosomal proteins. GST fusion proteins (150 pmol) immobilized on glutathione−Sepharose beads were incubated with 150−200 μg rat brain synaptosomes (P2 fraction) in HKA buffer with 2% CHAPS or 4% Triton X-100 and a mixture of protease inhibitors (Boehringer Mannheim) at 4°C for 12 hr. Samples were washed four times with HKA containing 0.1% Triton X-100, then boiled for 10 min in SDS sample buffer, electrophoresed (12% polyacrylamide gel), immunoblotted, and processed as described above. ECL signals were quantified with TINA software (Budapest, Hungary).

In vitro *binding of GST fusion proteins with syntaxin 1A*. The fusion proteins were synthesized and reacted with syntaxin as described (Jing et al., 1999). Briefly, purified GST fusion proteins (150 pmol) immobilized on glutathione−Sepharose beads were incubated with either 5 μl of the lysate containing ^{35}S-labeled syntaxin [syntaxin 1A translated on the template of *in vitro* synthesized RNAs using a translation rabbit reticulocyte lysate kit (Promega) according to the manufacturer's instructions] or 200 pmol of recombinant syntaxin peptide prepared from a GST fusion construct (amino acids 1−264) cleaved by thrombin (molar ratio 1:500) in 500 μl of PBS with 0.1% Triton X-100 and 0.5 mg/ml BSA for 1 hr at room temperature, with gentle rocking. After washing, the GST fusion proteins were eluted with 20 mM reduced glutathione in 30 μl elution buffer (120 mM NaCl, 100 mM Tris−HCl, pH 8) or not eluted, and then subjected to SDS−PAGE (12% polyacrylamide).

Oocyte plasma-membrane cortex preparation and confocal microscopy. Plasma-membrane cortex preparations and fluorescence labeling were performed as described (Singer-Lahat et al., 2000). Briefly, devitellinized oocytes were transferred to a plastic coverslip and incubated for 5 min in ND96 solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.5) supplemented with 1 mM $CaCl_2$, 2.5 mM sodium pyruvate, and 50 μg/μl gentamycin and containing 5 mM EGTA. Each oocyte was sucked into a Pasteur pipette, and the yolk was removed, leaving a clean plasma

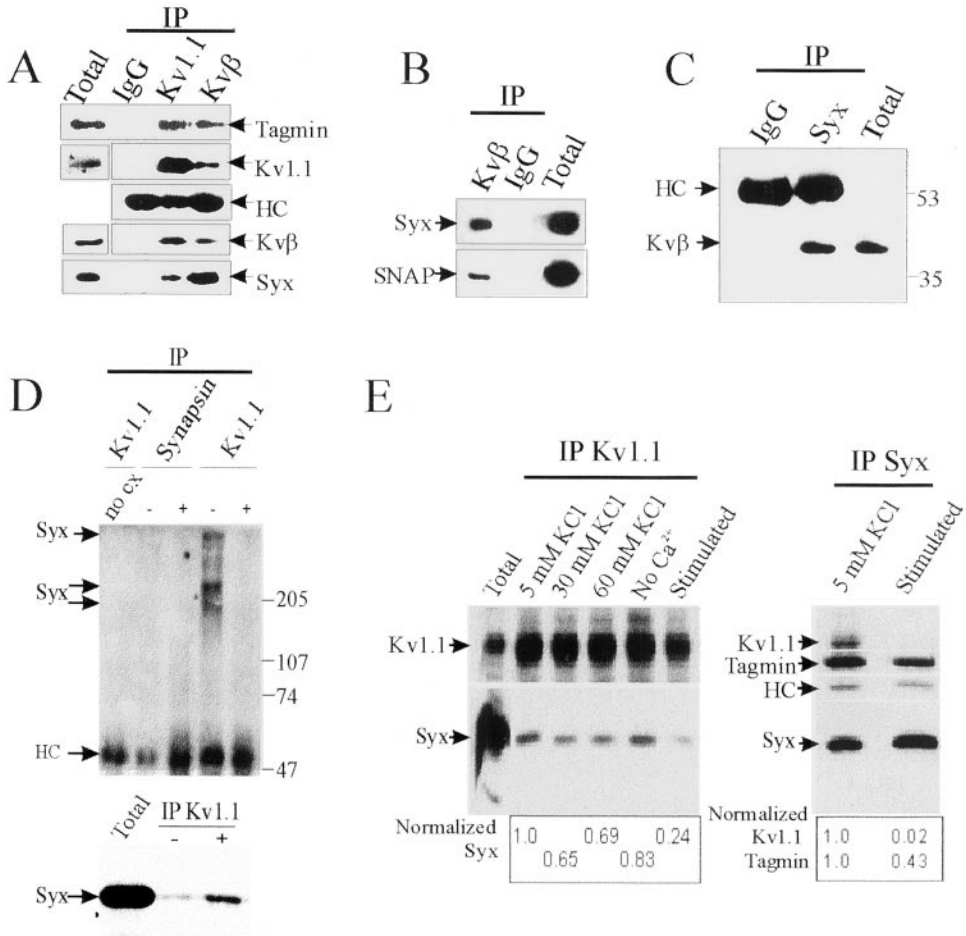

Figure 1. Kv1.1 and Kvβ proteins interact with syntaxin in fresh brain synaptosomes. *A–C*, The interaction with syntaxin also involves SNAP-25 and synaptotagmin. Fresh brain synaptosomal lysates were immunoprecipitated (*IP*) by Kv1.1, Kvβ, syntaxin 1A, or IgG (irrelevant) antibodies, as indicated above the *lanes*. The immunoprecipitated proteins were separated by SDS−PAGE, blotted, and detected by antibodies, as indicated at the *sides* of the *blots*. *Syx*, Syntaxin 1A (anti-HPC-1); *Tagmin*, synaptotagmin; *SNAP*, SNAP-25; *HC*, heavy chain of the antibodies used. Molecular weight markers are shown on the *right* in *C*. Each of the results shown in *A* and *C* is representative of four similar experiments performed using either 2% CHAPS or 1% Triton X-100. The result shown in *B* is representative of two similar experiments. For each IP reaction we used 200 μg of synaptosomes and loaded 0.5 or 45 μg of synaptosomes on *Total* (no immunoprecipitation was performed) lanes for blotting with syntaxin or Kvβ and Kv1.1, respectively. *D*, In intact fresh synaptosomes, interaction of Kv1.1 with syntaxin occurs *in situ*. Immunoprecipitations were performed with antibodies against synapsin and Kv1.1, after *in situ* cross-linking of intact synaptosomes. After solubilization by SDS, each reaction was performed with 100 μg of either DSP-treated or DMSO-treated synaptosomes (*no cx*). *Total* indicates that no immunoprecipitation was performed. In each reaction, the proteins were loaded on an 8.5% SDS gel before (−) or after (+) reduction with 100 mM DTT. The gel was blotted and processed for Western analysis using syntaxin antibodies (*top panel*). High molecular weight bands (marked by *arrows*) were detected. In an identical IP experiment, proteins were separated on 12.5% SDS gel and immunoblotted with syntaxin antibodies (*bottom panel*). Immunoreactivity with syntaxin was increased after reduction of the DSP-treated synaptosomes. *E*, Dynamic interaction between the Kv1.1 and syntaxin. Reciprocal coimmunoprecipitations by Kv1.1 (*left panel*) and syntaxin antibodies (*right panel*) were followed by SDS−PAGE, blotting, and detection by the indicated antibodies. Stimulation of the synaptosomes (incubation with 1.6 mM external Ca^{2+} and 60 mM external KCl; see Materials and Methods) before the immunoprecipitation was followed by a severalfold reduction in the interaction between syntaxin and Kv1.1 (compare *5 mM KCl* and *Stimulated lanes* in both panels). For control, synaptosomes were incubated with either high concentrations of external KCl alone (*30 mM KCl* and *60 mM KCl*) or with 2 mM EGTA (and 5 mM KCl) (*No* Ca^{2+}). The same pattern was observed in four independent experiments; quantification of syntaxin normalized to Kv1.1 (*left panel*) and quantification of Kv1.1 and synaptotagmin, each normalized to syntaxin (*right panel*), are indicated below the *lanes*.

membrane cortex patch attached to the coverslip with its cytoplasmic surface exposed to the bathing solution. After fixation of the membrane with 1% formaldehyde, the nonspecific sites were blocked by donkey IgG, whole molecule (Jackson ImmunoResearch, West Grove, PA). Primary and secondary antibodies were used to label the proteins of interest, as follows: syntaxin was labeled with mouse antibody and then with Alexa-conjugated anti-mouse IgG. The proteins Kv1.1, GIRK1, and SNAP-25 were labeled with rabbit antibody (1:250, Alomone) and Cy3 donkey anti-rabbit IgG. Results were analyzed by confocal laser scanning microscopy, using a Zeiss instrument.

Statistical analysis. Data are presented as means ± SEM. Student's *t* test was used to calculate the statistical significance of differences between two populations.

RESULTS

Kv1.1 and Kvβ proteins interact physically with syntaxin in rat brain synaptosomes

Because the Kv1.1 channel is presynaptic, we were interested in determining whether it interacts with partners of the exocytotic machinery in fresh rat brain synaptosomes. Using two different antibodies, one against the N terminus and the other against the C terminus of Kv1.1, we found that both syntaxin 1A (*Syx*) and synaptotagmin (*Tagmin*) coprecipitated with the Kv1.1 protein (Fig. 1*A*). As expected, Kvβ proteins were also coprecipitated. Using antibodies against Kvβ, we found that (along with Kvβ and

Kv1.1) syntaxin, synaptotagmin (Fig. 1*A*), and SNAP-25 (Fig. 1*B*) could also be coprecipitated. No cross-reactivity of the Kv1.1 antibody with other close members of the Kv family was expected because the serum was depleted of antibodies that react with closely related isoforms such as Kv1.2. Moreover, the coprecipitation could be blocked by preincubation of the antibodies with the peptide against which the antibodies were raised (data not shown). To verify the specificity of the coprecipitation, we performed the reciprocal experiments in which Kvβ (Fig. 1*C*) and Kv1.1 (Fig. 1*E*, *right panel*, *left lane*) were coprecipitated with syntaxin, using an antibody against syntaxin.

To exclude the possibility that Kv1.1 was interacting with components of the SNARE complex, which tend to reassemble after their solubilization, we performed a cross-linking experiment in intact fresh synaptosomes (under the conditions used to establish the interaction of muscarinic ACh receptors with SNARE proteins) (Linial et al., 1997) (Fig. 1*D*). Before solubilization and immunoprecipitation, the intact synaptosomes were treated with DSP, a lipid-soluble, homo-bifunctional, cross-linking reagent. Solubilization was performed under stringent conditions (1% SDS) to ensure that the interactions captured were authentic, and in very dilute conditions (up to 0.1 mg/ml total protein) to exclude the possible occurrence of interactions after solubilization. Under these conditions, syntaxin immunoreactivity was precipitated by Kv1.1 antibodies in the form of high molecular weight complexes (Fig. 1*D*, *top panel*) and was detected in a monomeric form after reduction of the DSP thiol groups (Fig. 1*D*, *bottom panel*). As expected, in control experiments (Fig. 1*D*, *top panel*) in which DSP was eliminated (*no cx*), or in which immunoprecipitation was performed using DSP-treated synaptosomes with synapsin antibodies, no syntaxin was detected (Rosahl et al., 1993). These results corroborated the results of the immunoprecipitation experiment (Fig. 1*A*) and showed that in intact fresh synaptosomes, Kv1.1 interacts with syntaxin *in situ*.

Our next objective was to determine whether the interaction with syntaxin is dynamic, i.e., whether it depends on the physiological state of the synaptosomes. This was done by performing the coimmunoprecipitation experiments using fresh synaptosomes that, before their homogenization, were subjected to increasing depolarization in the presence or absence of Ca^{2+} ions. In synaptosomes stimulated by depolarization and high Ca^{2+} concentrations (60 mM external KCl and 1.6 mM external Ca^{2+} concentration), the interaction between Kv1.1 and syntaxin was much weaker than in unstimulated synaptosomes (5 mM KCl, no Ca^{2+} added) or in synaptosomes subjected to depolarization alone (30 or 60 mM KCl, no Ca^{2+} added) (Fig. 1*E*). The interaction of syntaxin and synaptotagmin was also weaker after stimulation (Fig. 1*E*, *right panel*), as already reported (Ilouz et al., 1999). Under conditions of stimulation, the synaptosomes can release neurotransmitters (Linial 1997). These findings suggest that the interaction with syntaxin may be associated with neurotransmitter release and it is sensitive to changes mimicking the physiological stimulation conditions.

Another objective was to substantiate the notion that syntaxin interacts physically with the Kv channel subunits. To this end we restricted ourselves to working with a Kvβ subunit (Kvβ1.1), because larger amounts of coprecipitated syntaxin were obtained by immunoprecipitation with Kvβ antibodies than with Kv1.1 antibodies [(Fig. 1*A*) compare the corresponding lanes derived by simultaneous analyses in a single batch of synaptosomes]. Four approaches were used. First, a pull-down assay, using immobilized Kvβ1.1–GST (corresponding to the full-length protein) fusion protein and synaptosomal lysates (2% CHAPS or 4% Triton X-100), revealed a syntaxin-immunoreactive band when lysates were incubated in the presence of the recombinant Kvβ1.1 but not with the recombinant Kv1.1 cytoplasmic C terminus (GST–Kv1.1C, corresponding to amino acids 412–495) or with GST alone (Fig. 2*A*). The second approach was an *in vitro* binding assay using immobilized Kvβ1.1–GST fusion protein with either the recombinant cytoplasmic part of syntaxin (corresponding to amino acids 4–264) cleaved by thrombin from its corresponding GST fusion protein (Fig. 2*B*, *bottom panel*) or ^{35}S-labeled full-length syntaxin synthesized in reticulocyte lysate (data not shown). The results of both settings were similar and are summarized in Figure 2*B* (*top panel*), confirming a direct *in vitro* binding between syntaxin and Kvβ1.1, the amount of which was more than twofold larger than that between syntaxin and $L_{753-893}$ [corresponding to domain II–-III (amino acids 753–893) of the L-type Ca^{2+} channel]. The latter interaction was confirmed to be highly specific (Wiser et al., 1999). The third approach was an *in vitro* binding assay using immobilized GST–Kvβ1.1 with different concentrations of the cytoplasmic syntaxin. This assay demonstrated that under our binding conditions, binding is half-maximal at ~0.2–0.3 μM syntaxin and that ~8 pmol of syntaxin is bound per 10 pmol of Kvβ1.1, at a saturating concentration of syntaxin (Fig. 2*C*). The fourth approach was an *in vitro* assay of competitive binding (Fig. 2*D*) between GST–Kvβ1.1 and the hexahistidine-tagged (His_6) protein expressing segment II–III (amino acids 718–963) of the N-type Ca^{2+} channel (His_6–$N_{718-963}$; "synprint" peptide). This domain of the channel interacts strongly with syntaxin and was found to be physiologically relevant (Sheng et al., 1994, 1996). As a control we used His_6–$N_{718-859}$, corresponding to a shorter II–III segment that is unable to interact with syntaxin (Rettig et al., 1996). In this experiment, binding to syntaxin was performed in the presence of two concentrations of His_6–$N_{718-963}$. As the concentration of this peptide increased, a significant decrease was observed in the amount of bound syntaxin, whereas no such decrease was seen when the molar concentration of His_6–$N_{718-859}$ was even twofold larger. At a molar ratio of $N_{718-963}$/Kvβ1.1 = 0.4, the bound syntaxin was reduced to ~10% of its amount in the absence of the competitor. Thus, the interaction of Kvβ1.1 with syntaxin is blocked by $N_{718-963}$.

Syntaxin 1A associates with the Kv1.1/Kvβ 1.1 ($\alpha\beta$) channel in *Xenopus* oocytes

In an effort to relate functional interaction with the physical interaction between syntaxin and the Kv1.1 (α) channel, we used the heterologous expression system of *Xenopus* oocytes, in which biochemical and electrophysiological analyses can be performed simultaneously. First we examined whether syntaxin interacts physically with the Kv1.1/Kvβ1.1 ($\alpha\beta$) channel in oocytes. SDS–PAGE analysis of metabolically labeled proteins, in both the PM (manually dissected, see Materials and Methods) and the IF (consisting of cytoplasm and intracellular organelles) of oocytes, showed that syntaxin coimmunoprecipitates with $\alpha\beta$, using two different α antibodies (Fig. 3*A*). Note that Kvβ1.1 (β) immunoreactivity appears in the form of two bands; the lower band (the nature of which is unknown) (Levin et al., 1996a) migrates just above syntaxin. The stoichiometry of the interaction of the channel with syntaxin in plasma membranes was estimated from the molar ratio of coprecipitated syntaxin to coprecipitated β, with a given amount of α, which was 1.35 ± 0.54 (mean ± SEM of four experiments). The specificity of the interaction of the

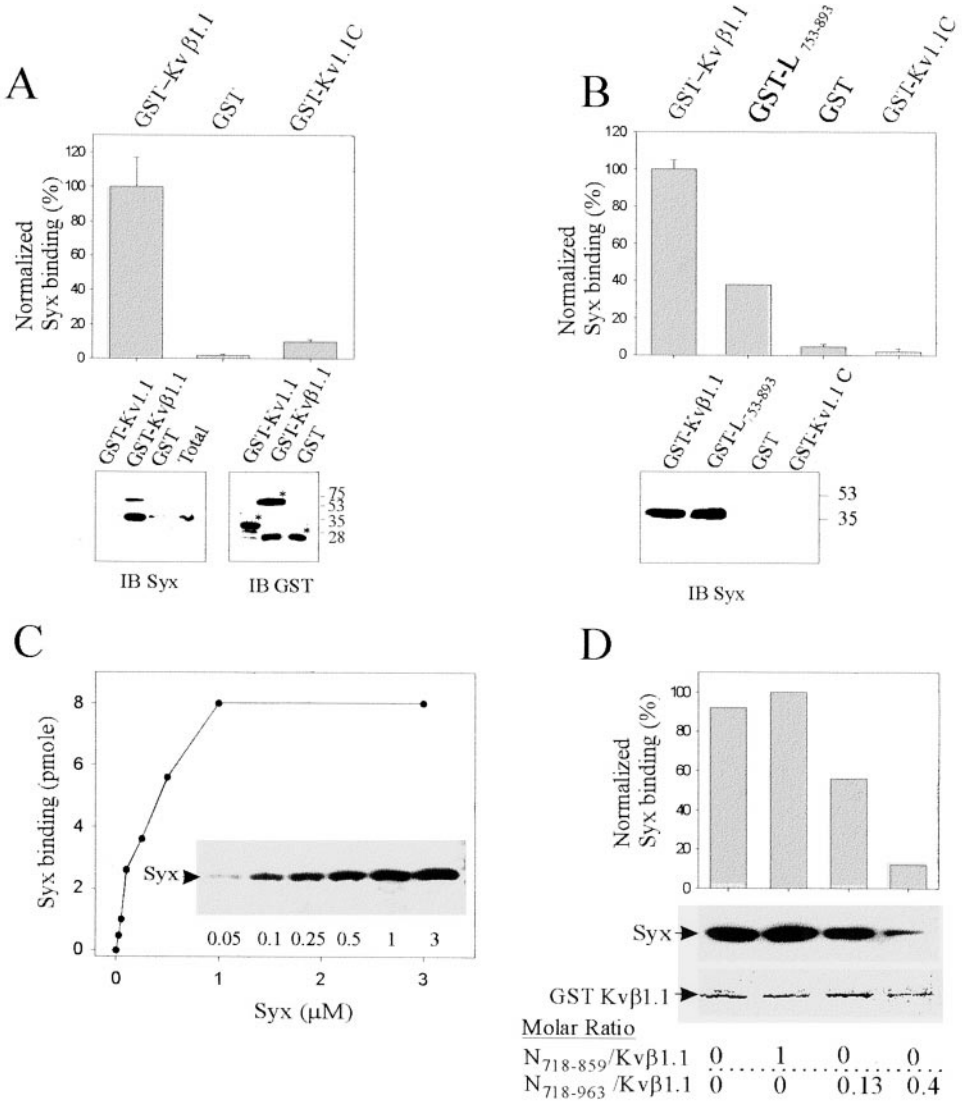

Figure 2. Interaction of recombinant full-length Kvβ1.1 with syntaxin 1A. *A*, GST−Kvβ1.1 fusion protein pulls down syntaxin 1A from brain synaptosomes. *GST−Kvβ1.1*, *GST−Kv1.1C* (corresponding to the C terminus of Kv1.1), or *GST* immobilized on GSH−agarose beads (each at 150 pmol) was incubated with 2% CHAPS synaptosomal lysate (200 μg) for 12 hr at 4°C. Precipitated proteins were separated by SDS−PAGE (12% polyacrylamide) and immunoblotted (*IB*) with either anti-syntaxin 1A antibody (*IB Syx*; *bottom left panel*) or anti-GST antibodies (*IB GST*; *bottom right panel*). Normalized relative ECL signal intensities of bound syntaxin (derived from *IB Syx*) for each of the GST proteins normalized to its relative amount (derived from *IB GST*) were derived from four experiments (in one of which we used 4% Triton X-100, instead of CHAPS lysate) (*top panel*). The predicted position of the fusion protein is indicated by an *asterisk*. *Numbers* on the *right* refer to the mobility of prestained molecular weight standards. *B*, Direct interaction between syntaxin and the recombinant Kvβ1.1. A 200 pmol cytosolic syntaxin (amino acid 4–264), cleaved from the corresponding GST fusion protein by thrombin, was incubated with 200 pmol of the indicated GST fusion proteins (as in *A*; *GST−$L_{753–893}$* corresponding to domain II−III of the L-type Ca^{2+} channel was included for reference) immobilized on GSH−agarose beads in a 1 ml reaction volume. Binding of syntaxin was detected by Western analysis using syntaxin antibody. *Top panel*, Relative values of syntaxin-binding intensities for each of the GST fragments (*bottom panel*) normalized to the corresponding Ponceau S staining intensities (data not shown). The values shown are the mean results of three experiments, in one of which we used 5 μl of *in vitro*-synthesized ^{35}S-labeled full-length syntaxin instead of the thrombinized syntaxin. GST−$L_{753–893}$ was used in only one experiment. *C*, Stoichiometry of the binding of syntaxin 1A to Kvβ1.1, derived from binding curves that show saturation. Thrombinized cytosolic fragment of syntaxin at the indicated concentrations was bound to immobilized GST−Kvβ1.1 (10 pmol) in a 1 ml reaction volume. Bound syntaxin was determined by SDS−PAGE and immunoblotting with syntaxin antibody (*inset*), and GST−Kvβ1.1 was determined by immunoblotting with an anti-GST antibody (data not shown). ECL signal intensities were quantitated with TINA software and converted to picomoles by the use of standard curves for the corresponding proteins. The data were averaged from two independent experiments. *D*, Binding of syntaxin to Kvβ1.1 is blocked by the synprint peptide $N_{718–963}$. Thrombinized cytoplasmic syntaxin was bound to immobilized GST−Kvβ1.1 (150 pmol) in the presence of either increasing $His_6-N_{718–963}$ concentrations or $His_6-N_{718–859}$ as control, as indicated. The *bar diagram* shows the normalized syntaxin binding values, derived as in *A*, according to the intensity of immunostaining for syntaxin (*Syx*) and GST−Kvβ1.1 (below *bars*). The molar ratio in each of the reactions between GST−Kvβ1.1 and the His_6-peptides is indicated below the corresponding bars (*bottom panel*).

channel with syntaxin in the plasma membranes was verified by reciprocal coimmunoprecipitation using antibodies against syntaxin (Fig. 3*B*). Also, in control coimmunoprecipitation experiments we could not detect any association between syntaxin and two G-protein-activated inwardly rectifying channels: GIRK1/4 (Kir3.1/3.4) (Fig. 4, *left panel*) or GIRK1/2(Kir3.1/3.2) (data not

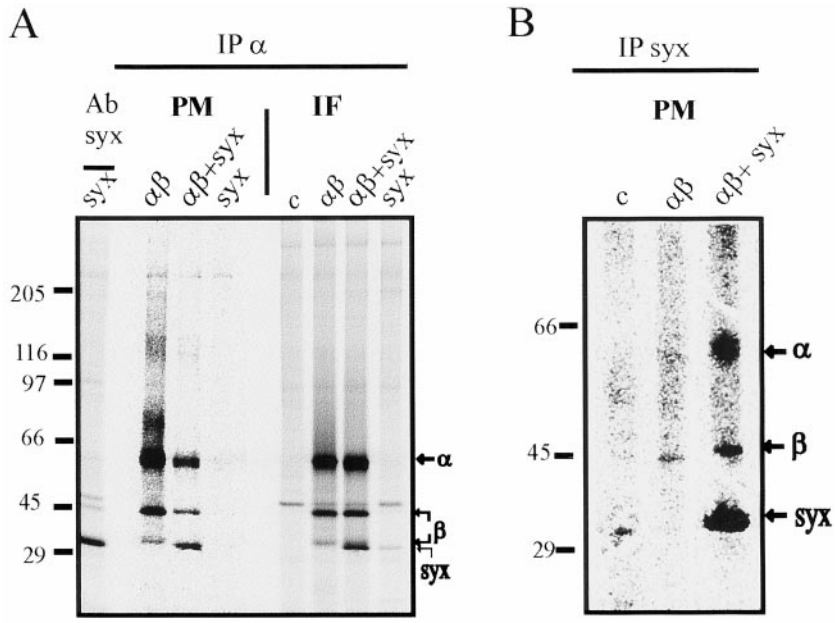

Figure 3. The Kv1.1/Kvβ1.1 (αβ) channel interacts physically with syntaxin 1A in oocytes. *A*, Digitized Phosphorimager scan of SDS−PAGE analysis of [^{35}S]Met/Cys-labeled αβ and syntaxin 1A proteins coprecipitated by α antibody (*IP α*) from homogenates of plasma membranes (*PM*) or internal fractions (*IF*) of oocytes that were uninjected (*c*), injected with α and β mRNAs only (αβ), coinjected with syntaxin 1A (2.5 ng/oocyte; αβ+*syx*), or injected with syntaxin alone (*syx*). The *left lane* shows syntaxin immunoprecipitated by syntaxin antibody from oocytes injected with syntaxin-1A mRNA alone to mark the migration of syntaxin. The protein samples were analyzed on a 5−15% gradient gel to separate between the lower band of β and the syntaxin band. *Arrows* indicate the relevant proteins. The results shown are from one of three independent experiments. *B*, Reciprocal coimmunoprecipitation in plasma membranes of oocytes from the same experiment, performed using a monoclonal syntaxin 1A antibody (*IP syx*).

shown), using antibody against GIRK1. This was despite the high expression of syntaxin in these oocytes, as verified in reciprocal experiments using syntaxin 1A antibody (Fig. 4, *right panel*).

αβ Colocalizes with syntaxin

The coimmunoprecipitation results indicated an interaction between the channel proteins and syntaxin. To further evaluate the extent of such interaction, we performed an immunocytochemical study of preparations of the plasma membrane cortex of oocytes (Singer-Lahat et al., 2000). A monoclonal antibody against syntaxin and a polyclonal antibody against α were used for double staining of the corresponding proteins in oocytes coexpressing αβ and syntaxin. The confocal fluorescence microscopic images are shown in Figure 5*A*. Oocytes coexpressing GIRK1/2 channels with syntaxin were used as a negative control (Fig. 5*B*). As a positive control, we examined the well established interaction between SNAP-25 and syntaxin 1A in oocytes coexpressing these proteins (data not shown). The results showed spatial coincidence of α and syntaxin staining, similar to that of SNAP-25 and syntaxin, indicating colocalization of syntaxin with the channel.

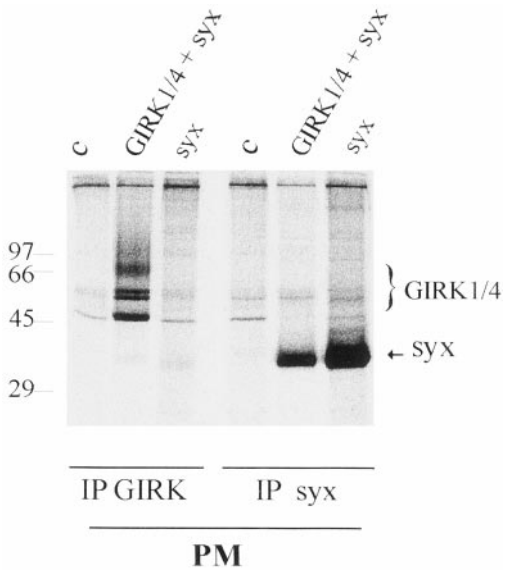

Figure 4. The GIRK1/4 channel does not interact physically with syntaxin 1A in oocytes. Digitized Phosphorimager scan of SDS−PAGE analyses of immunoprecipitation experiments using GIRK1 (*IP GIRK*) or syntaxin 1A (*IP syx*) antibodies from the plasma membranes (*PM*) of oocytes expressing [^{35}S]Met/Cys-labeled GIRK1 and GIRK4 (*GIRK1/4*), with or without syntaxin 1A (*syx*), as indicated above lanes.

Syntaxin 1A regulates inactivation of the αβ channel

In a previous study by our group, it was shown that the K^+ current of αβ channels expressed in oocytes injected with the corresponding mRNAs has a fast-inactivating component (I_i) and a substantial noninactivating sustained component (I_s) (Levin et al., 1996a). The extent of inactivation [defined by the proportion of *Ii* from the peak current (I_p), I_i/I_p] (Fig. 6*A*) increases up to saturation levels of $I_i/I_p = 0.5-0.8$, as the ratio of the injected β mRNA to α mRNA (β/α) is increased to saturation ratios of >50:1. Any modulation of the αβ channels identified by us so far (see introductory remarks) affected the extent but not the rate of inactivation. It appears that the same is true for modulation by syntaxin: coexpression of syntaxin (1.25 ng/oocyte mRNA) with the channel subunits increased the extent of inactivation without affecting the rate (Fig. 6*A*,*B*). The effect was dependent on the β/α mRNA ratio: >40% increase at the nonsaturating (ns) ratio of 4:1 and 20% increase at saturating (s) ratios >50:1 [αβ(ns) and αβ(s), respectively (Fig. 6*B*, *inset*)].

After a report that the sea urchin egg expresses the core components of the SNARE machinery, and after a syntaxin

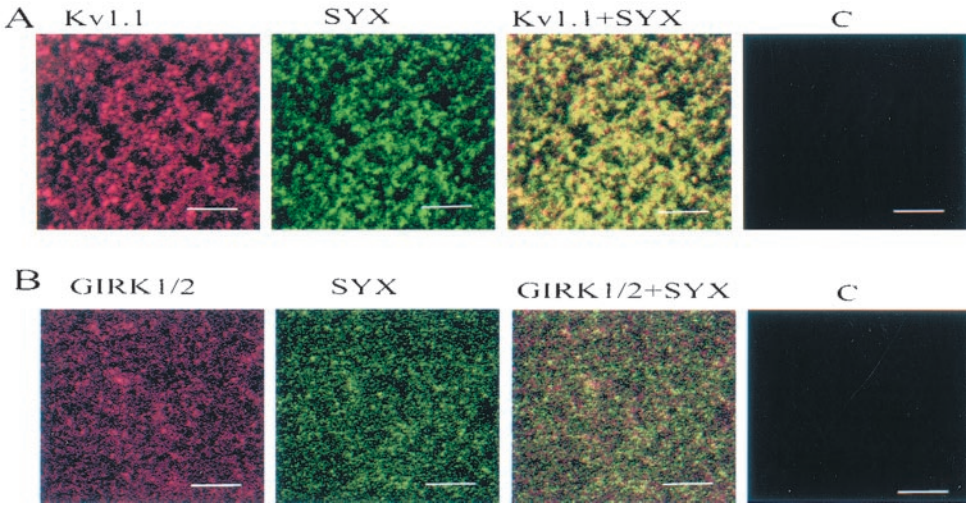

Figure 5. Kv1.1/Kvβ1.1 and SNAP-25, but not GIRK1/2, colocalize with syntaxin 1A in plasma-membrane patches of oocytes. Shown are representative confocal microscopic images of membrane-cortex patches from an oocyte coexpressing syntaxin 1A (*SYX*) with Kv1.1/Kvβ1.1 (*Kv1.1*, *top row*, *A*) or with GIRK1/2 (*bottom row*, *B*). Kv1.1 and GIRK1/2 proteins are shown in *red*, and syntaxin 1A is shown in *green*. The overlay image (*second panel* from *right*, *A*) of syntaxin with Kv1.1 depicts colocalization of the two proteins (shown in *yellow*). In contrast, no colocalization of syntaxin 1A and GIRK1/2 could be detected (*second panel* from *right*, *B*). In control oocytes (*c*, *right panels*) no labeling could be detected. Scale bars, 10 μm.

homolog was identified (Conner et al., 1997), we attempted to establish whether a *Xenopus* oocyte syntaxin isoform(s) exists. Western blot analysis revealed an endogenous protein band that migrated similarly to the exogenously expressed syntaxin 1A. As expected of an endogenous plasma membrane protein, this protein could be detected primarily in the plasma membrane, and very little or none was detectable in the internal fraction, which consists of cytoplasm and intracellular organelles (Fig. 6*C*). We then attempted to reduce the amount of this (unidentified syntaxin-like) endogenous protein to study its effect on the Kv channel. Accordingly, we designed AS-ODNs directed against the most highly conserved domains among syntaxins from different species (Dulubova et al., 1999). These AS-ODNs, referred to as AS-linker and AS-H3, correspond to stretches in the linker separating the helixes H2B and H3 and within the H3 helix of syntaxin, respectively. An ODN of the same length and scrambled nucleotide sequence (non-sense ODN) was used as control. The efficiency of the AS-ODNs in knocking down syntaxin was verified by testing them against expressed syntaxin 1A (Fig. 6*D*). Electrophysiological analysis of the effects of the AS-ODNs showed that injection of 30 pg of either one of them decreased the extent of inactivation, whereas 50 pg of the non-sense ODN had no effect (Fig. 6*A*,*E*). The average reduction by AS-linker in six oocyte batches was ~40% (Fig. 6*F*); in two other oocyte batches there was no reduction. Overall, the effect of a decrease in endogenous syntaxin was the opposite of that of overexpression of syntaxin (which increased the extent of inactivation).

Physical interaction between the channel and syntaxin in plasma membranes mediates the increase in extent of inactivation

Next, we investigated the possible existence of a causative relationship between the physical interactions of the channel proteins with syntaxin and the functional interaction that leads to increased channel inactivation. To address this issue we took advantage of the fact that the $N_{718-963}$ (synprint) peptide competed successfully with β for binding to syntaxin (Fig. 2*D*) and tried to acutely rescue the channel from the functional effects of syntaxin by microinjection of this peptide into oocytes coexpressing αβ with syntaxin in the plasma membrane. As a control we used the $N_{718-859}$ peptide, which does not compete for syntaxin binding (Fig. 2*D*). As shown in Figure 7, the increase in the extent of inactivation caused by coexpressed syntaxin could indeed be reversed by $N_{718-963}$. The control peptide had no effect, confirming that $N_{718-963}$ attenuates the effect of syntaxin by disrupting its interaction with αβ. The results of this experiment point to a link between the functional effect of syntaxin on the extent of inactivation and its physical interaction with the channel in the plasma membrane.

Syntaxin regulates the amplitudes of αβ and α channels

In addition to its effect on inactivation, coexpressed syntaxin also affected the amplitude of the αβ channel. The effect was dependent on the amount of syntaxin expressed. Accordingly, coinjection of syntaxin mRNA (1.25 ng/oocyte) decreased the amplitude of the αβ current (Fig. 8*A*,*C*). The amplitude of the delayed rectifier α current (through homomeric α channels) was also decreased (Fig. 8*B*,*C*). We noticed that, in contrast, syntaxin at very low concentrations (0.15 ng per oocyte) enhanced significantly ($p < 0.001$) both αβ and α amplitudes (Fig. 8*A*,*B*,*D*). No effect on the voltage dependence of channel activation was observed at any concentration of syntaxin (Fig. 8*A*, *inset*). Biochemical analysis showed that in the presence of relatively large amounts of syntaxin (injection of 1.25–5 ng per oocyte of syntaxin mRNA), the amounts of channel proteins in the plasma membrane were significantly decreased (Fig. 3*A*). To quantify this effect, the content of α in the plasma membrane was normalized to the corresponding internal fraction content, and in the presence of syntaxin was found to be only 0.54 ± 0.14 ($p < 0.05$) of the normalized plasma membrane content in the absence of syntaxin (in six of seven experiments). In one case, an electrophysiological experiment was performed concomitantly with a biochemical experiment (Fig. 3*A*), yielding a good correlation between the reduction in current amplitudes (by 64%) and the reduction in normalized plasma membrane α content (by 62%) in the presence of

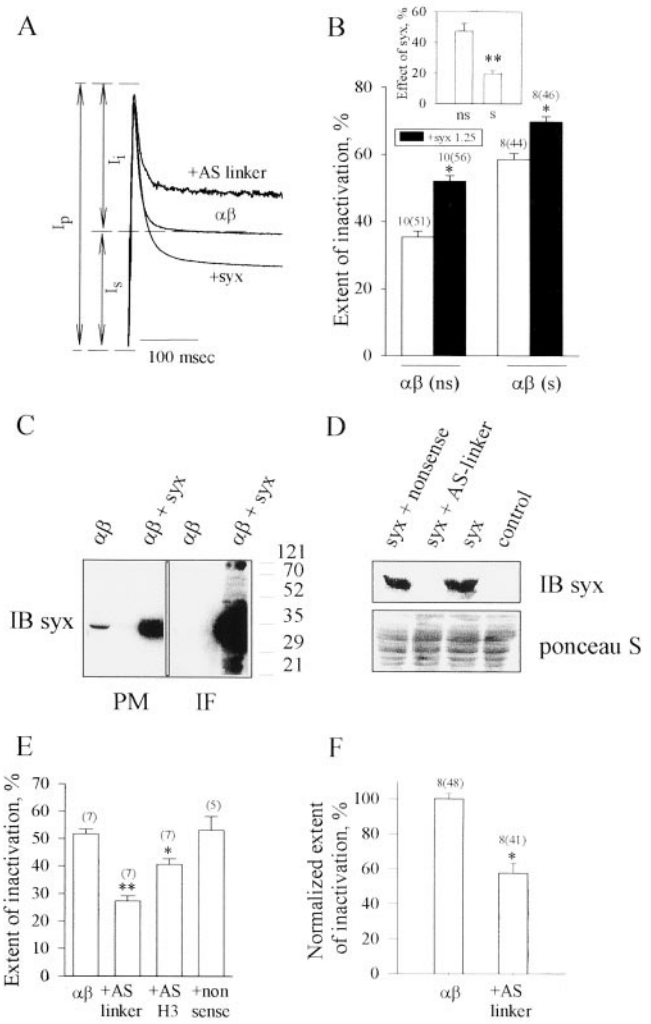

Figure 6. Inactivation of $\alpha\beta$ current in *Xenopus* oocytes is increased by overexpression of syntaxin and decreased by antisense ODN knock-down of syntaxin. *A*, Current traces evoked by depolarization to +50 mV from single oocytes of the same batch injected with α and β mRNAs, either alone ($\alpha\beta$) or together with 1.25 ng per oocyte of syntaxin 1A mRNA (*+syx*), or injected with the antisense ODN (*+AS linker*) 2 d before the assay. I_i, I_s, and I_p illustrate the inactivating, noninactivating, and total current components of $\alpha\beta$, respectively, as defined in Results. *B*, Normalized and averaged effects of syntaxin 1A (1.25 ng per oocyte) coinjected with nonsaturating (*ns*) or saturating (*s*) β/α mRNA ratios. Currents were recorded 3 d after the injection. *Inset* shows the corresponding effects of syntaxin. *C*, Western blot analysis of endogenous syntaxin. Homogenates of internal fractions (*IF*) or plasma membranes (*PM*), consisting of 5 or 25 oocytes, respectively, injected with $\alpha\beta$ with or without syntaxin 1A (*+syx*) were subjected to SDS–PAGE (8% polyacrylamide), transferred to nitrocellulose membranes, and immunoblotted (*IB*) for syntaxin. *Numbers* on the *right* refer to the mobility of prestained molecular weight standards. *D*, Western blot analysis of the effect of antisense ODNs on exogenous syntaxin. Homogenates of whole oocytes injected with syntaxin 1A, without or with either AS-linker (30 pg) or a non-sense ODN (30 pg), were immunoblotted for syntaxin (*top panel*) or stained with Ponceau S (*bottom panel*). *E*, *F*, Effects of antisense ODNs, injected 2 d before the assay, on the extent of inactivation in a single batch of oocytes injected with $\alpha\beta$ (*E*) and normalized and averaged over eight batches of oocytes (*F*). $^{**}p < 0.002$, $^{*}p < 0.02$. *Numbers* above the *bars* refer to the number of oocyte batches; *numbers* in *parentheses* refer to oocytes.

syntaxin. Notably, in oocytes expressing small amounts of syntaxin and in which the amplitudes of the $\alpha\beta$ channels were increased, no significant effect of syntaxin on the amount of α in plasma membranes was detected (data not shown).

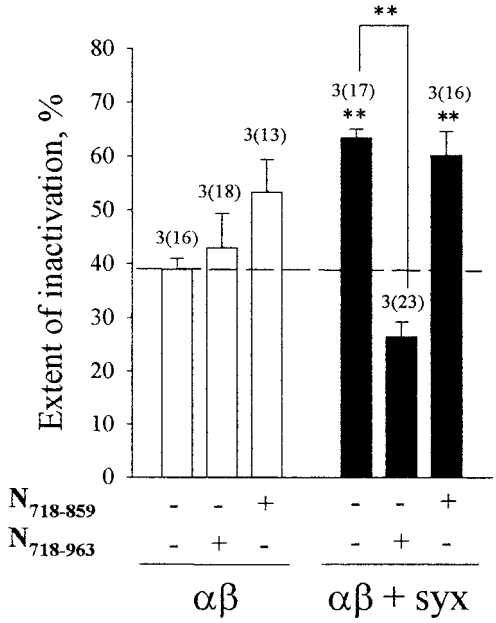

Figure 7. The synprint $N_{718-963}$ peptide, injected 20–120 min before electrophysiological assay of $\alpha\beta$ currents, reverses the effect of coexpressed syntaxin1A on the extent of inactivation. Before the assay, oocytes injected with $\alpha\beta$ at a nonsaturating β/α mRNA ratio (4:1) alone (*left panel*) or with syntaxin 1A mRNA (1.25 ng per oocyte) (*right panel*) were injected (+) or not injected (–) with 1 μM (final concentration in oocytes, assuming a volume of 1 μl) of either His-tagged $N_{718-963}$ (synprint) or His-tagged $N_{718-859}$ (control) peptides.

Taken together, the amplitude-reducing effect of the larger amounts of syntaxin is probably related, at least in part, to a decreased channel expression at the cell surface. The amplitude-increasing effect of smaller amounts of syntaxin might be attributable to changes in the intrinsic biophysical characteristics of the channel.

Notably, saturation of α with β increased the amplitudes by approximately twofold (Fig. 8*C*,*D*, compare $\alpha\beta$ with α). Concomitant biochemical experiments showed that the amount of plasma membrane channels as a fraction of the total expressed channels was larger by 3.3 ± 2.7-fold ($n = 3$) for $\alpha\beta$ channels than for α channels, pointing to chaperone-like properties for Kvβ1.1 in oocytes, as suggested previously for Chinese hamster ovary cells (Shi et al., 1996).

DISCUSSION

Physical interaction of a voltage-gated K^+ channel with syntaxin 1A occurs in brain synaptosomes, with functional consequences that can be detected in *Xenopus* oocytes

A significant role for syntaxin in mediating the regulation of K^+ channels has been inferred in plants in the regulation of unidentified inward and outward rectifier K^+ channels by the hormone abcisic acid (Leyman et al., 1999). The present study demonstrates, for the first time, direct physical and functional interactions of syntaxin 1A with a specific voltage-gated K^+ channel in rat brain. The channel consists of Kv1.1α subunits existing in a complex with Kvβ subunits. Physical interaction between syntaxin 1A and this channel in rat brain synaptosomes was demonstrated here by reciprocal coimmunoprecipitation and by cross-linking experiments. Most importantly, the physical interaction occurs, at least in part, within a molecular complex containing syntaxin,

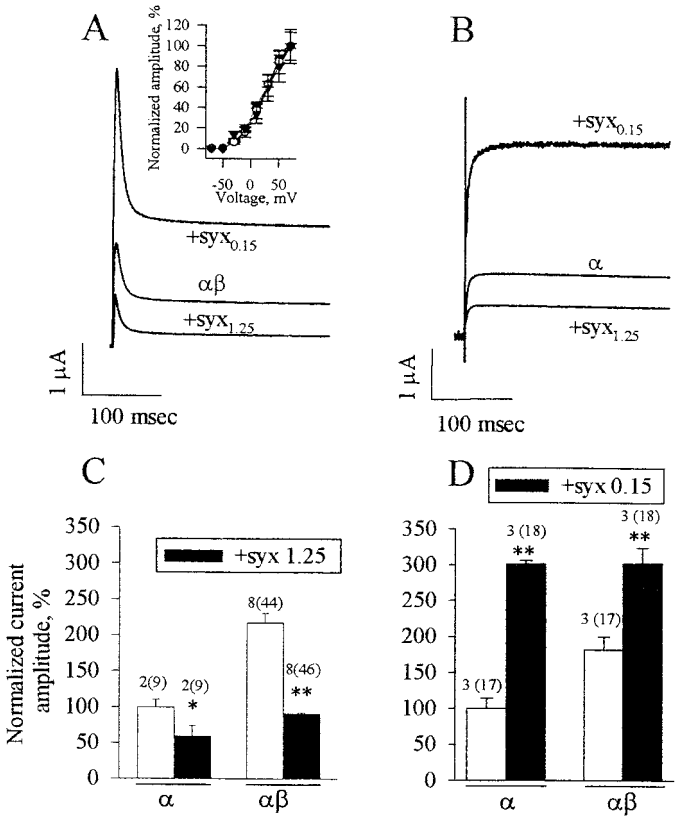

Figure 8. Syntaxin 1A has a biphasic effect on the amplitudes of α and $\alpha\beta$ channels in *Xenopus* oocytes. *A*, *B*, Current traces evoked by depolarization to +50 mV from single oocytes of the same batch injected with α alone (*B*) or with β mRNAs (*A*), with or without two concentrations (0.15 and 1.25 ng per oocyte) of syntaxin 1A mRNA. *Inset* in *A* shows activation curves of the $\alpha\beta$ currents with and without the two syntaxin concentrations (see Materials and Methods). *C*, Syntaxin at higher concentrations reduces amplitudes. Normalized and averaged effects of syntaxin 1A (1.25 ng per oocyte) coinjected with α mRNA alone or with β mRNA. *D*, Syntaxin at lower concentrations increases amplitudes. Normalized and averaged effects of syntaxin 1A (0.15 ng per oocyte) coinjected with α mRNA or with β mRNA. $^{**}p < 0.002$, $^{*}p < 0.02$. *Numbers* above the *bars* refer to the number of oocyte batches; *numbers* in *parentheses* refer to oocytes.

synaptotagmin, and SNAP-25 and can be altered by stimulation (achieved by a combination of depolarization and increased concentration of external Ca^{2+}) that induces neurotransmitter release. The physical interaction may be direct or mediated by another, yet unidentified, protein or proteins. These findings point to coupling of a voltage-gated K^+ channel to the exocytotic apparatus of neurons. Biochemical and electrophysiological studies in *Xenopus* oocytes, combined with *in vitro* binding experiments, demonstrated that the physical interaction between the Kv1.1/Kvβ1.1 channel and syntaxin regulates the fast inactivation of the channel.

Inactivation of the Kv1.1/Kvβ1.1 channel is regulated by direct interaction with syntaxin 1A

The heteromultimeric ($\alpha\beta$) K^+ current is of the fast inactivating A-type (Rettig et al., 1994), but it also possesses a substantial noninactivating current component (Levin et al., 1996a). In a previous study using *Xenopus* oocytes, our group identified several mechanisms that modulate inactivation of the $\alpha\beta$ current (see introductory remarks). Such modulations involved changes in the extent but not in the rate of inactivation. This finding suggested to us that the modulations might affect the equilibrium constant between two gating modes of the $\alpha\beta$ channel, the one inactivating and the other noninactivating (Levin et al., 1996; Singer-Lahat et al., 1999). This was indeed demonstrated by our group in the case of phosphorylation-induced modulation of the Kv1.1 subunit (Singer-Lahat et al., 1999).

In the present study, we identified syntaxin 1A as another regulator of the inactivation of $\alpha\beta$ channels expressed in *Xenopus* oocytes. The extent of this inactivation was increased by overexpression of exogenous rat brain syntaxin 1A and decreased by antisense knock-down of endogenous syntaxin. In addition, we identified a physical interaction between the channel and syntaxin and showed that it occurs in membranes of both rat synaptosomes (Figs. 1, 2) and oocytes (Fig. 3). This was indicated by the results of coimmunoprecipitation experiments in both preparations and supported by an analysis of immunocytochemical colocalization of plasma membrane cortex preparations of oocytes (Fig. 5). In an effort to establish a link between the functional interaction of syntaxin (manifested by an increased extent of channel inactivation) and the physical interaction of syntaxin with the channel, we tried to prevent the functional effect by disrupting the physical interaction (Fig. 7). Thus, in oocytes already expressing both channel proteins and syntaxin in the plasma membrane we could decrease the extent of inactivation to its former level by injecting the synprint peptide. This peptide was found previously to specifically block coimmunoprecipitation of native N-type Ca^{2+} channels with syntaxin 1A (for review, see Sheng et al., 1998) and was shown here, in an *in vitro* binding assay, to compete efficiently with the binding of syntaxin to β (Fig. 2). The result of this experiment strongly suggested that the synprint peptide reversed the effect of syntaxin on the extent of inactivation by disrupting the syntaxin–channel interaction, meaning that the enhanced inactivation caused by syntaxin was the result of cell-surface protein–protein interactions.

Notably, saturation of α with β, which by itself causes enhancement of the extent of inactivation, occluded the effect of syntaxin on inactivation (Fig. 6*B*). This finding, together with the findings that (1) β binds syntaxin directly, as shown by *in vitro* binding studies using recombinant proteins (Fig. 2), and (2) direct interaction of $\alpha\beta$ with syntaxin is responsible for the observed increase in inactivation (Fig. 7), raises the possibility that syntaxin, by binding to β, enhances the efficiency of this subunit, which contains the "ball and chain" machinery of fast inactivation (Rettig et al., 1994), to implement fast inactivation.

It seems reasonable to speculate that the syntaxin-induced modulation of inactivation is coupled to one or more of the several signal transduction mechanisms that were shown by us to modulate α and $\alpha\beta$ channels. In this respect, the modulation by syntaxin resembles that induced by G-protein $\beta\gamma$ subunits (Jing et al., 1999): both enhance the extent of inactivation, an effect that is occluded by saturation with β subunits, and involve physical interactions with the β subunit. Coupling of signalings by syntaxin and by G$\beta\gamma$ was demonstrated recently for Ca^{2+} channels (Jarvis et al., 2000).

Syntaxin regulates $\alpha\beta$ amplitudes

In addition to its enhancement of the inactivation of $\alpha\beta$ channels, syntaxin affected both α and $\alpha\beta$ amplitudes in a biphasic manner that depended on its concentration (Fig. 8). Thus, at low concentrations it increased the amplitudes, and at higher concentrations it decreased them. The decrease in amplitudes was accompanied by a reduction in the content of cell-surface channels and thus

could be explained, at least in part, in terms of this reduction. In this respect, syntaxin might function as a key component in the machinery responsible for trafficking of proteins to the plasma membrane: overexpression of syntaxin might impair the machinery by disrupting the optimal stoichiometry among its various protein components (Nagamatsu et al., 1996). The enhancement of amplitudes, however, seems to result from changes in intrinsic channel properties, because it was not accompanied by changes in the cell-surface channel content. The mechanism underlying this latter effect has yet to be determined.

Physiological significance

The functional consequences of the interaction of syntaxin with Kv channels may be physiologically relevant on both short and long time scales. In the short term, depolarizations of the presynaptic nerve terminal that are sensed by the voltage-dependent K^+ channels result in attenuation of the interaction of the channels with syntaxin, which is probably associated with components of the exocytotic apparatus [for discussion on the exocytotic apparatus and the presynaptic muscarinic ACh receptors, see Ilouz et al. (1999)]. Such events may alter the accessibility of the exocytotic apparatus to be activated by the physiological stimuli, thereby affecting properties of transmitter release. Alternatively, interaction of Kv with syntaxin may define the termination postfusion state of the release. In this context the Kv channel may serve as a sensor for the hyperpolarized state, and conformational changes may result in resetting of the release apparatus to its primed state.

The physiological significance in the long term may be deduced from the growing body of evidence suggesting that the expression of genes that encode certain proteins involved in neurosecretion might be modulated by induction of synaptic activity. For example, induction of long-term potentiation in rat dentate gyrus induces an increase in syntaxin 1B (Helme-Guizon et al., 1998). Also, activation of P/Q-type Ca^{2+} channels activates syntaxin 1A expression in cultured rat cerebellar granular cells (Sutton et al., 1999). The effect of syntaxin on a Kv channel, described in this study, is characterized by a biphasic dependence on syntaxin concentration: at low concentrations it causes an increase in K^+ efflux (because of increased amplitudes), and at higher concentrations it causes a reduction of K^+ efflux (because of decreased amplitudes and increased inactivation). Taken together, we suggest that the interaction of presynaptic Kv channels with the exocytotic machinery may serve to clamp a given synaptic efficacy. At low synaptic activity, the level of syntaxin being relatively low, interaction of the channel with syntaxin results in high K^+ efflux that serves to preserve low synaptic activity. On induction of enhanced synaptic activity, the level of expression of syntaxin increases, and its interaction with the channel results in low K^+ efflux, favoring enhanced synaptic activity. Furthermore, high levels of syntaxin expression are accompanied by downregulation of presynaptic voltage-gated Ca^{2+} channel activity (see introductory remarks), which may act in concert with the downregulation of K^+ channel activity to fine-tune synaptic efficacy.

Finally, it should be noted that both syntaxin 1A (Sesack and Snyder, 1995) and Kv1.1 (Sheng et al., 1993) are localized also to nonsynaptic regions of axons, raising the possibility of a role for syntaxin–channel interaction in axonal excitability.

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