The Gαo/i-coupled Cannabinoid Receptor-mediated Neurite Outgrowth Involves Rap Regulation of Src and Stat3*

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The study of the signaling pathways regulating neurite outgrowth in culture is important because of their potential role in neuronal differentiation in vivo. We have previously shown that the Gαo/i-coupled CB1 cannabinoid receptor (CB1R) activates Rap1 to induce neurite outgrowth. Gαo/i also activates the Src-Stat3 pathway. Here, we studied the relationship between the Gαo/i-Rap1 and Src-Stat3 pathways and the role of these signaling pathways in CB1R-mediated neurite outgrowth in Neuro-2A cells. The CB1 agonist HU-210 induced pertussis toxin-sensitive Src and Stat3 phosphorylation. Dominant negative (DN) mutants of Src and Stat3 blocked CB1R-induced neurite outgrowth. Constitutively active Rap 1B and RaI-activated Src and CB1R-induced Src phosphorylation was inhibited by Rap1-DN and RaI-DN, indicating that both Rap1 and RaI mediate downstream signaling from Gαo/i for Src activation. Rap1-activated RaI and RaI-DN blocked Rap-induced Src phosphorylation. Gαo/i-induced Stat3 activation was blocked by RaI-DN, whereas v-Src-induced Stat3 activation was not inhibited by RaI-DN, indicating that the CB1R, through Gαo/i mediates the sequential activation of Rap1 to RaI to Src to Stat3 in Neuro-2A cells. Downstream of Src, the CB1R also activated Rac1 and JNK, which enhanced CB1R-mediated Stat3 activation. Rac-DN blocked CB1R-induced activation of JNK. Pharmacological inhibition of JNK blocked Src and CB1R activation of Stat3, indicating that Rac and JNK are also involved in CB1R-mediated neurite outgrowth. Overall, this study demonstrated that Gαo/i-coupled CB1R triggers neurite outgrowth in Neuro-2A through the activation of a signaling network containing two pathways that bifurcate at Src and converge at Stat3.

The differentiation process in neurons is a complex phenomenon involving changes in electrophysiological properties as well as morphological features characterized by dendritic and axonal outgrowths, broadly termed neurite outgrowth. The capacity to elaborate processes has achieved its maximal complexity in neurons, which utilize dendritic arbors to regulate the number of synaptic contacts and thus define the frequency at which impulses spread and thereby establish regionally distinct functional outputs (1). The regulation of neurite outgrowth is tightly controlled because of its critical physiological function. There are many signals that control the growth and directionality of dendrites and axons (2, 3). Many intracellular signaling components that control neurite outgrowth have been studied (4). However, their connections into networks have not been fully defined. Several G protein-coupled receptors trigger neurite outgrowth. For example, D2 dopamine receptors regulate neurite outgrowth in cortical neurons (5). Serotonin-1 B receptors are known to enhance neurite outgrowth in thalamic neurons (6). These receptors are coupled to Gαi/o proteins, and Gαi/o one of the most abundant proteins in the neuronal growth cones, can induce neurite outgrowth (7). These studies indicate that Gαi/o-coupled receptors play an important role in controlling neurite outgrowth. However, the signaling pathways by which the Gαi/o signals trigger neurite outgrowth have not been determined. Recently, we demonstrated that the CB1 cannabinoid receptor, which is coupled to Gαi/o, mediates neurite outgrowth through activation of Rap1 by enhancing proteasomal degradation of Rap1GAP (8, 9). In a previous study, we showed that Gαi/o activates the c-Src-Stat3 pathway in NIH-3T3 fibroblast cells leading to cell transformation (10). Because c-Src is also an abundant protein in neuronal growth cones, we wondered as to the relationship between Rap and c-Src in Gαi/o neurite formation and outgrowth. In this study, we showed that CB1 receptor activation leads to the Rap-dependent phosphorylation of Src and Stat3, and this network plays a critical role in neurite outgrowth. The network includes the small GTPases, such as Ral and Rac, as well as protein kinases such as JNK3/p38 that are involved in this process. These studies showed, within a fully endogenous system, the presence of a two-armed complex network that contains multiple small GTPases converging on Stat3 to regulate neurite outgrowth.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—Neuro-2A cells were cultured in 44% Dulbecco’s modified Eagle’s medium, 44% F-12 medium, 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% glucose. Transfection was performed when cells were 75% confluent using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. The catalytic domain of Src was replaced by green fluorescent protein to create a dominant negative form of Src (provided by Dr. Pamela L. Schwartzberg, National Institutes of Health). A dominant negative mutant of Stat3 was created by mutation of the tyrosine phosphorylation site (Y-F) or mutation of the DNA binding site (DN EE(E)V/VV) (10). Dominant negative or constitutively active mutants for small G proteins (Rap1, Ral, and Rac) were purchased from the Guthrie laboratory (San Francisco, CA). 24 h after transfection, cells were plated onto 6- or 24-well plates and stimulated with the indicated concentrations of...
HU-210 (Sigma) for various times according to the experimental designs.

**Immunoblotting**—Cells were lysed in a buffer containing 1% Nonidet P-40, a protease inhibitor mixture (Sigma), and tyrosine/serine-threonine phosphorylation inhibitors, including sodium fluoride (30 mM), sodium orthovanadate (2 mM), and sodium pyrophosphate (10 mM). After determination of the protein concentration, the cell lysates were subjected to Western blot analysis using the following types of antibodies: anti-phospho-Stat3 monoclonal, anti-Stat3 polyclonal, anti-phospho-Src polyclonal (Cell Signaling Technology Inc.), and anti-total Src monoclonal (Upstate Biotechnology).

**Ral and Rac1 Activation Assays**—Activated Ral and Rac1 were measured by use of the RalGDS or Rho-GDS binding domain (Ral-GDS-RBD or RhoGDS-RBD) using the same method as described for Rap1 (9). Glutathione S-transferase (GST)-RalGDS-RBD and GST-RhoGDS-RBD fusion proteins prebound to glutathione beads were used to detect the active GTP-bound form of Ral and Rac1 in cell lysates in the presence of dithiothreitol and protease inhibitor mixtures. GTP-bound Ral and Rac1 were separated from beads by boiling the samples in loading buffer. The samples were then resolved by SDS-PAGE, transferred to nitrocellulose membrane, and subjected to Western blot analysis using antibodies for Ral and Rac1 (Santa Cruz Biotechnology).

**Stat3 Luciferase Assay**—Neuro-2A cells were transfected with the Ly6E Stat3-response luciferase reporter construct (10), Gαo or Src constructs, and with or without dominant negative mutant constructs for Ral and Rac in serum-free medium. The next day, cells were lysed in lysis buffer (Invitrogen), and luciferase activity was measured using a fluorometer. β-galactosidase activity was measured simultaneously as a control.

**Neurite Outgrowth Assay**—Neuro-2A cells were plated in 6-well plates. The cells displaying neurite outgrowth were those that had cellular projections of length two times greater than the cell diameter. For each culture condition, randomly chosen regions of the plate containing 100 cells were scored under a phase contrast microscope (Nikon TMS) (9). All assays were carried out in triplicate, and the indicated values are represented as mean ± S.E. (n = 3).
**Data Analysis**—The data are represented as mean ± S.E. For Western blot analysis, all experiments were repeated at least three times. Representative experiments are shown. Bands were quantified by densitometric analysis and expressed as mean ± S.E. Statistically significant differences were determined by unpaired Student’s t test. Significance was defined as *p* < 0.05.

**RESULTS**

HU-210 Induces Src and Stat3 Phosphorylation in Neuro-2A Cells by Activating the G<sub>a15</sub>-coupled CB1 Cannabinoid Receptor—Because we have previously shown that G<sub>a15</sub> mediates activation of the Src-Stat3 pathway in NIH-3T3 cells (10), we tested here whether activation of G<sub>a15</sub>-coupled CB1 receptor caused phosphorylation of Src/Stat3 in...
Neuro-2A cells. Treatment of Neuro-2A cells with HU-210 (a CB1 receptor agonist) led to a dose-dependent increase in the phosphorylation of Src and Stat3 (Fig. 1, A and B). This increase in phosphorylation was abolished by the CB1 receptor antagonist (SR1411716A) and by pretreatment with pertussis toxin, indicating that the effect is agonist-dependent and requires the Gi/o-coupled pathway (Fig. 1, C and D). These data indicate that Gi/o-coupled CB1 receptor activation leads to the activation of the Src-Stat3 pathway in Neuro-2A cells.

**Src/Stat3 Pathway Is Involved in the CB1 Receptor-mediated Neurite Outgrowth**—Because the CB1 receptor activates the Src/Stat3 pathway, we tested here whether Src/Stat3 activation is required for CB1 receptor-mediated neurite outgrowth. Neuro-2A cells were transfected with Src-DN or Stat3-DN to block the levels of activated Src and Stat3, respectively. Thereafter, the cells were stimulated with HU-210 at different concentrations as indicated (Fig. 2, A and B). We found that both Src-DN and Stat3-DN significantly inhibited HU-210-induced neurite outgrowth in a dose-dependent manner (Fig. 2, A and B). Transfection with a combination of Src-DN and Csk led to a more significant inhibition (Fig. 2C). These data indicate that the Src-Stat3 pathway is necessary for Gi/o-coupled CB1 receptor-mediated neurite outgrowth.

**Signal Flow from Rap1/Ral to Src/Stat3 Occurs upon Activation of the CB1 Receptor**—We have previously reported that the CB1 receptor-mediated neurite outgrowth requires Rap1 activation through proteasomal degradation of Rap1GAPII (9). Here we directly tested whether CB1 receptor-induced Src/Stat3 activation requires Rap1 activation. We also tested the role of Ral in CB1 receptor-induced Src phosphorylation, because it was found previously that Rap1 can activate Ral (11, 12) and Ral activates Src (13–15). We found that constitutively active mutants of Rap1 and Ral induced Src phosphorylation (Fig. 3A). We also found that constitutively active Rap1 and Ral in Neuro-2A cells blocked by Rac-DN (Fig. 5B). Finally, both Rap1-DN and Ral-DN abolished the effect of HU-210 on Src phosphorylation (Fig. 3C), indicating that both Rap1 and Ral are required for CB1 receptor-induced Src phosphorylation in Neuro-2A cells. Interestingly, Ral activation was not significantly affected by Src-DN (Fig. 4A). Also, Ral-DN did block Src-induced Stat3 activation, as it efficiently blocked HU and Gαi/o-induced Stat3 activation (Fig. 4B). These results indicate that Ral acts upstream of Src in the CB1 receptor/Gαi/o-induced signaling pathway in Neuro-2A cells.

**CB1 Receptor Activates Rac Downstream of Src in Neuro-2A Cells**—Rac1 was shown to play an important role in neurite outgrowth by regulating cytoskeletal structure (16, 17). We found that CB1 receptor activation led to the activation of Rac and this, in turn, was inhibited by Src-DN (Fig. 5A). v-Src also activated Rac in Neuro-2A cells (Fig. 5B). Furthermore, both v-Src and HU/Gαi/o-induced Stat3 activation were inhibited by Rac-DN (Fig. 5C), indicating that Rac acts downstream of Src in the CB1 receptor/Gαi/o-induced signaling pathway. Also, we confirmed that HU induced serine phosphorylation of Stat3, which is blocked by Rac-DN (Fig. 5D).

**Ral and Rac Mediate CB1 Receptor-induced Neurite Outgrowth**—Based on the above findings, both Ral and Rac1 are important mediators in the CB1 receptor-mediated signaling pathway. Therefore, we directly tested the role of Ral and Rac1 in the CB1 receptor-mediated neurite outgrowth. We found that Ral-DN (1 μg of plasmid in a 10-cm dish) completely blocked neurite outgrowth (Fig. 6A). Transfection of...
Rac-DN at 1 μg of plasmid in 10-cm dish caused significant cell toxicity. We found that the inhibitory effect of Rac-DN on neurite outgrowth depended on the amount of Rac-DN (100–300 ng) used for transfection (Fig. 6B). 90% of inhibition was observed at 300 ng of plasmid.

Role of Rac1-JNK in CB1 Receptor-mediated Stat3 Activation and Neurite Outgrowth—It is well known that Rac1 stimulates JNK activation (18, 19) and plays a critical role in maintaining cell-cell contacts as well as cytoskeletal structure (20). Here we examined the role of JNK in CB1 receptor-mediated neurite outgrowth. We found that treatment with HU-210 enhanced JNK phosphorylation (Fig. 7A). HU-210 also induced extracellular signal-regulated kinase phosphorylation (not shown) in Neuro-2A cells. Interestingly, the JNK/p38 inhibitor (SB-202190), but not the MAPK1,2 inhibitor (PD 098059), significantly inhibited HU-induced Stat3 activation (Fig. 7B) and neurite outgrowth (Fig. 7C). Furthermore, we confirmed that HU-induced JNK phosphorylation was blocked by Rac-DN, consistent with previous reports (18, 19). These data indicate a critical role of Rac1-JNK in Gαs/o-coupled CB1 receptor-mediated neurite outgrowth.

Temporal Relationship between the Small GTPases in CB1R Activation of Src and Stat3—The data from the persistently activated and dominant negative GTPases indicate a hierarchy in the organization of GTPases as signal flows from the CB1 receptor to Src to Stat3. Such a hierarchy predicts a temporal relationship between the GTPases. To determine this temporal relationship between the GTPases in CB1R network signaling, we conducted time course experiments for the activation of the various signaling components in HU-stimulated cells (Fig. 8). We found that the activation of Rap1 occurred by 5 min. Activation of Ral was also observable by 5 min and reached maximum by 10 min. Src activation occurred by 10 min. Src-activated Stat3 tyrosine phosphorylation was also observable by 10 min and lasted for more than 2 h. Rac activation was the slowest and occurred by 30 min. Overall, the time course experiments support the hierarchy of components deduced from the previous experiments, although the reason for the delay for coupling between Src and Rac is not entirely clear.

DISCUSSION

G Protein Network and Neuronal Differentiation—Neurite outgrowth is a cellular phenomenon that mimics neuronal differentiation and regeneration in the organism. The pathways regulating neurite outgrowth in culture are likely to play a role in the terminal differentiation of neurons in vivo. We have previously shown that CB1 receptor-coupled Gαs/o mediates neurite outgrowth through the activation of Rap1 (9). In this study, we elucidated the downstream network that mediates the CB1 receptor/Gαs/o signaling to induce neurite outgrowth in...
Neuro-2A cells. It should be emphasized that all of the components in the network were native, and the perturbations we introduced were to block signal flow at various levels in the network.

FIGURE 6. Ral and Rac mediate CB1 receptor-induced neurite outgrowth. Neuro-2A cells were transfected with either pcDNA3 as control vector or constitutively active Ral 1A (1 µg of plasmid in a 10-cm dish), Ral-DN (1 µg of plasmid in a 10-cm dish) (A) or Rac-DN (100–300 ng in a 10-cm dish) (B). The cells were serum-starved for 24 h and then treated with or without 1 µM HU-210 for 16 h at 37 °C. Neurites were scored as described under “Experimental Procedures.” Mean ± S.E. of three experiments in triplicate are shown. Statistically significant differences in HU-induced neurite outgrowth with or without Ral-DN or Rac-DN (compared with vector control) are indicated. **, \( p < 0.001 \) (\( n = 3 \)).

FIGURE 7. JNK mediates CB1 receptor-induced Stat3 activation and neurite outgrowth. A, Neuro-2A cells were serum-starved for 24 h and then treated with or without 1 µM HU-210 for 30 min at 37 °C. The cells were lysed for Western blot analysis using anti-JNK/SAPK polyclonal or anti-actin monoclonal antibodies (Cell Signaling). A representative blot is shown (similar results were seen in two additional experiments). B, cells were transfected with Stat3 luciferase reporter and β-galactosidase, and after 24 h, they were treated with 1 µM SB-202190 or 10 µM PD098059 for 2 h followed by treatment with or without 1 µM HU-210 for 30 min at 37 °C. Luciferase activity was measured as described under “Experimental Procedures.” C, cells were serum-starved for 24 h and then pretreated with 1 µM SB-202190 or 10 µM PD098059 for 2 h followed by treatment with or without 1 µM HU-210 for 16 h at 37 °C. Cells with neurites more than twice the cell body were scored as positive. For both B and C, mean ± S.E. of three experiments in triplicate are shown. Statistically significant differences in HU-induced effects with or without SB202190 are indicated. *, \( p < 0.001 \) (\( n = 3 \)). D, after the cells were transfected with pcDNA3 or Rac-DN as described for 24 h, they were treated with HU-210 (1 µM) for 30 min. Thereafter, the cells were lysed for Western blot using antibodies specific for phospho-JNK and tubulin. The corresponding densitometric data are shown below the blots. **, \( p < 0.001 \) when cells treated with HU-210 are compared with non-treated cells, \( n = 3 \).

We found that activation of the CB1 receptor/Gαi/o leads to the activation of a cascade of signaling components, including Rap1, Ral, Src, Rac, and JNK, resulting in both Tyr and Ser phosphorylation of Stat3.
This network is schematically depicted in Fig. 9. Experiments in this study demonstrated that each of these components in the signaling network play a critical role in regulating CB1 receptor/Gαi/o-induced neurite outgrowth.

We clearly demonstrated a relationship between small GTPases and Src-Stat3 pathways in Neuro-2A cells. The relationship between small GTPases and the Src-Stat3 pathway has also been seen in other cell systems. Some studies show that Ral activates c-Src directly (13–15), whereas other studies suggest that Src can activate the Ras/Ral signaling pathway probably through C3G phosphorylation (21, 22), indicating the possible presence of a positive feedback loop. This configuration needs to be further studied to understand how intracellular signal processing at the level of small GTPases can prolong Src activation. Rap1/Ras activate Ral through the sequestration of Ral GDS to the membrane (11, 12), and Ral mediates Ras-induced c-Jun phosphorylation (14). Rap1 was also shown to stimulate integrin-mediated adhesion and spreading through activation of Rac1 (24), although it is not known how this activation occurs. Our results would suggest that Ral and Src serve as intermediaries in this activation. Previously, several studies have suggested that Ral activates Rac1 through Vav2 to regulate cell cytoskeletal structure (25–27). Rac1 is known to activate JNK, whereas Ras activates MAPK1,2 (28).

Important cellular functions, such as proliferation and differentiation (such as neurite outgrowth), are regulated by multiple pathways. In different cell types, cell proliferation can be regulated by the Ras-MAPK pathway or the Src-Stat3 pathway. These two pathways may co-exist in the same cell. In the case of proliferation, in one extensively studied model cell line, the NIH-3T3 fibroblasts, both the Ras-MAPK and the Src-Stat3 pathways can trigger proliferation (29, 30). This appears to be true for neurite outgrowth as well. Most analyses that use PC12 cells to identify pathways regulating neuronal process formation have focused on the Ras/MAPK1,2 pathway. It is well established that activation of the MAPK cascade can induce neurite outgrowth (31, 32). However, our studies indicate that the Src-Stat3 pathway can also induce neurite outgrowth. In this case, Rac-JNK, rather than MAPK1,2, plays an important role in CB1 receptor/Gαi/o-induced neurite outgrowth.

Small GTPases that play a major role in actin organization have been found to be involved in the regulation of neuronal morphology, including neurite outgrowth. This study showed that Rap1, through Ral, activates Src. Rap1 also functions in Src-related tyrosine kinase-induced neurite outgrowth in PC12 cells (33).

The role of Ral in neurite outgrowth appears to be contextual. Ralguanine nucleotide exchange factors and Ral activation inhibits Ras-mediated neurite outgrowth in PC12 cells (34). This inhibition may be due to Ral interaction with Ral-GDS, which also functions in the Ras pathway. In contrast, here we found that Ral mediates the CB1 receptor/Gαi/o-induced neurite outgrowth in Neuro-2A cells where it connects Rap to Src. Thus the same signaling component can have different
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A signaling cascade that activates Rac1 (16). The rhoG-Elmo-Dock180 pathway is required for activation of Rac1 and neurite outgrowth induced by nerve growth factor (17). The downstream components, including the transcription factors in this pathway, are currently not fully known. Src also mediates epidermal growth factor-induced neurite outgrowth independent of MAPK1,2 phosphorylation (36). It is possible that these effects of Src are mediated through Stat3. Here we found that Src plays a key role in CB1 receptor/Gαi/o-mediated neurite outgrowth.

The evidence for a critical role for Stat3 in neurite outgrowth has, thus far, not been conclusive. Activation of the Stat3 signaling pathway, but not the Ras/MAPK1,2-dependent pathway, is required for interleukin-6-induced neurite outgrowth in PC12 cells (37). However, an earlier study reports that Stat3 activation has a negative regulatory effect on nerve growth factor-stimulated neurite outgrowth (38). The molecular mechanisms underlying these differences have not been characterized. Our studies indicated a crucial role for Stat3 in mediating signals from the endogenous CB1 receptor present in Neuro-2A cells. Future studies will have to delineate the specific upstream signals that utilize Stat3-mediated transcription to induce neurite outgrowth and the genes regulated by Stat3 that are responsible for neurite outgrowth.

Relationship between Heterotrimeric G Proteins and Small GTPases and the Ability to Control Multiple Cellular Machines—All heterotrimeric G protein pathways contain one or more small GTPases downstream of the heterotrimeric G protein subunits (39). In the network studied here, we found a cascade of three GTPases involved in the signal flow from the heterotrimeric G proteins to the effectors, which in this case was the transcriptional regulator Stat3. Although one role of the GTPases is to communicate signals to Stat3, it was immediately obvious that these GTPases, such as Rap and Rac, could have other major effects, such as regulating cytoskeletal dynamics. Thus, each of these small GTPases can function as nodes within the signaling network where receptor signals can be split and routed to multiple cellular machines, such as the transcriptional machinery and the cytoskeletal machinery (23). Such routing to multiple machines can allow for integrated complex responses, such as neurite outgrowth. An interesting question that arises from such a regulatory configuration is the relationship between the machines. In this context, further studies should focus on Stat3-regulated genes that modulate cytoskeletal dynamics to induce neurite outgrowth.

In conclusion, we demonstrated a complex signaling network containing the small GTPases (Rap1, Rac, and JNK) and protein kinases Src and JNK that regulate Stat3 to mediate CB1 receptor-Gαi/o-induced neurite outgrowth in Neuro-2A cells. Our study provides evidence for multiple signaling pathways involved in regulating neurite outgrowth in one type of cell system that endogenously expresses the receptor and all of the signaling components.

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**FIGURE 9. Schematic of CB1 receptor/Gαi/o signaling network.** The downstream signaling network of the Gαi/o-coupled CB1 receptor in regulating neurite outgrowth in Neuro-2A cells is summarized. Activation of CB1 receptor/Gαi/o leads to the activation of a cascade of signaling components, including Rap1, Rac, Src, and JNK, leading to the activation of Stat3 and neurite outgrowth.

**TABLE 1. Effects of cannabinoid on neurite outgrowth.**

| Effects | Source |
|---|---|
| Neurite outgrowth | CB1 receptor/Gαi/o |
| Activation of Rap1, Rac, and JNK | CB1 receptor/Gαi/o |
| Activation of Stat3 | CB1 receptor/Gαi/o |
| Neurite outgrowth independent of MAPK1,2 phosphorylation | CB1 receptor/Gαi/o |
| Negative regulatory effect on nerve growth factor-stimulated neurite outgrowth | CB1 receptor/Gαi/o |

**FIGURE 2. Diagram of the signaling network.** The network involves multiple signaling pathways that converge on the transcription factors in this pathway. The transcription factors, including the transcription factors in this pathway, are currently not fully known. Src also mediates epidermal growth factor-induced neurite outgrowth independent of MAPK1,2 phosphorylation (36). It is possible that these effects of Src are mediated through Stat3. Here we found that Src plays a key role in CB1 receptor/Gαi/o-mediated neurite outgrowth.

**FIGURE 3. Schematic of the signaling cascade.** The signaling cascade that activates Rac1 (16). The rhoG-Elmo-Dock180 pathway is required for activation of Rac1 and neurite outgrowth induced by nerve growth factor (17). The downstream components, including the transcription factors in this pathway, are currently not fully known. Src also mediates epidermal growth factor-induced neurite outgrowth independent of MAPK1,2 phosphorylation (36). It is possible that these effects of Src are mediated through Stat3. Here we found that Src plays a key role in CB1 receptor/Gαi/o-mediated neurite outgrowth.

**FIGURE 4. Diagram of the signaling network.** The network involves multiple signaling pathways that converge on the transcription factors in this pathway. The transcription factors, including the transcription factors in this pathway, are currently not fully known. Src also mediates epidermal growth factor-induced neurite outgrowth independent of MAPK1,2 phosphorylation (36). It is possible that these effects of Src are mediated through Stat3. Here we found that Src plays a key role in CB1 receptor/Gαi/o-mediated neurite outgrowth.

**FIGURE 5. Schematic of the signaling cascade.** The signaling cascade that activates Rac1 (16). The rhoG-Elmo-Dock180 pathway is required for activation of Rac1 and neurite outgrowth induced by nerve growth factor (17). The downstream components, including the transcription factors in this pathway, are currently not fully known. Src also mediates epidermal growth factor-induced neurite outgrowth independent of MAPK1,2 phosphorylation (36). It is possible that these effects of Src are mediated through Stat3. Here we found that Src plays a key role in CB1 receptor/Gαi/o-mediated neurite outgrowth.

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