TrkB Activation by Brain-derived Neurotrophic Factor Inhibits the G Protein-gated Inward Rectifier Kir3 by Tyrosine Phosphorylation of the Channel*

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G protein-activated inwardly rectifying potassium channels (Kir3) are widely expressed throughout the brain, and regulation of their activity modifies neuronal excitability and synaptic transmission. In this study, we show that the neurotrophin brain-derived neurotrophic factor (BDNF), through activation of TrkB receptors, strongly inhibited the basal activity of Kir3. This inhibition was subunit dependent as functional homomeric channels of either Kir3.1 or Kir3.4 were significantly inhibited, whereas homomeric channels composed of Kir3.2 were insensitive. The general tyrosine kinase inhibitors genistein, Go¨ 6976, and K252a but not the serine/threonine kinase inhibitor staurosporine blocked the BDNF-induced inhibition of the channel. BDNF was also found to directly stimulate channel phosphorylation because Kir3.1 immunoprecipitated from BDNF-stimulated cells showed enhanced labeling by anti-phosphotyrosine-specific antibodies. The BDNF effect required specific tyrosine residues in the amino terminus of Kir3.1 and Kir3.4 channels. Mutations of either Tyr-12, Tyr-67, or both in Kir3.1 or mutation of either Tyr-32, Tyr-53, or both of Kir3.4 channels to phenylalanine significantly blocked the BDNF-induced inhibition. The insensitive Kir3.2 was made sensitive to BDNF by adding a tyrosine (D41Y) and a lysine (P32K) upstream to generate a phosphorylation site motif analogous to that present in Kir3.4. These results suggest that neurotrophin activation of TrkB receptors may physiologically control neuronal excitability by direct tyrosine phosphorylation of the Kir3.1 and Kir3.4 subunits of G protein-gated inwardly rectifying potassium channels.

Neurotrophins are a family of growth factors that include nerve growth factor, BDNF,1 NT3, and NT-4/5 (1) and activate receptor tyrosine kinases (Trk) to regulate neuronal survival and differentiation during brain development (2). Neurotrophins also rapidly modulate neuronal excitability to regulate synaptic plasticity in the hippocampus (3–7), plasticity of spinal cord neurons in models of chronic pain (8), and excitability of cortical neurons (9). The mechanisms of these neuronal effects on excitability are not yet known; however, BDNF was shown to rapidly modulate sodium channels in the CA1 region of the hippocampus (3) and to enhance synaptic currents in hippocampal postsynaptic neurons (6). These studies suggest that BDNF has direct effects on ion channel properties to modulate synaptic activity.

The neurotrophin receptors are transmembrane tyrosine kinases, and BDNF activation of the TrkB receptor is known to initiate a cascade of phosphorylation events that activate a complex of signaling proteins (10). Tyrosine kinases directly phosphorylate ion channels to provide rapid regulation of neuronal excitability (11–18). Tyrosine kinase activation by G protein-coupled receptors (19) also suppresses delayed rectifying potassium channels by phosphorylation of a tyrosine residue in the amino terminus of Kv1.2 (20). Similarly, phosphorylation of serine residues in the amino terminus of a different delayed rectifying potassium channel Kv3.4 causes channel inactivation (21). Additionally, tyrosine phosphorylation of other potassium channels may regulate neuronal excitability. Because G protein-coupled receptor activation of Kir3 type potassium channels is one of the major mechanisms controlling neuronal excitability, we explored the hypothesis that BDNF regulation of Kir3 may control neuronal excitability by modulation of these channels. Our results show that BDNF inhibits basal Kir3 channel activity and define specific tyrosine phosphorylation sites in the amino terminus of Kir3 that are important for channel inhibition caused by TrkB activation.

EXPERIMENTAL PROCEDURES

Complementary DNA Clones and mRNA Synthesis—cDNAs for the Kir3.1 (GIKR 1) (GenBank accession no. U01071) and Kir3.2 (GIKR 2) (GenBank accession no. U11859) were obtained from Drs. Cesar Leb-arca and Henry Lester. Kir3.4 (GIKR 4) was provided by Dr. John Adelman (GenBank accession no. X83584). TrkB (GenBank accession no. M55293) was obtained from Dr. Mark Bothwell. Rat κ opioid receptor (KOR) was obtained from Dr. David Grandy (GenBank accession no. D16829). Point mutations to produce functional homomeric channels, Kir3.1(F137S) (22), Kir3.2(S146T), and Kir3.4(S143T) (23), were produced. Mutations were introduced by polymerase chain reaction amplification using Pfu Turbo DNA polymerase with complementary oligonucleotide primers incorporating the desired mutation. Positive clones were confirmed by automated sequencing. Plasmid templates for constructs were linearized prior to in vitro mRNA synthesis (24) using mMessage Machine (Ambion Inc.).

Oocyte Maintenance and Injection—Healthy stage V and VI oocytes were harvested from marine anesthetized Xenopus laevis (Xenopus Express, Gainesville, FL) and defolliculated enzymatically as described (25). The oocytes were maintained at 18 °C in standard oocyte buffer and
were bathed in normal oocyte saline buffer (ND96) containing 2 mM KCl. Oocytes were injected with 10 ng of Kir3.1, 1 ng of Kir3.4, and 0.08 ng of TrkB mRNA. After 4 days of protein expression, groups of 25 oocytes received either ND96 or BDNF treatment (400 ng/ml) for 15 min and were then frozen at −70 °C. Oocyte membranes were extracted with 100 μl of solubilization buffer (1% SDS, 25 mM Tris, pH 7.4, 150 mM NaCl, 2 mM EDTA), plus general protease inhibitors (1 μg/ml leupeptin, 2 μg/ml apronin, 200 μM phenylmethylsulfonyl fluoride) and the tyrosine phosphatase inhibitor perthovanadate (1 μM). The preheated (55 °C) solubilization solution was applied to frozen intact oocytes for oocyte membrane solubilization and agitated until the outer surface pigment layer was removed. This unpublished method was developed by Abraham Kovoor while a member of the Chavkin laboratory. To immunoprecipitate Kir3.1, the oocyte membrane protein was incubated with 35 μg of an affinity-purified antibody directed against carboxyl-terminal residues 482–498 of rKir3.1 (26). The membrane/antibody complex was precipitated with 7.5 μg of Protein A-Sepharose, washed three times with radioimmunoassay buffer (25 mM Tris, pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100), and washed once with 15 mM Tris, pH 7.4, each containing protease inhibitors and perthovanadate as described above. The immunoprecipitate was extracted using sample buffer (50 mM Tris, pH 6.8, 500 mM β-mercaptoethanol, 2% SDS, 0.1% bromphenol blue, 10% glycerol). The protein samples were resolved by SDS-polyacrylamide gel electrophoresis, transferred to 0.2-μm nitrocellulose membranes, and then blocked overnight in 5% nonfat dry milk in Tris-buffered saline. Membranes were first blotted with a bioitin-conjugated PY:20 anti-phosphotyrosine antibody (1:7500 dilution, ICN Biomedical), followed by a horseradish peroxidase-conjugated avidin secondary antibody (ICN Biomedical). Immunoreactivity was visualized using ECL detection methods (NEN Life Science Products). The results shown were replicated three times.

Materials—Genistein, staurosporine, phorbol 12-myristate 13-acetate (PMA), and Me2SO were from Sigma. Gö 6976 and K252a were obtained from Calbiochem, San Diego, CA. U0126,980 was obtained from Research Biochemicals International. EFu Turbo was from Strategene, La Jolla, CA. PMA, Gö 6976, genistein, and K252a were dissolved in Me2SO; the final concentration of Me2SO applied to the oocytes was ~0.02%. BDNF (Amgen, Thousand Oaks, CA) was dissolved in water and stored at −70 °C until use.

Statistical Analysis—Data are presented as means ± S.E. Statistical analysis was carried out using an unpaired t test. A probability of p < 0.05 was considered statistically significant.

RESULTS

BDNF Effects on Kir3 Channels—Brief treatment of Xenopus oocytes expressing TrkB and Kir3 heteromultimers composed of either Kir3.1/3.2 or Kir3.1/3.4 with BDNF (200 ng/ml) produced a strong depression of the channel current (Fig. 1). BDNF suppressed Kir3.1/3.2 by 62 ± 1% (n = 17) and Kir3.1/3.4 by 70 ± 1% (n = 15). The same oocyte was then perfused with BDNF (200 ng/ml) for 10–15 min, then retested with 24 mM KCl. The basal current in ND96 was subtracted from both curves. B, potassium currents were measured in separate oocytes either pretreated with 200 ng/ml BDNF for 10–15 min or not pretreated. Responses were then normalized as a percentage of the mean response produced in untreated oocytes from the same batch and injection day. Bars represent mean ± S.E. from three independent experiments (n = 4–6). C, the inhibitory effects of BDNF on Kir3.1/3.4 were characterized by constructing a dose-response curve. Groups of oocytes received either no treatment or BDNF (5, 50, 200, or 500 ng/ml) (n = 3–4, each dose). Potassium currents in 24 mM KCl buffer were normalized as a percentage of the average response obtained from untreated oocytes of the same group.
Tyrosine Phosphorylation Inhibits Kir3

Fig. 2. Tyrosine kinase inhibitors G0 6976, genistein, and K252a blocked the BDNF-induced suppression of Kir3 channel currents, whereas the Ser/Thr kinase inhibitor staurosporine did not. Oocytes injected with TrkB, Kir3.1, and Kir3.2 mRNA were treated with either ND96 or kinase inhibitor for 25–30 min and then treated with ND96 (Basal) or 200 ng/ml BDNF in the continued presence of inhibitor for an additional 10–15 min. Data are the percentage of mean Kir3 current measured in untreated oocytes from the same batch. Pretreatment with the tyrosine kinase inhibitors G0 6976 (100 nM), genistein (100 μM), or K252a (20 μM) for 25 min significantly blocked the BDNF effect (p < 0.05; n = 8–10), whereas staurosporine (1 μM) did not (n = 7) (p > 0.05). The effects of BDNF on oocytes after treatment with G0 6976, genistein, or K252a were not significantly different from control oocytes treated with inhibitor alone. Bars represent mean ± S.E. from two independent experiments. As a control for the effectiveness of staurosporine, control oocytes were treated with 100 nM PMA for 10 min. PMA produced significant inhibition of the Kir3.1/3.2 current, and the PMA-induced inhibition was blocked by pretreatment with 1 μM staurosporine (n = 7). (*, p < 0.05).

3.4 by 70 ± 1% (n = 16). As shown in the current-voltage curve (Fig. 1A, inset), BDNF reduced the total conductance through Kir3.1/3.2 channels; similar results were found for Kir3.1/3.4 (data not shown). The effect of BDNF required co-expression of the TrkB receptor (Fig. 1B). The dose-response analysis (Fig. 1C) showed that the potency of BDNF in this system (EC50 = 20 ng/ml) was consistent with prior findings (27).

TrkB activation results in an increase in tyrosine kinase activity and the subsequent activation of a cascade of kinase effectors (10). To determine if the suppression of the Kir3 current was initiated by the tyrosine kinase activity, oocytes were pretreated for 25 min with the nonspecific tyrosine kinase inhibitor G0 6976 (100 μM) (28), genistein (100 μM) (29), or K252a (20 μM) (30) prior to treatment with BDNF and inhibitor.

Pretreatment with G0 6976 completely blocked the effects of BDNF. Similarly, genistein or K252a pretreatment also completely blocked the effects of BDNF treatment. In contrast, the serine/threonine kinase inhibitor staurosporine (1 μM) was ineffective (Fig. 2). The results suggest that the BDNF-induced suppression of the Kir3 current required activation of the tyrosine kinase, but not an intervening serine/threonine phosphorylation event.

The BDNF-induced suppression could either result from a direct phosphorylation of Kir3 or an indirect effect on channel activation mechanisms. The Kir3 channels normally assemble as heteromers. To advance our analysis of the mechanism, we next determined if the channels formed from subunits of single Kir3 isoforms were sensitive to the BDNF effect. To do this, we used the strategy developed by Logothetis and colleagues (22, 23) to generate functional homomeric Kir3 channels. Mutations in the putative pore of these channels, Kir3.1(F137S) and Kir3.4(S143T) greatly increased expression and activity of the channel homomers. We generated a point mutation in Kir3.2(S146T), and we found that this change also increased current compared with wild-type Kir3.2, which normally forms a homomeric channel. Moreover, this point mutation of Kir3.2(S146T) served as a control, as this change was similar to Kir3.1(F137S) and Kir3.4(S143T). The homomeric Kir3.1(F137S) was as strongly inhibited by BDNF treatment as was the Kir3.1/3.2 heteromeric channel (Fig. 3A). The homomeric Kir3.4(S143T) was also highly sensitive to BDNF treatment. In contrast, neither wild-type Kir3.2 nor Kir3.2(S146T) were affected by BDNF treatment. The channel type selectivity

Fig. 3. BDNF and PMA effects on different combinations of Kir3 channel subunits. A, the effects of BDNF treatment were measured in oocytes injected with 0.004 ng of TrkB mRNA plus the following: 0.05 ng of Kir3.1 and 0.05 ng of Kir3.2, 1.0 ng of wild type Kir3.2 alone, 1.0 ng of Kir3.1(F137S), Kir3.2(S146T), or Kir3.4(S143T). In each case, the effects of BDNF treatment were compared with untreated oocytes injected with the same mRNA. The effects of treatment with 200 ng/ml BDNF on Kir3.1/3.2 served as a positive control. BDNF treatment effectively reduced the Kir3 current for each channel except the Kir3.2 and Kir3.2(S146T). For the Kir3.1 and Kir3.2 heteromultimer, the current was suppressed 65 ± 5% (n = 10) by BDNF treatment compared with untreated oocytes. Kir3.1(F137S) was suppressed 61 ± 4% (n = 16) by BDNF, a result not significantly different from the BDNF effect on the Kir3.1 and Kir3.2 combination. Kir3.2(S146T) was only reduced by 16 ± 5% (n = 61) after BDNF treatment. Because the wild type Kir3.2 also forms homomeric channels in Xenopus oocytes, we tested its sensitivity to BDNF. For wild type Kir3.2, the potassium current after BDNF treatment was 100 ± 3% (n = 10) of control. BDNF treatment suppressed Kir3.4(S143T) current by 85 ± 2% (n = 11). B, in contrast to the selective effects of BDNF on Kir3 channel subtypes, activation of protein kinase C by PMA reduced the potassium currents mediated by each of the Kir3 channels. Oocytes were pretreated for 15 min in 100 nM PMA, and then the potassium current in 86 mM K+ buffer was measured. Significant inhibition of the basal response was observed in all three homomeric channels: PMA treatment suppressed Kir3.1(F137S) by 65 ± 13% (n = 9), suppressed Kir3.2(S146T) by 78 ± 6% (n = 7), and suppressed Kir3.4(S143T) by 91 ± 2% (n = 14). Each group was compared with its own control with that day’s matched group. Bars represent mean ± S.E. from two independent replicates.
Tyrosine Phosphorylation Inhibits Kir3

FIG. 4. Immunodetection of tyrosine phosphorylation of Kir3.1 after BDNF treatment. The 45-kDa protein band corresponding to the immunoprecipitated Kir3.1 showed a substantial increase in phosphotyrosine immunoreactivity following BDNF treatment.

Evident from this experiment supports the hypothesis that the BDNF-induced suppression of the current was caused by a direct modification of the channel.

Because protein kinase C activation also suppresses Kir3 conductance (31, 32), we compared the effects of BDNF with those of PMA. Control oocytes expressing Kir3.1(F137S), Kir3.2(S146T), or Kir3.4(S143T) homomeric channels were compared with matched oocytes preincubated in PMA (100 nM, 10–15 min). PMA produced significant inhibition of the potassium current for all three channels (Fig. 3B). The lack of selectivity of PMA effects contrasts sharply with BDNF. Thus, we conclude that the channel subtype-specific effects of TrkB activation were not mediated by protein kinase C activation. This is consistent with the lack of effect of staurosporine described above.

To test the hypothesis that the activated TrkB had a direct effect on the Kir3 channel, cells expressing TrkB and Kir3.1/3.4 were either pretreated with BDNF (400 ng/ml, 15 min) or pretreated in vehicle. Membrane proteins were solubilized, and equivalent amounts of protein were immunoprecipitated with an affinity-purified antibody against Kir3.1 (26). A 45-kDa protein band corresponding to the immunoprecipitated Kir3.1 showed a substantial increase in phosphotyrosine immunoreactivity following BDNF treatment. The 45-kDa band was not evident in the lane loaded with an equal amount of Kir3.1 isolated from untreated oocytes expressing Kir3.1/Kir3.4 and TrkB (Fig. 4). These results suggest that BDNF-induced TrkB activation causes direct tyrosine phosphorylation of the Kir3.1 subunit of the channel.

Identification of Potential Phosphorylation Sites—Potential sites of tyrosine phosphorylation in the Kir3 sequence were first studied by making an amino-terminal truncation of Kir3.4(S143T) (Fig. 5). Using a polymerase chain reaction-based amplification, we prepared a cDNA template for mRNA encoding a Kir3.4(S143T) lacking amino acid residues 1–57. Oocytes expressing the truncated Kir3.4(S143T) produced strong potassium currents that were not significantly (p > 0.05) suppressed by BDNF treatment. After BDNF treatment, the mean current was 90 ± 11% (n = 8) of control compared with untreated oocytes expressing truncated Kir3.4(S143T) (Fig. 6A). Two tyrosine residues within the Kir3.4 affected by the amino-terminal truncation and are noted as potential phosphorylation sites A and B (Fig. 5B). Using a sequence alignment program (BCM Search Launcher, Baylor College of Medicine, Houston, TX), we compared the sequence motifs surrounding site A of Kir3.1, Kir3.2, and Kir3.4. Sequence alignment analysis indicated that Kir3.1 and Kir3.4 both have tyrosine residues in the amino terminus that align with aspartic acid 41 of Kir3.2 (Fig. 5B). A tyrosine was present in all three channels in site B (Fig. 5B). To test whether either or both tyrosine residues of Kir3.1 and Kir3.4 were sites of BDNF-induced tyrosine phosphorylation, tyrosine residues 12 and 67 in the Kir3.1 sequence and 32 and 53 in the Kir3.4 sequence were mutated to phenylalanine. In contrast to the Kir3.4(S143T), BDNF did not significantly (p < 0.05) inhibit the conductance of Kir3.4(S143T/Y32F) (Fig. 6A). After BDNF treatment, the current was 80 ± 14% (n = 14) of the untreated current, not significantly different from control currents 100 ± 23% (n = 8) (p > 0.05). The same loss of BDNF sensitivity was observed with channels having tyrosine 32 mutated to aspartic acid to produce Kir3.4(S143T/Y32D) (data not shown). Mutation of the other tyrosine in the amino terminus of Kir3.4(S143T/Y53F) also significantly (p < 0.05) reduced the sensitivity to BDNF; BDNF treatment caused only a reduction to 74 ± 5% (n = 11) of the control, untreated currents. As expected, the deletion of both amino-terminal tyrosines to produce Kir3.4(S143T/Y32F/Y53F) also significantly blocked (p < 0.05) the effect of BDNF treatment 112 ± 8% (n = 15) (Fig. 6A). These results suggest that tyrosine residues in both of these sites in the amino terminus of Kir3.4 (S143T) are critical for sensitivity to BDNF and are likely sites of tyrosine phosphorylation on Kir3.4.

For the Kir3.1(F137S) homomeric channel, mutation of tyrosine 12 to phenylalanine, Kir3.1(F137S/Y12F), completely blocked the BDNF effect 95 ± 12% (n = 23) (Fig. 6B). Mutation of tyrosine 67, Kir3.1(F137S/Y67F), effectively reduced the BDNF effect. After BDNF treatment, the current through Kir3.1(F137S/Y67F) was 78 ± 4% (n = 16) (p < 0.05). Deletion of both tyrosines from the amino terminus to produce Kir3.1(F137S/Y12F/Y67F) also completely blocked the BDNF effect; current was 75 ± 7% (n = 12) after treatment with BDNF, a result not significantly different than the single point mutations (p < 0.05) (Fig. 6B). The results suggest that TrkB-induced phosphorylation of either Tyr-12 or Tyr-67 in Kir3.1 mediated the BDNF effect on the channel. Removal of the tyrosines 12 and 67 of Kir3.1 did not block the substantial increase in phosphotyrosine immunoreactivity after BDNF treatment (data not shown). Because Kir3.1 has 14 potential...
Tyrosine Phosphorylation Inhibits Kir3

To determine the basis for Kir3.2(S146T) insensitivity to BDNF, the sequences of Kir3.1 and Kir3.4 were compared (Fig. 5B). An aspartic acid in the BDNF-insensitive channel Kir3.2(S146T) (Asp-41) aligned with the tyrosine in the BDNF-sensitive Kir3.4(S146T) (Tyr-32). Unexpectedly, the mutation of aspartic acid to tyrosine to produce Kir3.2(S146T/D41Y) did not confer sensitivity to BDNF (Fig. 7). The presence of proline in site A of Kir3.2 instead of a lysine as in Kir3.4 (Fig. 5B) was a potentially significant difference between the two channels. Consistent with other tyrosine phosphorylation motifs (33), the positively charged lysine Kir3.4 (Lys-23) may be permissive for a tyrosine phosphorylation event on Kir3.4. In confirmation of this hypothesis, the conductance of the amino terminus combined with a nearby cluster of positively charged residues seem to be required.

A reduction in channel conductance may be caused either by reduced current through existing channels or by loss of channel from the plasma membrane. To distinguish between these two alternatives, we tested the effects of channel activation by G protein-coupled receptor stimulation. We expressed the rat κ opioid receptor (KOR), TrkB, Kir3.1, and Kir3.4 heteromultimers (34, 35). Pretreatment with BDNF inhibited the channel conductance as shown previously (Fig. 8). In contrast, the stimulation of Kir3 current by a maximally effective concentration of the κ receptor by U69,593 was not inhibited by BDNF pretreatment (Fig. 8). The results suggest that BDNF-induced phosphorylation shifts the channel from a basally active to a basally inactive state but does not prevent Gβγ activation of the channel.

**FIG. 7.** The effect of addition of tyrosine and alteration of the surrounding charge in the amino terminus of Kir3.2(S146T). A, oocytes were injected with 0.004 ng of TrkB and the following: 0.05 ng of Kir3.4(S146T), 0.05 ng of Kir3.4/S146T/Del 1–57, 1 ng of Kir3.4(S143T/Y32F), 1 ng of Kir3.4(S143T/Y53F), or 1 ng of Kir3.4(S143T/Y32F/Y53F). Oocytes were pretreated for 10–15 min with BDNF (200 ng/ml). Bar graph summarizes the effects of BDNF compared with untreated controls from the same batch (*, p < 0.05) (+ t test with value compared with untreated, control oocytes: p < 0.05). As a control for the BDNF, experiments with the insensitive Kir3.2 (S146T) were performed on the same day as experiments with BDNF-sensitive Kir3.1/3.2 channels.

**FIG. 6.** The effect of mutation of Tyr in the amino terminus of Kir3.4(S143T) and Kir3.1(F137S). A, oocytes were injected with 0.004 ng of TrkB and the following: 0.05 ng of Kir3.4(S143T), 0.05 ng of Kir3.4/S143T/Del 1–57, 1 ng of Kir3.4(S143T/Y32F), 1 ng of Kir3.4(S143T/Y53F), or 1 ng of Kir3.4(S143T/Y32F/Y53F). Oocytes were pretreated for 10–15 min with BDNF (200 ng/ml). Bar graph summarizes the effects of BDNF compared with untreated controls from the same batch. B, oocytes were injected with 0.004 ng of TrkB and the following: 0.05 ng of Kir3.1(F137S), 1 ng of Kir3.1(F137S/Y12F), 1 ng of Kir3.1(F137S/Y67F), or 1 ng of Kir3.1(F137S/Y12F/Y67F). Oocytes were presoaked for 10–15 min BDNF (200 ng/ml). Bar graph summarizes the effect of BDNF on homomeric channels compared with its own control from that day’s matched group. Data are means ± S.E. from 4–7 oocytes and two to three independent experiments (*, p < 0.05) (Φ t test with value compared with untreated, control oocytes: p < 0.05).
Tyrosine Phosphorylation Inhibits Kir3

should be phosphorylated following TrkB activation. By introducing a second tyrosine phosphorylation site in the amino terminus of Kir3.2 and adding an essential basic charge near the phosphorylation site, Kir3.2 became sensitive to BDNF inhibition. We suggest that the change in charge created by the consensus site mutant enhanced the ability of tyrosine kinase to phosphorylate the Kir3.2(D41Y) tyrosine mutation. This reinforces the idea that tyrosine kinase phosphorylation is dependent upon charge interactions surrounding the phosphorylation site (33, 38). Alternatively, Kir3.2 may be insensitive because the phosphorylation site (Fig. 5B) does not interact with a complementary domain required for channel inhibition. A clearer understanding of the biophysical basis for the phosphotyrosine inhibition of the channel conductance is required before we can understand why Kir3.2 is insensitive to BDNF inhibition.

As is evident from the surrounding sequences, all three BDNF-sensitive Kir3 channels have charged amino acids (aspartic acid or arginine) on the amino side of the tyrosine and a hydrophobic residue on the carboxyl side. This general motif is characteristic of other sites phosphorylated by protein tyrosine kinases (33, 38); however, the motif is not sufficiently specific to allow the identification of the kinase involved. Although the specific kinase mediating the Kir3 inhibition was not defined in this study, the Src tyrosine kinase, Fyn, is present in oocytes, associates with TrkB and mediates some of the tyrosine phosphorylation events following TrkB activation (39).

The conductance of the G protein-gated inwardly rectifying potassium channel is controlled by a wide range of regulatory factors, as would be expected for a ubiquitous channel having a key role in the regulation of neuronal excitability. Kir3 is activated by Gβγ, phosphotyidylinositol 4,5-bisphosphate (PIP2), and sodium binding and inhibited by protein kinase phosphorylation (40). Logothetis and colleagues suggest that PIP2 binding generally regulates the inwardly rectifying potassium channels; the Kir3 channel interaction with PIP2 additionally requires both sodium and Gβγ binding to the carboxyl terminus of the channel (41). Our results show that U69,593 activation of the α opioid receptor, which acts by increasing Gβγ concentration, overcomes the basal inhibition of the conductance caused by BDNF treatment. This result shows that the tyrosine phosphorylated Kir3 channel still can be activated by PIP2, sodium, and Gβγ binding. We suggest that BDNF-induced phosphorylation of Kir3 causes a conformational change in the channel that reduces the ability of intrinsic activators to basally activate the channel. The observation that Gβγ, increased by α receptor activation, can overcome the BDNF effect suggests that the inhibition by phosphorylation is independent of the activation by endogenous regulators PIP2, sodium, and Gβγ.

Evidence suggests that growth factors have diverse effects on ion channels. For example, nerve growth factor regulates both the number and distribution of delayed rectifying K+ channels in PC 12 cells (42). However, growth factors can also have extremely selective effects on ion channels. The growth factors FGF1 and FGF2 have opposing effects in the regulation of Kir in cardiac myocytes via distinct second messenger pathways (43). Our data demonstrate that the growth factor BDNF selectively modulates Kir3 through a unique pathway. That growth factors can control both ion channel distribution and regulation suggests that BDNF modulation of Kir3 may be a determinant in synaptic transmission. The results in this study suggest an additional mechanism for BDNF regulation of ion channel conductance, an important mechanism regulating neuronal excitability. Our analysis utilized a malleable expression system to define a novel regulatory mechanism and predict a physiological relationship between neurotrophin action and potassium channel function.

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Fig. 8. The effect of BDNF on Gβγ activation of the α opioid receptor. A, the effects of BDNF treatment were measured in oocytes injected with 1.0 ng of the α opioid receptor, 0.004 ng of TrkB mRNA, 0.05 ng of Kir3.1, and 0.05 ng of Kir3.4. Panel gives representative current traces from oocytes expressing KOR, Kir3.1/3.4, and TrkB during perfusion with 24 mM KCl followed by KOR activation by the selective agonist U69,593 (500 nM). B, bar graph summarizing the effects of BDNF on the channel response to 24 mM KCl and the additional response caused by KOR activation with the selective agonist U69,593 (500 nM) compared with matched controls not treated with BDNF. Responses were then normalized as a percentage of the mean response to either KCl or U69,593 (500 nM) produced in untreated oocytes from the same batch and injection day. Data are from 5–6 oocytes and two independent experiments.
Tyrosine Phosphorylation Inhibits Kir3

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