Reactive Oxygen Species Are Required for 5-HT-Induced Transactivation of Neuronal Platelet-Derived Growth Factor and TrkB Receptors, but Not for ERK1/2 Activation

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Abstract

High concentrations of reactive oxygen species (ROS) induce cellular damage, however at lower concentrations ROS act as intracellular second messengers. In this study, we demonstrate that serotonin (5-HT) transactivates the platelet-derived growth factor (PDGF) type β receptor as well as the TrkB receptor in neuronal cultures and SH-SY5Y cells, and that the transactivation of both receptors is ROS-dependent. Exogenous application of H₂O₂ induced the phosphorylation of these receptors in a dose-dependent fashion, similar to that observed with 5-HT. However the same concentrations of H₂O₂ failed to increase ERK1/2 phosphorylation. Yet, the NADPH oxidase inhibitors diphenyleneiodonium chloride and apocynin blocked both 5-HT-induced PDGFβ receptor phosphorylation and ERK1/2 phosphorylation. The increases in PDGFβ receptor and ERK1/2 phosphorylation were also dependent on protein kinase C activity, likely acting upstream of NADPH oxidase. Additionally, although the ROS scavenger N-acetyl-l-cysteine abrogated 5-HT-induced PDGFβ and TrkB receptor transactivation, it was unable to prevent 5-HT-induced ERK1/2 phosphorylation. Thus, the divergence point for 5-HT-induced receptor tyrosine kinase (RTK) transactivation and ERK1/2 phosphorylation occurs at the level of NADPH oxidase in this system. The ability of 5-HT to induce the production of ROS resulting in transactivation of both PDGFβ and TrkB receptors may suggest that instead of a single GPCR to single RTK pathway, a less selective, more global RTK response to GPCR activation is occurring.

Introduction

Serotonin (5-HT) is a tryptophan-derived signaling molecule best known for its role as a neurotransmitter [1]. In the central nervous system (CNS), it is involved with a variety of functions including circadian rhythm, mood, memory, and cognition [2–4]. The role of 5-HT in CNS pathology is of particular interest given the fact that there are several examples of clinically used drugs that target the 5-HT system for the treatment of depression, schizophrenia, and other CNS diseases [2,5]. 5-HT binds and activates seven different receptor subtypes including six G protein-coupled receptors (GPCRs) comprising subtypes 1-2 and 4-7, and 5-HT₁A, a ligand-gated ion channel [6].

The platelet-derived growth factor type β (PDGFβ) receptor is an important receptor tyrosine kinase (RTK) for the development of the CNS [7,8]. Four isoforms of PDGF ligands exist as hetero- or homodimers that bind to the extracellular ligand-binding domains of the receptor [9]. Ligand binding results in the dimerization and activation of the receptor, which triggers intracellular kinase domain-mediated trans-autophosphorylation of several tyrosine residues [7]. Multiple intracellular signaling pathways are initiated that result primarily in the promotion of cell growth [7], however the roles of PDGF signaling in the developed CNS have not been fully elucidated. In addition to direct ligand activation, RTKs like the PDGFβ receptor can be activated in a ligand-independent manner through a process known as transactivation. Transactivation of RTKs is initiated by the activation of GPCRs by ligands such as 5-HT [10,11], dopamine [12], angiotensin II [13], sphingosine-1-phosphate [14], lysophosphatidic acid [15], and leukotrienes [16]. The magnitude of activation of the PDGFβ receptor during transactivation (as measured by tyrosine phosphorylation) is typically much less than ligand-induced activation [10]. This may explain why ligand-induced activation results in rapid down-regulation of RTKs such as the PDGFβ receptor [9].
whereas down-regulation of transactivated PDGFB receptors has not been observed [10,17].

The receptor tyrosine kinase TrkB is activated by brain-derived neurotrophic factor (BDNF) and neurotrophin-4 as well as neurotrophin-3 [18]. TrkB receptors can also be transactivated by adenosine A3 receptors and many of the proteins involved in that pathway are similar to those required for 5-HT-induced transactivation of the PDGFB receptor [10,19,20]. One of the main components of the neurotrophic factor hypothesis of depression suggests that a reduction of neurotrophic factor signaling, including BDNF, contributes to synaptic dysregulation and neuronal dysfunction [18]. Conversely, the older monoamine hypothesis of depression posits that imbalances in serotonergic systems contribute to depression, with serotonin being the key dysregulated neurotransmitter [21]. A clearer understanding of the signaling relationships between the serotonergic, neurotrophic factor, and neuronal growth factor systems may provide insights into how these two hypotheses of depression could be reconciled.

We have previously shown that 5-HT-induced PDGFB receptor transactivation involves Gq-coupled 5-HT receptors including 5-HT1A receptors in SH-SY5Y cells [10]. This pathway was sensitive to PLC inhibition and intracellular, but not extracellular, calcium chelation [10]. Previous studies have suggested that ERK1/2 is phosphorylated as a downstream reactive oxygen species (ROS) in the transactivation of RTKs was sensitive to PLC inhibition and intracellular, but not extracellular, calcium chelation [10]. The current study investigates the role of reactive oxygen species (ROS) in the transactivation of RTKs in neurons. We demonstrate that PDGFB and TrkB receptors can be transactivated by 5-HT in neuronal cultures and that the transactivation of these RTKs requires ROS and NADPH oxidase activity, however 5-HT-induced ERK1/2 activation is not ROS-dependent.

Materials and Methods

Reagents and Antibodies

5-HT (5-hydroxytryptamine hydrochloride), N-acetyl-l-cysteine (L-α-acetamido-β-mercaptopropionic acid), diphenyleleniodonium chloride, AG 1296 (6,7-dimethoxy-2-phenylquinoxaline), Go 6983 (3-[1-[3-(dimethylamino)propyl]-5-methoxy-1H-indol-3-yl]-4-(1H-indol-3-yl)-1H-pyrrole-2,5-dione) and pertussis toxin were purchased from Santa Cruz (Santa Cruz, CA). Antibodies against β-actin, TrkB, PDGFβ receptor, and phospho-PDGFB receptor Y1021 were also purchased from Santa Cruz. Antibodies against phospho-TrkB Y816, ERK1/2 and phospho-ERK1/2 were purchased from Cedarlane.

SH-SY5Y cultures

SH-SY5Y cells were obtained as a generous gift from Dr. Shilpa Buch, University of Nebraska. Cultures were grown in complete growth media consisting of DMEM and Ham’s F12 in a 1:1 ratio, 10% fetal bovine serum (Sigma, Oakville, ON), and penicillin/streptomycin. Cultures were maintained in a humidified atmosphere of 95% air and 5% CO2 at a temperature of 37°C, with media changes every 3-5 days. For experimentations, cells were plated without antibiotics, and prior to drug treatments, cells were serum starved for 24 h.

Primary mouse cortical neuron cultures

CD-1 mouse embryos (Harlan, Indianapolis, IN) were removed at E17 to E19 and transferred to chilled dissection media (33 mM glucose, 58 mM sucrose, 30 mM HEPES, 5.4 mM KCl, 0.44 mM KH2PO4, 137 mM NaCl, 0.34 mM Na2HPO4, 4.2 mM NaHCO3, 0.03 mM phenol red, pH 7.4, 320-335 mOsm/kg). The brains were removed, and the cortex was dissected and trypsinized with 0.25% trypsin for 20 min at 37°C. Cells were then strained and plated on poly-l-lysine-coated culture dishes and grown at 37°C in a humidified atmosphere of 95% air and 5% CO2. Cells were plated with plating media (DMEM, supplemented with 10% fetal bovine serum, 10% horse serum) for the first 2-4 h until cells attached. Media were then replaced with feeding media consisting of Neurobasal medium and B-27 supplement (Life Technologies, Burlington, ON) without serum, and half of the media volume per well was changed twice per week. Drug treatments were performed 7-8 days after plating the cells. To prevent the overgrowth of non-neuronal cells, a mitotic inhibitor (81 μM 5-fluoro-2-deoxyuridine and 200 μM uridine added to media) was added for 24 h once cells reached confluence. All animal experiments were performed in strict accordance with the guidelines and policies on the Use of Animals at the University of Waterloo, and all efforts were made to minimize discomfort. The protocol was approved by the Waterloo Office of Research Ethics Animal Care Committee (Animal Utilization Project Proposal 09-17, 2009-2013).

Western blotting and data analysis

Following drug treatments, cells were washed once with ice-cold PBS. Chilled lysis buffer (20 mM Tris-HCl at pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 30 mM sodium pyrophosphate; 1 mM β-glycerophosphate; 1 mM sodium orthovanadate; 1% NP-40; supplemented with Halt Protease and Phosphatase Inhibitor (Thermo, Fisher, Pittsburgh, PA) prior to use) was added and lysates were homogenized and centrifuged at 13,000 x g for 20 min at 4°C. Supernatant protein concentration was determined using the BCA protein assay (Thermo, Fisher) and samples were normalized. Loading buffer (240 mM Tris-HCl at pH 6.8, 6% w/v SDS, 30% v/v glycerol, 0.02% w/v bromophenol blue, 50 mM DTT, 5% w/v β-mercaptoethanol) was added to samples, which were then heated for 15 min at 75°C. SDS-PAGE was used to separate proteins followed by transfer of proteins to nitrocellulose or PVDF membranes. 5% non-fat milk in Tris-buffered saline plus 0.1% Tween (TBS-T) was used to block membranes for 1 h at room temperature or overnight at 4°C. Membranes were then incubated with primary antibody for 1 h at room temperature or overnight at 4°C. Membranes were washed three times with TBS-T, and then incubated with secondary antibody conjugated to horse radish peroxidase (HRP) for 1 h at room temperature. Membranes were washed three additional times.
with TBS-T. Proteins were visualized with western chemiluminescent substrate (Millipore, Billerica, MA) on a Kodak 4000MM Pro Imaging Station. Kodak Molecular Imaging software was used for densitometric analyses of images and data statistics were evaluated with GraphPad Prism software with statistical significance set at p < 0.05. After imaging, membranes were stripped and re-probed with other antibodies.

### MTT cell viability assay

SH-SYSY cells were seeded at equal concentrations and grown to 90% confluency, followed by overnight serum starvation. After H$_2$O$_2$ treatments, media was changed to serum-free, phenol red-free DMEM/F12 and cultures were returned to the cell culture incubator for 24-48 h to allow mitochondrial enzyme deactivation in non-viable cells. MTT reagent (thiazolyl blue tetrazolium bromide: 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; Sigma) was then added to cell culture media, and plates were returned to the cell culture incubator for 2-4 h for the reaction to occur. Cells were then lysed and resulting crystals dissolved in solubilization buffer (0.1 M HCl, 10% Triton X-100 in propan-2-ol) on a gyratory plate shaker. Plates were read at 570 nm absorbance and background absorbance at 690 nm was subtracted from these values.

### Results

#### H$_2$O$_2$ increases PDGFβ receptor phosphorylation

We have previously shown that 5-HT increases PDGFβ receptor phosphorylation in both the neuroblastoma-derived SH-SYSY cell line and primary mouse cortical neuron cultures [10]. Based on transactivation pathways described in other cell types [11,24], we postulated that reactive oxygen species (ROS) are involved in the 5-HT-induced transactivation of neuronal PDGFβ receptors. Since H$_2$O$_2$ can cross the cell membrane [25,26], we analyzed a dose response of exogenously applied H$_2$O$_2$ to SH-SYSY cells for 5 min and observed peak tyrosine 1021 phosphorylation of PDGF receptor at a concentration of 0.1 µM (Figure 1A). This concentration was also sufficient to cause transactivation of PDGFβ receptors in primary mouse cortical neuron cultures (Figure 1B). To determine if 5-HT-induced transactivation of PDGFβ receptors involved the generation of endogenous ROS, we pretreated the cells with the ROS scavenger, N-acetyl-L-cysteine, followed by 100 nM 5-HT for 5 min (Figure 1C) (we previously determined that this concentration and incubation time of 5-HT resulted in maximal PDGFβ receptor transactivation in these cells [10]). N-acetyl-L-cysteine (1000 µM) was able to abrogate PDGFβ receptor phosphorylation, suggesting that ROS are indeed involved in 5-HT-induced PDGFβ receptors transactivation. Because H$_2$O$_2$ can cause cell damage and death at high concentrations, we verified that the low concentrations of H$_2$O$_2$ used here (particularly, the concentration of 0.1 µM) that induced PDGFβ receptor phosphorylation) were not adversely affecting cell viability. As determined by the MTT cell viability assay, we found that the cells were unaffected by H$_2$O$_2$ treatment after 30 min (Figure 1A) or overnight treatment (Figure 2B) at concentrations less than 100 µM.

### The role of NADPH oxidase in PDGFβ receptor transactivation

To investigate the source of ROS, we considered NADPH oxidase since it has been previously implicated in growth factor receptor transactivation in fibroblasts and keratinocytes [27,28]. Treatment with the NADPH oxidase inhibitors, diphenyleneiodonium chloride (1 µM and 10 µM) or apocynin (100 µM) blocked PDGFβ receptor transactivation by 5-HT (Figure 3A and 3B). In addition, NADPH oxidase components have been previously demonstrated to be activated by protein kinase C (PKC) [29], either directly or via Rap1A and Rac1/2 [30,31]. We have previously demonstrated that the PDGFβ receptor transactivation pathway initiated by 5-HT involves phospholipase C (PLC) activity and intracellular calcium [10], both of which could lead to the activation of calcium-dependent PKC isoforms [32]. When cells were pretreated with the PKC inhibitor Go 6983 (0.1 µM), 5-HT failed to transactivate the PDGFβ receptor (Figure 3C). These findings, coupled with our previous results, suggest that 5-HT treatment leads to the activation of PKC via PLC and intracellular calcium release, the assembly and activation of NADPH oxidase complex, the production of ROS, and ultimately the phosphorylation of PDGFβ receptor.

#### 5-HT also transactivates TrkB receptors

In addition to PDGF receptors, 5-HT receptors have been shown to trigger transactivation of fibroblast growth factor and epidermal growth factor receptors [33,34], but it is unknown if 5-HT can transactivate TrkB receptors, and whether ROS may be involved. Thus, we first determined whether TrkB phosphorylation is increased after H$_2$O$_2$ application. Indeed, similar to the PDGFβ receptor, TrkB phosphorylation at Y816 was increased in a dose-dependent manner with a maximum concentration of 0.1 µM H$_2$O$_2$ (Figure 4A). To determine if 5-HT could transactivate the TrkB receptor, we performed a time course of 5-HT application and, similar to the results with PDGFβ receptor transactivation, we observed maximum phosphorylation of the TrkB receptor after 5 min (Figure 4B). Given the similarity to PDGFβ receptor transactivation and the effect of H$_2$O$_2$ on TrkB receptor phosphorylation, we investigated whether 5-HT-induced TrkB receptor transactivation also required ROS. Indeed, pretreatment with N-acetyl-L-cysteine also blocked 5-HT-induced TrkB receptor transactivation (Figure 4C). Analogous to the 5-HT-PDGFB receptor transactivation pathway [10], 0.1 µg/ml pertussis toxin also blocked 5-HT-induced TrkB receptor phosphorylation (Figure 4D), indicating a dependence on a Gα$_i/coupled$ 5-HT receptor. Although our previous data showed that the PDGF receptor kinase inhibitor AG 1296 blocked PDGFβ receptor transactivation by 5-HT [10], it did not block TrkB receptor transactivation (Figure 4E), suggesting that TrkB transactivation was not dependent on changes in PDGFβ receptor activity.
The pathways for GPCR activation of ERK1/2 and RTK transactivation diverge at NADPH oxidase

ERK1/2 is activated downstream of several RTKs and GPCRs, and RTK transactivation pathways have been proposed as a mechanism for GPCR to ERK signaling [12,22,23]. We have previously shown that the pathways for 5-HT-induced ERK1/2 phosphorylation and PDGFB receptor transactivation are parallel: both involve Go, PLC, and intracellular calcium signaling [10]. However, these pathways must diverge at some point because PDGFB receptor phosphorylation is not required for 5-HT-induced changes in ERK1/2 activity [10]. Given the results described above, we sought to determine whether 5-HT-induced ERK1/2 phosphorylation similarly involved ROS and NADPH oxidase.
Figure 2. H$_2$O$_2$ concentrations sufficient for inducing PDGFβ receptor phosphorylation do not result in cell death. SH-SY5Y cells were treated with 0, 0.1, 1, 10, 100, or 1000 µM H$_2$O$_2$ for (A) 30 min, or (B) overnight. Following treatment with MTT reagents and lysis, cell viability was measured and compared to control (VEH) values. (Data are representative of 4 independent experiments. * = p < 0.05 compared to vehicle-treated cells, one-way ANOVA, Tukey post-test).

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When SH-SY5Y cells were treated with H$_2$O$_2$, no significant increase in ERK1/2 phosphorylation was observed at any concentration tested (Figure 5A). H$_2$O$_2$ treatment also failed to induce ERK1/2 phosphorylation in primary cortical neurons (data not shown). Furthermore, in contrast to its ability to block 5-HT-induced PDGFβ and TrkB receptor phosphorylation, pretreatment with N-acetyl-L-cysteine had no effect on 5-HT-induced ERK1/2 phosphorylation (Figure 5B). However, the
NADPH oxidase inhibitors, diphenyleneiodonium chloride and apocynin, as well as the PKC inhibitor Go 6983, blocked 5-HT-induced ERK1/2 phosphorylation (Figure 5C-E). This suggests that the divergence point for ERK1/2 phosphorylation and RTK transactivation occurs at or after NADPH oxidase, but upstream of ROS production (Figure 6).

Discussion

The current report adds to a growing number of studies that have implicated ROS in the transactivation of RTKs [11,35,36]. There are several similarities in the pathways described for both 5-HT and ROS-induced increases in RTK phosphorylation. In both pathways, the phosphorylation of TrkB and PDGFR receptors follows a similar dose response, and achieves a similar maximum fold change in phosphorylation compared to baseline. This, along with the ability of the ROS scavenger N-acetylcysteine to abrogate transactivation, suggests that ROS is a component of 5-HT-initiated transactivation pathways, and possibly other transactivation pathways as well. One of the striking differences between transactivation and direct ligand activation of the PDGF β receptor is that the application of high concentrations of PDGF-BB can induce 10 to 100-fold increases in receptor phosphorylation [10] whereas for both 5-HT- and H$_2$O$_2$-
mediated transactivation of PDGFβ receptor, the maximum observed increase in phosphorylation is only 1.5-2 fold.

Although we have identified ROS as being required for the transactivation of PDGFβ and TrkB receptors, the mechanism whereby ROS ultimately leads to increases in the phosphorylation state of the RTKs remains unknown. Some studies suggest that low levels of ROS act as second messengers capable of participating in intracellular signaling pathways [37,38]. ROS have the ability to oxidize catalytic cysteine residues in tyrosine phosphatase enzymes, such as the RTK phosphatase SHP-2, and the result of this oxidation is phosphatase inactivation [39,40]. These phosphatases possess a microenvironment that lowers the pK_a of the catalytic cysteine residue from the expected value of 8.5 to less than 5.5, sufficient for the thiol group to exist as a thiolate ion at physiological pH and to be sensitive to H_2O_2-induced oxidation [37]. This phosphatase inactivation is readily reversible and short-lived [39], which may explain why, if phosphatase inactivation is involved in RTK transactivation, the transactivation is transient [10]. Additional evidence supporting a role for SHP-2 in transactivation suggests that a knockdown of SHP-2 results in a greater basal phosphorylation of the epidermal growth factor receptor [39]. Since inhibition of PDGFβ receptor kinase activity in our system also abrogated 5-

![Figure 5](image-url)

**Figure 5.** 5-HT induced ERK1/2 phosphorylation diverges from the transactivation pathway at or after NADPH oxidase. (A) SH-SY5Y cells were treated with 0.01 to 100 µM H_2O_2 for 5 min. Following drug treatments, cell lysates were evaluated by Western blot analysis as described in Materials and Methods. Data were normalized to total ERK1/2 protein expression and are expressed as the fold change (average ± S.E.M.) in phospho-ERK immunoreactivity compared to vehicle-treated cells. (B) SH-SY5Y cell cultures were pretreated with vehicle or 10, 100 or 1000 µM of the ROS scavenger N-acetyl-L-cysteine (NAC) for 45 min followed by treatment with vehicle or 100 nM 5-HT for 5 min and lysates were evaluated as in “A”. Cell cultures were also pretreated with vehicle or the NADPH oxidase inhibitor diphenyleneiodonium chloride (DPI) (C) or apocynin (D) for 45 min followed by treatment with vehicle or 100 nM 5-HT for 5 min, and results were analyzed for phospho-ERK1/2 as described above. Representative blots of phospho-ERK1/2 and total ERK1/2 at 42 and 44 kDa are shown. (Data are representative of 4-6 independent experiments. * = p < 0.05 compared to vehicle-treated cells; # = p < 0.05 compared to 5-HT-treated cells, one-way ANOVA, Tukey post-test).

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HT-induced PDGFβ receptor transactivation [10], we suspect that an increase in basal phosphorylation mediated by the receptor’s own kinase activity is responsible for the increase in phosphorylation observed, rather than through the action of a different kinase.

Since H₂O₂ has been implicated in the transactivation pathway of several RTKs, including PDGFβ and TrkB receptors shown here, it is conceivable that the physiological relevance of ROS in transactivation may ultimately consist of phosphorylating multiple RTKs via phosphatase inactivation, rather than specific single GPCR to single RTK pathways. If so, the sum of multiple small increases in RTK activation could lead to a greater increase in overall cellular RTK activity and the activation of their intracellular signaling pathways. The identification of ROS in transactivation pathways may also be an endogenous protective mechanism whereby an initial, mild cell stress and production of ROS protects the cell against subsequent more severe insults (and higher, toxic levels of ROS) by promoting the mitogenic effects of multiple RTKs. However, some studies show NADPH oxidase assembles and functions in the cytoplasm, possibly in a vesicle or endoplasmic reticulum [49,50], which would result in intracellular ROS accumulation [51–53].

Our study failed to detect H₂O₂-induced increases in ERK1/2 phosphorylation, an observation that contradicts previous work showing that exogenously applied H₂O₂ results in ERK1/2 phosphorylation [54–56]. However, those reports used H₂O₂ concentrations between 0.1 and 2 mM – at least 100-fold higher than the concentrations used here. The low concentrations of H₂O₂ used in this study compared to other systems may not be sufficient to induce ERK1/2 phosphorylation, suggesting ROS is not required for ERK1/2 activation. This is further corroborated by the ROS scavenger N-acetyl-L-cysteine being able to block RTK phosphorylation, but not ERK1/2 phosphorylation, induced by 5-HT. Conversely, the NADPH oxidase inhibitors apocynin and diphenyleneiodonium chloride were able to inhibit ERK1/2 activation. These drugs may be preventing the assembly of the oxidase or chemically modifying the subunits [57,58], suggesting that the complete, functional oxidase is necessary for both PDGFβ receptor transactivation and ERK1/2 activation.

Figure 6. Mechanism of PDGFβ and TrkB receptor transactivation. Gqα-coupled GPCRs such as 5-HT₁A initiate transactivation signaling, which gets relayed through Ga or Gβγ subunits. PLC activation results in intracellular calcium release and activation of PKC. The NADPH oxidase subunits subsequently assemble and produce ROS. Active NADPH oxidase is required for both 5-HT-induced RTK and ERK1/2 phosphorylation but only endogenous ROS (or exogenous H₂O₂) is involved in RTK transactivation.

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activation. Since the subunit Rac1 has been shown to activate MEK and subsequently ERK1/2 [31,59], it is conceivable that these drugs may be inhibiting the activity of subunits such as Rac1 and thus prevents both NADPH oxidase function and the phosphorylation and activation of ERK1/2.

We also show for the first time that 5-HT is capable of transactivating TrkB receptors. Like PDGFβ receptor transactivation [10], TrkB transactivation is sensitive to pertussis toxin, therefore it is dependent on Gαi[14], and may represent a general mechanism for the dependency of transactivation on Gαi[14], and may represent a general mechanism for pertussis toxin, therefore it is dependent on Gαi[14], and may represent a general mechanism for cross-talk between 5-HT receptors and multiple RTKs may suggest that transactivation is a global pathway responsible for mitogenic or protective effects. In addition, the idea that serotonergic stimuli can activate neurotrophic factor and neuronal growth factor receptors brings together two major hypotheses for the pathophysiology of depression. Given that monoamine and neurotrophic hypotheses both propose a dysregulation in their respective signaling pathways as causes for clinical depression [21,61], it is possible that 5-HT-induced transactivation may improve symptoms by activating both serotonergic and neurotrophic signaling in the CNS.

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Author Contributions

Conceived and designed the experiments: JSK MAB. Performed the experiments: JSK MSV. Analyzed the data: JSK. Contributed reagents/materials/analysis tools: MAB. Wrote the manuscript: JSK MSV JJH MAB.

References

1. Steiner JA, Carneiro AM, Blakely RD (2008) Going with the flow: trafficking-dependent and -independent regulation of serotonin transport. Traffic 9: 1393-1402. doi:10.1111.j.1600-0854.2008.00757.x. PubMed: 18445122.
2. Filip M, Bader M (2009) Overview on 5-HT receptors and their role in physiology and pathology of the central nervous system. Pharmacol Rep 61: 761-777. PubMed: 19903999.
3. Monti JM, Jantsch H (2008) The roles of dopamine and serotonin, and of their receptors, in regulating sleep and waking. Prog Brain Res 172: 625-646. doi:10.1016/S0079-6123(08)00925-1. PubMed: 18772593.
4. Geldenhuys WJ, Van der Schyf CJ (2009) The serotonin 5-HT6 receptor: a viable drug target for treating cognitive deficits in Alzheimer’s disease. Expert Rev Neurother 9: 1073-1085. doi:10.1586/er.09.51. PubMed: 19589055.
5. Alex KD, Pehck EA (2007) Pharmacologic mechanisms of serotoninergic regulation of dopamine neurotransmission. Pharmacol Ther 113: 296-320. doi:10.1016/j.pharmacotherapy.2006.08.004. PubMed: 17049611.
6. Hannon J, Hoyer D (2008) Molecular biology of 5-HT receptors. Behav Brain Res 195: 198-213. doi:10.1016/j.bbr.2008.03.020. PubMed: 18571247.
7. Heldin CH, Ostman A, Rönntrand L (1998) Signal transduction via platelet-derived growth factor receptors. Biochim Biophys Acta 1378: F79-113. PubMed: 9739761.
8. Alvarez RH, Kantarjian HM, Cortes JE (2006) Biology of platelet-derived growth factor and its involvement in disease. Mayo Clin Proc 81: 1241-1257. doi:10.4065/81.9.1241. PubMed: 16970222.
9. Heldin CH, Westermark B (1999) Mechanism of action and in vivo role of platelet-derived growth factor. Physiol Rev 79: 1283-1316. PubMed: 10508235.
10. Kruk JS, Vaseli MS, Liu H, Heikkila JJ, Beazely MA (2013) 5-HT1A(1) receptors transactivate the platelet-derived growth factor receptor type beta in neuronal cells. Cell Signal 25: 133-143. doi:10.1016/j.cellsig.2012.09.021. PubMed: 23006663.
11. Liu Y, Li M, Warburton RR, Hill NS, Fanburg BL (2007) The 5-HT transporter transactivates the PDGFbeta receptor in pulmonary artery smooth muscle cells. FASEB J 21: 2725-2734. doi:10.1096/fj.06-6805com. PubMed: 17504974.
12. Oak JN, Lavine N, Van Tol HH (2001) Dopamine D(4) and D(2L) Receptor Stimulation of the Mitogen-Activated Protein Kinase Pathway Is Dependent on trans-Activation of the Platelet-Derived Growth Factor Receptor. Mol Pharmacol 60: 92-103. PubMed: 11408604.
13. Heeneman S, Haendeler J, Saito Y, Ishida M, Berk BC (2000) Angiotensin II induces transactivation of two different populations of the platelet-derived growth factor beta receptor. Key role for the p66 adaptor protein Shc. J Biol Chem 275: 15926-15932. doi:10.1074/jbc.M909616199. PubMed: 10748142.
14. Tanimoto T, Lungu AO, Berk BC (2004) Sphingosine 1-phosphate transactivates the platelet-derived growth factor beta receptor and epidermal growth factor receptor in vascular smooth muscle cells. Circ Res 94: 1050-1058. doi:10.1161/01.RES.0000126404.41421.BE. PubMed: 15044318.
15. Goppelt-Stuebe M, Fickel S, Reiser CO (2000) The platelet-derived-growth-factor receptor, not the epidermal-growth-factor receptor, is used by lysophosphatidic acid to activate p42/44 mitogen-activated protein kinase and to induce prostaglandin G/H synthase-2 in mesangial cells. Biochem J 345 2: 217-224. doi:10.1042/0264-6021:3450217.
16. McMillan B, Mitchell D, Shatlock R, Martin F, Brady HR et al. (2002) Lipoxin, leukotriene, and PDGF receptors cross-talk to regulate mesangial cell proliferation. FASEB J 16: 1817-1819. PubMed: 12223454.
17. Vaseli MS, Kruk JS, Liu H, Heikkila JJ, Beazely MA (2012) Activation of 5-HT7 receptors increases neuronal platelet-derived growth factor beta receptor expression. Neurosci Lett 511: 65-69. doi:10.1016/j.neulet.2012.01.016. PubMed: 22285262.
18. Rantamäki T, Castrén E (2008) Targeting TrkB neurotrophin receptor to treat depression. Expert Opin Ther Targets 12: 705-715. doi:10.1517/14728222.12.6.705. PubMed: 18479217.
19. Lee FS, Chao MV (2001) Activation of Trk neurotrophin receptors in the absence of neurotrophins. Proc Natl Acad Sci U S A 98: 3555-3560. doi:10.1073/pnas.0705267104. PubMed: 11248116.
20. Wiese S, Jablonka S, Holtmann B, Orel N, Rajagopal R et al. (2007) Adenosine receptor A2A-R contributes to motoneuron survival by transactivating the tyrosine kinase receptor TrkB. Proc Natl Acad Sci U S A 104: 17210-17215. doi:10.1073/pnas.0705267104. PubMed: 17940030.
21. Hirschfeld RM (2000) History and evolution of the monamine hypothesis of depression. J Clin Psychiatry 61 Suppl 6: 4-6. PubMed: 10775017.
22. Clark MA, Gonzalez N (2007) Angiotensin II stimulates rat astrocyte mitogen-activated protein kinase activity and growth through EGFr and PDGF receptor transactivation. Regul Pept 144: 115-122. doi:10.1016/j.regpep.2007.07.001. PubMed: 17688958.
23. Gill RS, Hsung MS, Sum CS, Lavine N, Clark SD et al. (2010) The dopamine D4 receptor activates intracellular platelet-derived growth...
factor receptor beta to stimulate ERK1/2. Cell Signal 22: 285-290. doi: 10.1016/j.cellsig.2009.08.031. PubMed: 19782189.

24. Chen K, Thomas SR, Albano A, Murphy MP, Keaney JF Jr. (2004) Mitochondrial function is required for hydrogen peroxide-induced growth factor receptor transactivation and downstream signaling. J Biol Chem 279: 35079-35086. doi:10.1074/jbc.M404859200. PubMed: 15180891.

25. Onno Y, Gallin JI (1985) Diffusion of extracellular hydrogen peroxide into intracellular compartments of human neutrophils. Studies utilizing the inactivation of myeloperoxidase by hydrogen peroxide and azide. J Immunol 135: 2138-2144. PubMed: 3938829.

26. Bienert GP, Schjoerring JK, Jahn TP (2006) Membrane transport of hydrogen peroxide. Biochim Biophys Acta 1758: 994-1003. doi: 10.1016/j.bbamem.2006.02.015. PubMed: 16566894.

27. Catarzi S, Giannoni E, Faviili F, Meacci E, Iantomassi T et al. (2007) Sphingosine-1-phosphate stimulation of NADPH oxidase activity: relationship with platelet-derived growth factor receptor and c-Src kinase. Biochim Biophys Acta 1770: 872-883. doi:10.1016/j.bbaben.2007.01.008. PubMed: 17349748.

28. Tseng HY, Liu ZM, Huang HS (2012) NADPH oxidase-produced superoxide mediates EGFR transactivation by c-Src in arsenic trioxide-stimulated human keratinocytes. Arch Toxicol 86: 935-945. doi: 10.1007/s00204-012-0586-9. PubMed: 22352027.

29. El-Benna J, Dang PM, Gougerot-Pocidalo MA (2008) Priming of the neutrophil NADPH oxidase activation: role of p47(phox)-phosphorylation and NOX2 mobilization to the plasma membrane. Semin Immunopathol 30: 279-289. doi:10.1007/s00281-008-0118-3. PubMed: 18536919.

30. Sheppard FR, Kelher MR, Moore EE, McLaughlin NJ, Banerjee A et al. (2005) Structural organization of the neutrophil NADPH oxidase: phosphorylation and translocation during priming and activation. J Leukoc Biol 78: 1025-1042. doi:10.1189/jlb.0804442. PubMed: 16204621.

31. Zhang J, Anastasiadis PZ, Liu Y, Thompson EA, Fields AP (2004) Protein kinase C (PKC) betall induces cell invasion through a Ras/MEK-PKC iota/Rac 1-dependent signaling pathway. J Biol Chem 279: 22118-22123. doi:10.1074/jbc.M404774200. PubMed: 15037605.

32. Mellor H, Parker PJ (1998) The extended protein kinase C superfamily. Biochem J 332 (Pt 2): 281-292. PubMed: 9601053.

33. Tsuchioka M, Takebayashi M, Hisaoka K, Maeda N, Nakata Y (2008) Serotonin (5-HT) induces glial cell line-derived neurotrophic factor receptor activation. Cell Signal 23: 354-362. doi: 10.1016/j.cellsig.2010.02.006. PubMed: 20231105.

34. Raad H, Paclet MH, Boussetta K, Kovriyskiy M, Vore F et al. (2009) Regulation of the phagocyte NADPH oxidase activity: phosphorylation of gp91phox/NOX2 by protein kinase C enhances its diaphorase activity and binding to Rac2, p67(phox), and p47(phox). FASEB J 23: 1011-1022. doi:10.1096/fj.08-114553. PubMed: 19028840.

35. Pipeleyn A, Dang PH, Gougerot-Pocidalo MA, El-Benna J (2002) Phosphorylation of p47(phox) sites by PKC alpha, beta II, delta, and zeta: effect on binding to p22(phox) and on NADPH oxidase activity. Biochemistry 41: 7743-7750. doi:10.1021/bi011953a. PubMed: 12056906.

36. Bayraktutan U, Blayney L, Shah AM (2000) Molecular characterization and localization of the NAD(P)H oxidase components gp91-phox and p22-phox in endothelial cells. Arterioscler Thromb Vasc Biol 20: 1903-1911. doi: 10.1161/01.ATV.20.8.1903. PubMed: 10939010.

37. Li JM, Shah AM (2002) Intracellular localization and preassembly of the NADPH oxidase complex in cultured endothelial cells. J Biol Chem 277: 19952-19960. doi:10.1074/jbc.M110073200. PubMed: 11893732.

38. Dusting GJ, Selemidis S, Jiang F (2005) Mechanisms for suppressing NADPH oxidase in the vascular wall. Mem Inst Oswaldo Cruz 100 Suppl 1: 97-103. doi:10.1590/S0074-02762005000100018. PubMed: 15852105.

39. Kieniewska P, Piechota A, Skibska B, Gorcza A (2012) The NADPH oxidase family and its inhibitors. Arch Immunol Ther Exp 60: 277-294. doi:10.1007/s00005-012-0178-z. PubMed: 22669046.

40. Moldovan L, Moldovan N, Sohn RH, Parikh SA, Goldschmidt-Clermont PJ (2000) Redox changes of cultured endothelial cells and actin dynamics. Circ Res 86: 549-557. doi:10.1161/01.RES.86.6.549. PubMed: 10720417.

41. Hu Y, Kang C, Philip RJ, Li B (2007) PKC delta phosphatases p55 and p56(p21) are essential for ERK activation in response to H2O2. Cell Signal 19: 410-418. doi:10.1016/j.cellsig.2006.07.017. PubMed: 16963324.

42. Kim YK, Bae GU, Kang JK, Park JW, Lee EK et al. (2006) Cooperation of H2O2-mediated ERK activation with Smad pathway in TGF-beta1 induction of p21WAF1/Cip1. Cell Signal 18: 236-243. doi:10.1016/j.cellsig.2005.04.008. PubMed: 15979845.

43. Mbong N, Anand-Srivastava MB (2012) Hydrogen peroxide enhances the expression of Glial proteins in aortic vascular smooth cells: role of growth factor receptor transactivation. Am J Physiol Heart Circ Physiol 302: H1591-H1602. doi:10.1152/ajpheart.00627.2011. PubMed: 22268112.

44. O'Donnell BV, Tew DG, Jones OT, England PJ (1993) Studies on the inhibitory mechanism of iodonium compounds with special reference to neutral NADPH oxidase. Biochem J 290(1): 41-49.

45. Stefanska J, Pawlickcz R (2008) Apocynin: molecular aptitudes. Mediat Inflamm. 2008: 2008:106507. PubMed: 19096513.

46. Eblen ST, Slack JK, Weber MJ, Catling AD (2002) Rac-PAK signaling of growth factor receptor transactivation. Am J Physiol Heart Circ Physiol 283: H1591-H1602. doi:10.1152/ajpheart.00627.2011. PubMed: 22268112.

47. Casrtn E, Rantamäki T (2010) The role of BDNF and its receptors in developmental plasticity. Dev Neurobiol 70: 289-297. doi:10.1002/dneu.20758. PubMed: 21096717.